The Histone Acetyltransferase TIP60 Interacts with c-Myb and Inactivates Its Transcriptional Activity in Human Leukemia*

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Background: Transcription factors regulate their target genes by recruiting histone-modifying proteins. Results: Histone acetyltransferase TIP60 binds transcription factor c-Myb to suppress c-Myb activity. Conclusion: c-Myb activity is regulated by TIP60. Significance: These findings broaden the understanding of how c-Myb is regulated and suggest that dysregulated or mutated TIP60 may contribute to leukemogenesis.

The histone acetyltransferase TIP60 is a coregulator of transcription factors and is implicated in tumorigenesis. In this study, we explored potential regulatory relationships between TIP60 and the c-Myb oncprotein in hematopoietic cells. We first showed that TIP60 is a c-Myb interacting protein and that the interaction is dependent on the TIP60 acetyltransferase domain and c-Myb transactivation domain. We then found that coexpression of TIP60 decreases the transcriptional activation ability of c-Myb in functional reporter assays. A ChIP assay also revealed that TIP60 binds to the c-Myb target gene c-Myc promoter in a c-Myb-dependent manner. Consistently, knockdown of Tip60 expression by siRNA increased endogenous c-Myc expression. Furthermore, coimmunoprecipitation of Jurkat cell lysates revealed that c-Myb is associated with histone deacetylases HDAC1 and HDAC2, known to interact with TIP60 and repress transcription. Finally, we compared Tip60 expression in six primary AML samples with three normal CD34+ cell samples using quantitative RT-PCR. Tip60 expression was significantly (~60%) lower in the AML samples. In summary, these studies demonstrate that TIP60 negatively modulates c-Myb transcriptional activity by recruiting histone deacetylases in human hematopoietic cells, leading us to hypothesize that TIP60 is a normal regulator of c-Myb function and that dysregulated or mutated TIP60 may contribute to c-Myb-driven leukemogenesis.

TIP60 (originally identified as Tat-interactive protein, 60 kDa) is a member of the MOZ, Ybf2/Sas3, Sas2, and TIP60 (MYST) family of histone acetyltransferases (HAT) (1), characterized by the presence of a conserved MYST domain that comprises both the C2HC nucleosome-binding domain and the putative HAT domain (2). TIP60 plays diverse physiological roles in many processes. It forms stable nuclear complexes that promote histone acetylation in nucleosomes (3). TIP60 associates with a growing number of transcription factors, either activating gene expression through its intrinsic HAT activity (4–6), or acting as a corepressor by the recruitment of histone deacetylases (HDAC) (7), or through interactions with the transcriptional repressor ZEB (zinc finger E box-binding protein) (8). TIP60 also interacts with the kinases ataxia-telangiectasia mutated (ATM) and DNA-dependent protein kinase catalytic subunit (DNA-Pkcs) and participates in DNA damage responses (9). In addition, a recent large-scale RNAi screen revealed that TIP60 is required for the maintenance of embryonic stem cell identity (10). Furthermore, loss of TIP60 is linked with a growing number of cancer types (11).

The proto-oncogene c-Myb is the normal cellular homologue of v-Myb, the transforming oncogene of avian myeloblastosis (AMV) and avian leukemia virus E26 (12). Located on chromosome arm 6q in humans, the transcript of c-Myb encodes a transcription factor, c-Myb, which recognizes the consensus nucleotide sequence 5'-PyAAC(G/Py)G-3' (13). c-Myb plays a major role in regulating G1/S transition in cycling hematopoietic cells (14), and functions as a transactivator of a number of important cellular genes such as mim-1, c-Myc, cdk2, Bcl-2, c-kit, Neuromedin U (NmU), CCNB1 (Cyclin B1), and GATA-3 (15).

c-Myb has long been postulated to play an important role in leukemogenesis on the basis of studies which have shown that activation of c-Myb by truncation at either the carboxyl or amino terminus, or both can induce acute myeloid leukemias in avian species and mouse or B-cell lymphomas in chicken (16, 17). Aberrant expression of c-Myb has also been associated with human malignancies, including colon carcinoma and leukemia, in particular human acute T-cell leukemia (18), though its role in disease causation has been less precisely defined. In addition, c-Myb is an essential factor for homeobox- and p210 BCR-ABL-mediated transformation of hematopoietic cells (19, 20). Further evidence that c-Myb is involved in tumorigenesis is found in its ability to drive MLL-associated leukemogenesis.
TIP60 Suppresses c-Myb Function

(21). We speculated that c-Myb functions with other proteins in a complex regulatory network that includes TIP60. In this study, we report that c-Myb activity is indeed subject to regulation by TIP60 and that this mechanism of regulation is likely of physiologic relevance for leukemia development.

