Published in final edited form as:

Science. 2011 August 5; 333(6043): 765–769. doi:10.1126/science.1201662.

The Leukemogenicity of AML1-ETO Is Dependent on Site-Specific Lysine Acetylation

Lan Wang¹, Alexander Gural¹, Xiao-Jian Sun², Xinyang Zhao¹, Fabiana Perna¹, Gang Huang¹, Megan A. Hatlen¹, Ly Vu¹, Fan Liu¹, Haiming Xu¹, Takashi Asai¹, Hao Xu¹, Tony Deblasio¹, Silvia Menendez¹, Francesca Voza¹, Yanwen Jiang³, Philip A. Cole⁴, Jinsong Zhang⁵, Ari Melnick³, Robert G. Roeder², and Stephen D. Nimer^{1,*}

¹Molecular Pharmacology and Chemistry Program, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA

²Laboratory of Biochemistry and Molecular Biology, Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

³Division of Hematology/Oncology, Department of Medicine, Weill Cornell Medical College, New York, NY 10065, USA

⁴Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

⁵Department of Cancer and Cell Biology, University of Cincinnati College of Medicine, 3125 Eden Avenue, Cincinnati, OH 45267, USA

Abstract

The chromosomal translocations found in acute myelogenous leukemia (AML) generate oncogenic fusion transcription factors with aberrant transcriptional regulatory properties. Although therapeutic targeting of most leukemia fusion proteins remains elusive, the posttranslational modifications that control their function could be targetable. We found that AML1-ETO, the fusion protein generated by the t(8;21) translocation, is acetylated by the transcriptional coactivator p300 in leukemia cells isolated from t(8;21) AML patients, and that this acetylation is essential for its self-renewal-promoting effects in human cord blood CD34⁺ cells and its leukemogenicity in mouse models. Inhibition of p300 abrogates the acetylation of AML1-ETO and impairs its ability to promote leukemic transformation. Thus, lysine acetyltransferases represent a potential therapeutic target in AML.

Histone-modifying enzymes can regulate the binding of specific chromatin-binding proteins to histone marks and can change the affinity of the histones for DNA (1, 2). These enzymes also affect nonhistone proteins, and posttranslational modifications of transcription factors such as p53 or AML1 (which is required for definitive hematopoietic development) can regulate their protein-protein interactions and their activity (1–3). For instance, p300 binds to the C terminus of AML1 and acetylates its N terminus, promoting its activating function (4, 5). When AML1 is fused to ETO (a nuclear protein that interacts with co-repressor

Copyright 2011 by the American Association for the Advancement of Science; all rights reserved.

*To whom correspondence should be addressed. nimer@mskcc.org.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1201662/DC1

Materials and Methods

Figs. S1 to S7

molecules) by the t(8;21) translocation, the p300-binding region of AML1 is lost from the AML1-ETO fusion protein (hereafter abbreviated as A-E).

Studies of hematopoietic development and specific gene promoter function indicate that A-E is generally considered to act as a transcription repressor via recruitment of co-repressors (such as NCoR and SMRT) and histone deacetylases (6–8). Although A-E also up-regulates target gene expression (9, 10), information about the mechanisms of gene activation or the importance of the up-regulated genes is sparse. We have shown that the NHR1 domain in A-E binds HEB, a class I basic helix-loop-helix protein (i.e., an E protein) (11). To explore the importance of this domain to the leukemia-promoting properties of A-E, we compared the effects of “wild-type” A-E with those of the A-E Δ NHR1 construct (which lacks amino acids 245 to 436) on the self-renewal and differentiation potential of human CD34⁺ hematopoietic stem/progenitor cells (HSPCs) isolated from cord blood.

After confirming the expression of Flag-tagged A-E and A-E Δ NHR1 by Western blotting (fig. S1A), we examined HSPC self-renewal with the use of CAFc (cobblestone area-forming cell) assays (12, 13). Unlike A-E, A-E Δ NHR1 did not increase the number of CAFcs present at week 5 (Fig. 1A and fig. S3C), nor did it promote the maintenance of CD34⁺ HSPCs growing in liquid culture (5.1% for A-E Δ NHR1 versus 22.1% for A-E and 3.7% for the MIGR1 control); these findings indicate the essential role of the NHR1 (amino acids 245 to 436) domain in the self-renewal-promoting effects of A-E in this model (Fig. 1B and fig. S1B).

To determine whether the NHR1 domain is required for the inhibitory effect of A-E on differentiation, we grew A-E Δ NHR1-transduced cells in liquid culture with erythroid or myeloid differentiation-promoting cytokines for 9 days. Both the A-E- and A-E Δ NHR1-expressing cells showed a similar decrease in glycophorin A, CD71, and CD11b expression (fig. S1C). Thus, loss of the NHR1 domain affects the self-renewal signals provided by A-E, but not the delay (or block) in myelo-erythroid differentiation.

To investigate how the NHR1 domain of A-E promotes self-renewal, we used microarray-based expression assays to compare the transcriptome of the A-E- and A-E Δ NHR1-transduced human HSPCs (versus control MIGR1-transduced HSPCs). Several potential regulators of HSC self-renewal (*Id1*, *p21*, and *Egr1*) were up-regulated by A-E but not by A-E Δ NHR1 (14–16) (fig. S3E). Similarly, A-E Δ NHR1 was much less transactivating than A-E on the *M-CSFR* promoter (Fig. 1C) (17). In contrast, the differentiation-promoting genes, *C/EBP α* and *Pu.1*, that are down-regulated by A-E are still down-regulated by A-E Δ NHR1 (18, 19) (fig. S3E), and both the *IL-3* and *GM-CSF* promoters were similarly down-regulated by A-E and A-E Δ NHR1 (Fig. 1C). These results suggest that the NHR1 domain is required for transcriptional activation but not repression by A-E, and is required for the effects of A-E on self-renewal but not differentiation.

We next examined whether A-E directly binds p300. Baculovirus-expressed A-E and p300 directly interact in vitro (fig. S6B), and an antibody to ETO coimmunoprecipitated the endogenous A-E and p300 proteins in human Kasumi-1 leukemia cells, which do not express ETO (fig. S6B). We generated several A-E or ETO deletion mutants in order to map the p300-binding domain in A-E (Fig. 2A and fig. S1D). Both A-E and ETO bound p300, and although deletions of the NHR2 domain in either ETO or A-E had no effect on p300 binding, deletion of the NHR1 domain (amino acids 245 to 436) completely abrogated this binding (Fig. 2A and fig. S1D). Furthermore, the A-E exon 9a protein, an alternatively spliced form of A-E that lacks NHR3 and NHR4 (depicted in fig. S2A), also interacts with p300 (Fig. 2B). Given that ETO can bind both co-repressor molecules and p300, ETO may

function as a fast-response adaptor protein, inducing transcriptional activation or repression depending on the signaling pathways activated in the cell.

To determine whether p300 is important for the up-regulation of A-E target gene expression, we knocked down p300 in Kasumi-1 cells with the use of two different short hairpin RNAs (shRNAs); we found significant decreases in the levels of *Id1*, *p21*, and *Egr1* mRNA but no change in the levels of *C/EBPα* or *PU.1* mRNA (Fig. 2C), indicating that p300 is essential for A-E-mediated transcriptional activation. To identify genes potentially regulated by A-E and p300, we performed ChIP-seq (chromatin immunoprecipitation sequencing) assays using antibodies to