EXPERIMENTAL PROCEDURES

Vectors and Reagents—We are grateful to Dr. H. Lee for pCMV2-FLAG-wild-type TIP60, Dr. C. Robson for pcDNA-FLAG-HAT-deficient TIP60 (Q377E/G380E, F-TIP60m), Dr. E. Seto for the HDAC2 plasmid, and Dr. R. Tsai for the myc-tagged p53 vector. pcDNA3-HA-c-Myb and deletions were described previously (21).

AML Samples—The AML samples and CD34+ cells purified from healthy donors were obtained from the Stem Cell and Xenograft Core of the University of Pennsylvania.

Cell Culture—Human embryonic kidney 293T (HEK293T) cells were maintained in DMEM (Invitrogen) supplemented with 10% FBS (HyClone Laboratories, Logan, UT). HL-60, K562, and Jurkat cells were grown in RPMI 1640 medium supplemented with 10% FBS and 2 mM glutamine. K562-MERT clones expressing the DNA-binding domain of c-Myb fused to a Drosophila engrailed transrepressor and a modified mouse estrogen receptor have been described elsewhere (22).

Quantitative Real-time PCR—Total RNAs were extracted using the RNeasy mini kit (Qiagen, Valencia, CA), and complementory DNA synthesis was performed using the Superscript™ reverse transcriptase (Invitrogen) following the manufacturer’s instructions. Quantitative PCR was carried out in an ABI 7900 HT sequence detection system using TaqMan master mix and the protocol of the manufacturer (Applied Biosystems, Foster City, CA). All data were normalized using the endogenous GAPDH control. The primers for detection of the expression c-Myc, TIP60 were from Qiagen. The primers for GAPDH and c-Myb have been described previously (23).

Western Blot Analyses—Cells were lysed in radioimmunoprecipitation assay buffer (1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) with the addition of the protease inhibitors. 50 μg of extracts were loaded onto polyacrylamide gels for electrophoresis, and proteins were transferred to Immobilon-P transfer membranes (Millipore, Bedford, MA). Western blot analyses were revealed using the enhanced chemiluminescence method (Amersham Biosciences, Fairfield, CT). Antibodies used in this study were anti-FLAG (Sigma); anti-TIP60; anti-Actin; anti-Myc (9E10); anti-AcK (acetylated lysine); anti-HDAC1, 2, 3, 4, 5, 7 (Cell Signaling Technology); and anti-c-Myb (Millipore).

ChIP Assay—ChIPs were performed with the chromatin immunoprecipitation assay kit (Upstate Biotechnology) using the protocol recommended by the manufacturer. ChIP DNA was detected using quantitative PCR or standard PCR and staining with ethidium bromide after electrophoresis in 2% agarose gels with c-Myc promoter-specific primers that flanked the c-Myb binding site (forward, 5′-AAAAGGGGAAAGAGGAC-CTGG-3′; reverse, 5′-CCTAAAGGGGCAAGTGAGAG-3′) or GAPDH promoter-specific primers as a control (forward, 5′-TACTAGCCTTTTACGGGC-3′; reverse, 5′-TCGAA-CAGGACGCAGAGGCGA-3′). ChIP assays were also completed with anti-acetyl-histone H4 as a positive control and normal mouse or rabbit IgG as a negative control.

Coimmunoprecipitation—K562 cells (2 × 10⁶), Jurkat cells (2 × 10⁶), or HEK293T cells (2 × 10⁶) transfected with various combinations of expression plasmids (4 μg each) by using nucleofection or FuGENE 6 were harvested, washed twice with ice-cold PBS, and lysed in 800 μl of lysis buffer (20 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 0.5% Nonidet P-40) containing 1 mM sodium orthovanadate, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 1% protease inhibitor mixture (Sigma-Aldrich) for 15 min on ice. Cell lysates were centrifuged at 13,000 × g for 10 min at 4 °C, and the protein content was determined using the bicinchoninic acid assay (Pierce). Total cell lysate (500 μg) was precleared with protein G-agarose beads and mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C for 1 h. c-Myb, FLAG-tagged, and Myc-tagged proteins were then immunoprecipitated using the anti-c-Myb anti-FLAG or anti-Myc mAb combined with the protein G-agarose beads. The agarose beads were then washed four times with the washing buffer (20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1% Nonidet P-40) and subjected to SDS-PAGE. The proteins were transferred to an Immobilon-P transfer membrane (Millipore), and the blots were probed with antibodies as indicated.

Dual Luciferase Assay—HEK293T transfactions and all luciferase assays were performed as described in (12).

Knockdown Experiments—K562 (1 × 10⁶) cells were transfected with 1 μg of either control siRNA (D-001210-01, Dharmacon), human TIP60 siRNA (sc-37966, Santa Cruz Biotechnologies), human HDAC1 siRNA (sc-29343, Santa Cruz Biotechnologies), human HDAC2 siRNA (sc-29345, Santa Cruz Biotechnologies), human HDAC3 siRNA (sc-35538, Santa Cruz Biotechnologies) or siRNA for c-Myc targeting 5′-UAU-CAGUUUGCUACGGAAGC-3′ with an Amaxa (Gaithersburg, MD) nucleofector.

In Vitro Acetylation Reaction—Acetylation assays were carried out according to the published method (24). Reaction mixture (50 μl) contained 50 mM Tris HCl (pH 8.0), 10% glycerol (v/v), 0.1 mM EDTA, 1 mM DTT, 10 mM Na butyrate, [3H]acetyl coenzymeA or 500 nM acetyl CoA (GE Healthcare), in vitro translated FLAG-Tip60, or FLAG-c-Myb, or a combination of the two proteins (produced from 2 μg of plasmid for each by a standard in vitro translation reaction from Promega). The mixture was then incubated at 30 °C for 60 min and analyzed on SDS/PAGE afterward. Acetylated proteins were detected by autoradiography. The expression of FLAG-TIP60 and FLAG-c-Myb were confirmed by Western blot analysis using anti-FLAG antibody. Myc-tagged p53 was used as the positive control substrate for acetylation by TIP60.

Statistical Analysis—The data are expressed as the mean ± S.D. The significance of differences between the groups compared was determined by Student’s t test. A p value of less than 0.01 or 0.05 was considered statistically significant.

RESULTS

TIP60 Interacts with c-Myb—TIP60 has been reported to associate with a growing number of transcription factors, modulating their transcriptional activities. To explore whether TIP60 can regulate c-Myb transcription activity, we first inves-
not interact with F-TIP60m (Fig. 1A) and was also absent in cells lacking F-TIP60. Furthermore, the endogenous c-Myb and TIP60 proteins interact in hematopoietic cell lines. Thus, endogenous TIP60 was present in immunoprecipitates of endogenous c-Myb from either K562 (data not shown) or Jurkat cell nuclear extracts (Fig. 1B), demonstrating that these proteins interact at physiological levels of protein expression. Together, these results provide strong evidence of an interaction between TIP60 and c-Myb in hematopoietic cells. To address whether TIP60 interacts directly with c-Myb, both proteins were in vitro translated with or without a FLAG tag. The translated proteins were then combined in an in vitro binding assay. Under these conditions, c-Myb again coimmunoprecipitated with F-TIP60 but was not present in immunoprecipitates from the empty vector control sample (Fig. 1C), suggesting that TIP60 directly interacts with c-Myb.

The c-Myb Transcription Activation Domain and TIP60 MYST Domain Are Required for the Association of c-Myb and TIP60—c-Myb comprises a DNA binding domain, a transcription activation domain, and a C-terminal negative regulatory domain. To determine which subdomain of c-Myb is responsible for the interaction with TIP60, we cotransfected a panel of c-Myb expression constructs together with TIP60 expression vectors in HEK293T cells. As summarized in Fig. 2A (Western blot analyses shown in B and C), deletion of the DNA binding domain or the negative regulatory domain has no effect on the ability of c-Myb to bind TIP60. In contrast, c-Myb fragments containing the transcription activation domain strongly interacted with TIP60, indicating that TIP60 interacts with the transcription activation domain within a region spanning residues 194 to 325. As mentioned above, mutations in the MYST domain within TIP60 disrupted the interaction between c-Myb and TIP60, suggesting that the MYST domain is necessary for its binding to c-Myb. This prompted us to test whether MYST is sufficient for the association. To this end, we used four additional FLAG-tagged truncations of TIP60, F-TIP60N (residues 1–258), which includes the chromodomain F-TIP60C (residues 259–513), F-TIP60M (residues 259–389), which spans the MYST domain, and F-TIP60C (residue 389–513). These constructs were cotransfected with c-Myb into HEK293T cells, and immunoprecipitations were carried out with anti-FLAG antibody. c-Myb coimmunoprecipitated when the MYST domain was present (Fig. 2E). This result further suggests that, although the chromodomain of TIP60 appears to be dispensable, the MYST domain of TIP60 is necessary and sufficient for the interaction between c-Myb and TIP60.

c-Myb Is Not a Substrate of TIP60—Because TIP60 acetylates and regulates the function of non-histone transcription factors such as c-Myc and p53 (1), we tested whether TIP60 could acetylate c-Myb, a posttranslational event known to modulate c-Myb activity. F-c-Myb and F-TIP60 proteins were generated by in vitro translation (Fig. 3A, left panel) and the translated proteins were mixed for the acetylation reactions in the presence of [3H]-acetyl-CoA. As shown in Fig. 3A, bands corresponding to the size of radioactively labeled TIP60 proteins were apparent (lanes 4 and 7), but a corresponding acetylated band for c-Myb was not detected, indicating that c-Myb is not a

FIGURE 1. c-Myb interacts with TIP60. A, HEK293T cells were cotransfected with HA-c-Myb and FLAG-tagged empty vector or FLAG-tagged TIP60 (F-TIP60) or a FLAG-tagged TIP60 mutant (F-TIP60m), respectively. 500 μg of cell lysates prepared from the transfected cells were immunoprecipitated with α-FLAG antibody M2-agarose beads. c-Myb proteins in the lysates and in the immunoprecipitates were detected with Western blot analyses using α-c-Myb antibody. TIP60 and TIP60 mutant protein in the immunoprecipitates were revealed with Western blot analyses using α-TIP60 antibody or α-TIP60m antibody, respectively. Data from one representative of three experiments are shown. B, in vitro translated HA-c-Myb and F-TIP60 proteins were mixed as indicated and immunoprecipitated with α-FLAG M2-agarose and c-Myb protein in the input, and immunoprecipitates were detected with Western blot analyses using α-c-Myb antibody and TIP60 protein in the input, and immunoprecipitates were revealed with Western blot analyses using α-FLAG antibody.
substrate of TIP60 under these assay conditions. F-TIP60 was also self-acetylated (lanes 4 and 7). However, when assayed under these conditions, p53 was readily acetylated by TIP60 (Fig. 3B, lane 3). We also tested whether TIP60 could promote acetylation of c-Myb in vivo. The FLAG-c-Myb expressing construct was cotransfected with F-TIP60 into K562 cells, and c-Myb and TIP60 proteins were immunoprecipitated with anti-FLAG-agarose beads. The acetylated proteins in the immunoprecipitates were examined by Western blot analyses with pan-anti-acetyl-lysine antibody. Again, only acetylated TIP60 protein could be detected in this approach (Fig. 3B, lanes 5 and 7), further confirming that c-Myb is not a favorable substrate for the acetyltransferase TIP60 in vivo. In contrast, TIP60 enhanced the acetylation of p53 when both Tip60 and p53 were cotransfected into K562 cells (Fig. 3D, lane 2 and 3).

**TIP60 Inhibits c-Myb Transactivation Activity**—We next examined the effect of TIP60 on the ability of c-Myb to activate transcription of known target genes. Using transient transfection and a dual luciferase assay we evaluated the effects of TIP60 on the transcriptional activation of the Myb-responsive mim-1 promoter in HEK293T cells. Coexpressing TIP60 with c-Myb decreased the ability of c-Myb to activate the mim-1-driven luciferase reporter by ~60% compared with activation in the absence of TIP60 (Fig. 4), and the inhibition was dependent on the amount of TIP60, suggesting that TIP60 inhibits c-Myb...
activity in a dose-dependent manner. The physiologic significance of this observation was further explored. A ChIP assay revealed that both c-Myb and TIP60 bind to the c-Myc promoter, another known c-Myb target gene, in K562 cells (Fig. 5B). Previously, we established an inducible c-Myb dominant negative (MybDN) cell line (K562-MERT) through stable transfection of a conditionally active c-Myb DNA binding domain fused to the Drosophila engrailed transrepressor and a modified mouse estrogen receptor into K562 human leukemia cells. The MybDN remains inactive unless presented with a modified ligand for the estrogen receptor, 4-hydroxy-tamoxifen. As shown in Fig. 5C, inactivation of endogenous c-Myb in K562-MERT cells reduced the occupancy of TIP60 in its preferred site in the c-Myc promoter. As expected, loss of c-Myb activity resulted in a reduction of histone H4 acetylation in c-Myc promoter (Fig. 5C) and a decrease in the level of c-Myc expression (D). Finally, we verified that TIP60 could regulate the expression of c-Myc by siRNA knockdown experiments. Using c-Myb, Tip60, and appropriate control siRNAs we achieved specific knockdowns of c-Myb and Tip60 (≏H1101180–90%, and ≏H1101170–80%, respectively). Consistent with prior reports, c-Myc expression decreased ≏H1101160% when c-Myb was targeted, and modestly but significantly (p ≏H110210.05) increased ≏H1101150% when Tip60 was targeted (Fig. 5E), confirming that TIP60 negatively regulates c-Myc expression. Similar activation of c-Myc expression was observed with a lentiviral Tip60 shRNA (data not shown). In addition, the levels of histone H4 acetylation in the c-Myc promoter were induced upon loss of TIP60 (Fig. 5F). Taken together, our data suggest that TIP60 might be recruited by c-Myb to suppress the expression of c-Myb target genes.

The c-Myb/TIP60 Complex Interacts with HDAC1/HDAC2—To begin to dissect how TIP60 suppresses c-Myb, we focused our attention on HDACs because the transcription suppression properties conferred by TIP60 have largely been attributed to the actions of its associated HDACs, including HDAC1, HDAC2, and HDAC7 (7). Coimmunoprecipitation of Jurkat cell or K562 cell lysates revealed that c-Myb associates with HDAC1 and HDAC2, but not HDAC4 and HDAC7 (Fig. 6A). To test whether the observed association of HDAC1/2 with c-Myb in cells is direct, pull-down assays were carried out to...
examine the interaction of in vitro translated HA-c-Myb and FLAG-HDAC1/2, but c-Myb could not be detected in the immunoprecipitates of FLAG-HDAC1/2 (data not shown). To determine whether TIP60 could facilitate the interaction of c-Myb and HDAC2, a further coimmunoprecipitation binding assay using FLAG-tagged TIP60, HA-tagged c-Myb, and Myc tagged HDAC2 expressed in transfected HEK293T cells indicated that c-Myb easily coprecipitated with HDAC2 in the presence of TIP60 (Fig. 6B). To investigate whether TIP60 facilitates the binding of HDAC2 to the c-Myb target site in the c-Myc promoter, we performed ChIP assays using anti-c-Myb, anti-TIP60, and anti-HDAC2 antibodies upon knockdown Tip60 by siRNA in K562 cells. We found that loss of Tip60 had no effect on the expression of c-Myb and HDAC2 (Fig. 6C). However, it reduced the occupancy of HDAC2 in the c-Myc promoter (Fig. 6D), suggesting that TIP60 is required for the recruitment of HDAC2 to the c-Myb target site. Last, we tested whether HDAC1/2 knockdown affects c-Myc expression in K562 cells. Consistent with a previous report (25), c-Myc expression increased upon loss of either HDAC1 or HDAC2 but not HDAC3 (Fig. 6E). Taken together, these data suggest that TIP60 directly associates with c-Myb and may inhibit the transcriptional activity of c-Myb by recruiting histone deacetylases to the complex.
Tip60 Is Induced during Leukemia Cell Differentiation and Down-regulated in Primary AML Samples—We next investigated whether Tip60 is implicated in leukemogenesis by analyzing Tip60 expression in a differentiating leukemia cell line and primary leukemia samples. Previous studies have shown that HL-60 cells can be induced to differentiate toward granulocytes by treatment with DMSO, which is also associated with a decline in c-Myc and c-Myb expression (Fig. 7, A and B) and histone deacetylation at the c-Myc promoter (data not shown). Moreover, the down-regulation kinetics of c-Myc is faster than c-myb. Interestingly, DMSO induction resulted in the elevated expression of Tip60 (Fig. 7C) and the increased occupancy of Tip60 in the c-Myc promoter (D). Taken together, our data suggest that Tip60 might play a causative role in the rapid reduction of c-Myc during DMSO-induced differentiation of HL-60 cells.

It has been reported that loss of Tip60 expression contributes to breast tumor progression. We hypothesize Tip60 might be also silenced in AML cells. To test this, we examined the expression of Tip60 in six primary AML samples and three normal CD34+ cell samples using quantitative RT-PCR. Tip60 expression was significantly (−60%) lower in the AML samples (p = 0.024) (Fig. 7E). We also analyzed the expression of c-Myb and c-Myc in the controls and patients. Consistent with previous studies (18, 26), the c-Myb as well as c-Myc genes, were higher expressed in AML samples in comparison to normal controls on the mRNA level (p = 0.013 and p = 0.021).

DISCUSSION

Specific Recruitment of Chromatin Modifiers by the Transcription Factor c-Myb is Relevant for Its Function in Transcriptional Regulation. Notably, c-Myb-associated p300/CBP, MLL, and Mi-2 chromatin-modifying proteins are required for the activation of c-Myb target genes (21, 27, 28). Meanwhile, corepressors TIF1, mSin3A, c-Ski, and N-CoR bind to c-Myb and block the trans-activating activity of c-Myb, suppressing the potential oncogenic properties of c-Myb (29). In this work we have identified the acetyltransferase TIP60 as a novel c-Myb interacting protein. TIP60 mainly interacts with c-Myb via its C-terminal MYST domain, which binds to the transactivation domain of c-Myb.

Our first hypothesis for the functional implication of this interaction was that TIP60 could function in a similar manner as the p300/CBP acetyltransferase, enhancing c-Myb DNA
binding capacity and transactivation potential by directly acetylating the c-Myb negative regulatory domain. However, in the in vitro as well as in vivo acetylation assays, no acetylated c-Myb proteins were detected, whereas TIP60 robustly acetylated p53 and itself. This suggests that the interaction of c-Myb and TIP60 does not induce the acetylation of c-Myb, although we cannot rule out the possibility that acetylated c-Myb is present at levels below the sensitivity of our assays.

Furthermore, and in contrast to the effect of p300/CBP, TIP60 suppressed c-Myb-dependent activation in all functional assays we performed. Overexpression of TIP60 inhibited c-Myb mediated-activation, whereas knockdown of endogenous TIP60 enhanced the expression of a c-Myb-activated target gene c-Myc. It therefore appears that c-Myb interacts with TIP60 to recruit a repressive complex. This conclusion was supported by the observation that c-Myb forms a complex with the corepressors histone deacetylases 1 and 2 (HDAC1/2) through TIP60. In the absence of TIP60, HDAC2 failed to interact with c-Myb and occupy the promoter of the c-Myb target gene c-Myc. In fact, the observed induction of c-Myc expression upon knockdown HDAC1/2 by siRNAs suggests that, by associating with the c-Myb-TIP60 complex, HDAC1/2 may be directed to the cognate binding sites of c-Myb, where effects on expression of c-Myb target genes may be silenced.

The TIP60-p400 complex represses gene expression, and histone 3 lysine-4 tri-methylation is required for efficient bind-

FIGURE 7. The expression of Tip60 in differentiated leukemia cells and primary leukemia samples. A–D, HL60 cells were treated with 1.25% DMSO for 5 days. The expression of c-Myc (A), c-Myb (B), and Tip60 (C) were measured by quantitative RT-PCR at indicated time points using GAPDH as a control. A ChIP assay was performed to assess the occupancy of Tip60 on the c-Myc gene locus of HL-60 cells treated with DMSO relative to day 0 (D). **, p < 0.01. E–F, down-regulation of Tip60 and up-regulation of c-Myb and c-Myc expression in AML leukemia cells. RNA samples prepared from normal bone marrow donors (1, 2, and 3) and AML patients (774, 935, 964, 970, 972, and 973) were subjected to quantitative RT-PCR for Tip60 (E) c-Myb and c-Myc (F) mRNA expression levels. Error bars represent S.D. of assays performed in triplicate.
ing to target genes of TIP60 in stem cell development (10). Furthermore, prior H3K4 methylation primes a subset of non-expressed genes for histone acetylation in vivo (30). Our recent study demonstrated that c-Myb forms a complex with MLL, positively affecting histone 3 lysine-4 tri-methylation status (21). Taken together, these studies suggest that chromatin remodeling by the c-Myb-associated MLL complex may strengthen the interaction between c-Myb and TIP60, which negatively modulates c-Myb, preventing the overactivation of c-Myb target genes.

As a coregulator of diverse transcription factors, TIP60 is involved in pathways that regulate transcription, genomic stability, cell growth, and apoptosis to either promote or suppress tumorigenesis, depending on the context (31). TIP60 promotes prostate cancer cell proliferation by facilitating translocation of the androgen receptor to the nucleus (32). On the other hand, TIP60 is also a haploinsufficient tumor suppressor in human breast cancer (33). An Eμ-Myc transgenic mouse model suggested that TIP60 also suppresses Myc-induced lymphoma (11). The finding that TIP60 associates and suppresses c-Myb, a transcription factor important for transactivating genes required for lineage commitment, cell proliferation, and differentiation, as well as hematopoietic cell transformation, suggests that TIP60 may inhibit c-Myb-dependent leukemia development. TIP60 also interacts with C/EBPα as a coactivator (4). C/EBPα is a transactivator involved in hematopoietic lineage decisions and in proliferation control and is required for the synergistic activation of myeloid genes and for myeloid cell maturation (34, 35). The expression of both C/EBPα and TIP60 increase during retinoic acid-induced differentiated U937 cells (4). Our results that Tip60 expression is elevated during DMSO-induced differentiation of HL60 cells and reduced in primary AML samples further support our hypothesis that TIP60 acts as tumor suppressor during leukemia transformation. In accordance with previous studies, the expression of c-Myb and c-Myc in AML patient samples was found to be elevated as compared with that in normal bone marrow cells. These data further support our notion that TIP60 may be involved in the regulation of c-Myc by c-Myb.

In summary, our observations and findings indicate a mechanism by which oncogenic c-Myb activity is controlled by a tumor suppressor activity associated with TIP60. A deeper understanding of the mechanisms and signaling pathways that control the interaction of c-Myb and Tip60 may provide new therapeutic targets for the treatment of acute leukemia. Also, elucidating the underlying processes that lead to the loss of Tip60 expression during leukemogenesis may shed light on a possible new pathway for leukemia prevention.

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REFERENCES

1. Patel, J. H., Du, Y., Ard, P. G., Phillips, C., Carella, B., Chen, C. J., Rakowski, C., Chatterjee, C., Lieberman, P. M., Lane, W. S., Blobel, G. A., and Mc-Mahon, S. B. (2004) The c-MYC oncoprotein is a substrate of the acetyltransferases hGCN5/PCAF and Tip60. *Mol. Cell. Biol.* 24, 10826–10834
2. Kamine, J., Elangovan, B., Subramanian, T., Coleman, D., and Chinnadurai, G. (1996) *Virology* 16, 357–366
3. Murr, R., Loizou, J. I., Yang, Y. G., Cuenin, C., Li, H., Wang, Z. Q., and Herceg, Z. (2006) Histone acetylation by Trap-Tip60 modulates loading of repair proteins and repair of DNA double-strand breaks. *Nat. Cell Biol.* 8, 91–99
4. Baradia, D., Trivedi, A. K., Zada, A. A., Greif, P. A., Mulaw, M. A., Christopeit, M., Hiddemann, W., Bohlander, S. K., and Behre, G. (2008) Proteomic identification of the MYST domain histone acetyltransferase TIP60 (HATATIP) as a co-activator of the myeloid transcription factor C/EBPa. *Leukemia* 22, 800–807
5. Baek, S. H., Ohgi, K. A., Rose, D. W., Koo, E. H., Glass, C. K., and Rosenfeld, M. G. (2002) Exchange of N-CoR corepressor and Tip60 coactivator complexes links gene expression by NF-kB and β-amyloid precursor protein. *Cell* 110, 55–67
6. Gaughan, L., Brady, M. E., Cook, S., Neal, D. E., and Robson, C. N. (2001) Tip60 is a co-activator specific for class I nuclear hormone receptors. *J. Biol. Chem.* 276, 46841–46848
7. Xiao, H., Chung, J., Kao, H. Y., and Yang, Y. C. (2003) Tip60 is a corepressor for STAT3. *J. Biol. Chem.* 278, 11197–11204
8. Gavaravarapu, J., and Kamne, J. (2000) Tip60 inhibits activation of CREB protein by protein kinase A. *Biochem. Biophys. Res. Commun.* 269, 758–766
9. Ikura, T., Ogryzko, V. V., Grigoriev, M., Groisman, R., Wang, J., Horikoshi, M., Scully, R., Qin, J., and Nakatani, Y. (2000) Involvement of the Tip60 histone acetylase complex in DNA repair and apoptosis. *Cell* 102, 463–473
10. Fazzio, T. G., Huff, J. T., and Panning, B. (2008) An RNAi screen of chromatin proteins identifies Tip60-p400 as a regulator of embryonic stem cell identity. *Cell* 134, 162–174
11. Gorrini, C., Squarrito, S., Luise, C., Syed, N., Perna, D., Wark, L., Martino, F., Sardella, D., Verrecchia, A., Bennett, S., Confalonieri, S., Cesaroni, M., Marchesi, F., Gasco, M., Scanziani, E., Capra, M., Mai, S., Nucofo, P., Crook, T., Lough, J., and Amati, B. (2007) Tip60 is a haplo-insufficient tumor suppressor required for an oncogene-induced DNA damage response. *Nature* 448, 1603–1607
12. Klempnauer, K. H., Gonda, T. J., and Bishop, J. M. (1982) Nucleotide sequence of the retroviral leukemia gene v-myb and its cellular progenitor c-myb: the architecture of a transduced oncogene. *Cell* 31, 453–463
13. Biedenkapp, H., Borgmeyer, U., Sippel, A. E., and Klempnauer, K. H. (1988) Viral myb oncogene encodes a sequence-specific DNA-binding activity. *Nature* 335, 835–837
14. Gewirtz, A. M., Anfossi, G., Venturelli, D., Valpreda, S., Sims, R., and Calabretta, B. (1989) GI/S transition in normal human T-lymphocytes requires the nuclear protein encoded by c-myb. *Science* 245, 180–183
15. Ramsay, R. G., and Gonda, T. J. (2008) MYB function in normal and cancer cells. *Nat. Rev. Cancer* 8, 523–534
16. Nason-Burchenal, K., and Wolff, L. (1993) Activation of c-myc is an early bone-marrow event in a murine model for acute promonocytic leukemia. *Proc. Natl. Acad. Sci. U.S.A.* 90, 1619–1623
17. Barletta, C., Pellicci, P. G., Kenyon, L. C., Smith, S. D., and Dalla-Favera, R. (1987) Relationship between the c-myb locus and the 6q-chromosomal aberration in leukemias and lymphomas. *Science* 235, 1064–1067
18. Clappier, E., Cucuqini, W., Kalota, A., Criquette, A., Cayuela, J. M., Dik, W. A., Langerak, A. W., Montpellier, B., Nadel, B., Walraen, P., Delattre, O., Aurias, A., Leblanc, T., Dombret, H., Gewirtz, A. M., Baruchel, A., Sigaux, F., and Soulier, J. (2007) The C-MYB locus is involved in chromosomal translocation and genomic duplications in human T-cell acute leukemia (T-ALL), the translocation defining a new T-ALL subtype in very young children. *Blood* 110, 1251–1261
19. Hess, J. L., Bittner, C. B., Zeisig, D. T., Bach, C., Fuchs, U., Borkhardt, A., Fraamton, J., and Slany, R. K. (2006) c-Myc is an essential downstream target for homebox-mediated transformation of hematopoietic cells. *Blood* 108, 297–304
20. Lidonnici, M. R., Corradi, F., Waldron, T., Bender, T. P., and Calabretta, B. (2008) Requirement of c-Myc for p210(BCR/ABL)-dependent transfor-
mation of hematopoietic progenitors and leukemogenesis. Blood 111, 4771–4779
21. Jin, S., Zhao, H., Yi, Y., Nakata, Y., Kalota, A., and Gewirtz, A. (2010) c-Myb binds MLL through menin in human leukemia cells and is an important driver of MLL-associated leukemogenesis. J. Clin. Invest. 120, 593–606
22. Shetzline, S. E., Rallapalli, R., Dowd, K. J., Zou, S., Nakata, Y., Swider, C. R., Kalota, A., Choi, J. K., and Gewirtz, A. M. (2004) Neuromedin U: a Myb-regulated autocrine growth factor for human myeloid leukemias. Blood 104, 1833–1840
23. Zhao, H., Kalota, A., Jin, S., and Gewirtz, A. (2009) The c-myb proto-oncogene and microRNA-15a comprise an active autoregulatory feedback loop in human hematopoietic cells. Blood 113, 505–516
24. Gu, W., Roeder, R. (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell 90, 595–606
25. Senese, S., Zaragoza, K., Minardi, S., Muradore, I., Ronzoni, S., Passafaro, A., Bernard, L., Draetta, G. F., Alcalay, M., Seiser, C., and Chiocca, S. (2007) Role for histone deacetylase 1 in human tumor cell proliferation. Mol. Cell. Biol. 27, 4784–4795
26. Müller-Tidow, C., Metzelder, C., Metzelder, S. K., Buerger, H., Packeisen, J., Ganser, A., Heil, G., Kübler, K., Adigüzel, G., Schwäble, J., Steffen, B., Ludwig, W. D., Heinecke, A., Büchner, T., Berdel, W. E., and Serve, H. (2004) Expression of the p14ARF tumor suppressor predicts survival in acute myeloid leukemia. Leukemia 18, 720–726
27. Tomita, A., Towatari, M., Tsuzuki, S., Hayakawa, F., Kosugi, H., Tamai, K., Miyazaki, T., Kinoshita, T., and Saito H. (2000) c-Myb acetylation at the carboxyl-terminal conserved domain by transcriptional co-activator p300. Oncogene 19, 444–451
28. Saether, T., Berge, T., Leddaas, M., Matre, V., Alm-Kristiansen, A. H., Dahle, O., Aubry, F., and Gabrielsen, O. (2007) The chromatin remodeling factor Mi-2alpha acts as a novel co-activator for human c-Myb. J. Biol. Chem. 282, 13994–14005
29. Nomura, T., Tanikawa, J., Akimaru, H., Kanei-Ishii, C., Ichikawa-Iwata, E., Khan, M. M., Ito, H., and Ishii, S. (2004) Oncogenic activation of c-Myb correlates with a loss of negative regulation by TIF1beta and Ski. J. Biol. Chem. 279, 16715–16726
30. Wang, Z., Zang, C., Cui, K., Schones, D. E., Barski, A., Peng, W., and Zhao K. (2009) Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. Cell 138, 1019–1031
31. Squatrito, M., Gorrini, C., and Amati B. (2006) Tip60 in DNA damage response and growth control: many tricks in one HAT. Trends Cell Biol. 16, 433–442
32. Shiota, M., Yokomizo, A., Masubuchi, D., Tada, Y., Inokuchi, J., Eto, M., Uchiumi, T., Fujimoto, N., and Naito, S. (2010) Tip60 promotes prostate cancer cell proliferation by translocation of androgen receptor into the nucleus. Prostate 70, 540–554
33. Kim, J. H., Kim, B., Cai, L., Choi, H. J., Ohgi, K. A., Tran, C., Chen, C., Chung, C. H., Huber, O., Rose, D. W., Sawyers, C. L., Rosenfeld, M. G., and Baek, S. H. (2005) Transcriptional regulation of a metastasis suppressor gene by Tip60 and β-catenin complexes. Nature 434, 921–926
34. Reddy, V. A., Iwama, A., Iotzova, G., Schulz, M., Elsasser, A., Vangala, R. K., Tenen, D. G., Hiddemann, W., and Behre, G. (2002) Granulocyte inducer C/EBPβ inactivates the myeloid master regulator PU.1: possible role in lineage commitment decisions. Blood 100, 483–490
35. Friedman, A. (2002) Runx1, c-Myb, and C/EBPβ couple differentiation to proliferation or growth arrest during hematopoiesis. J. Cell. Biochem. 86, 624–629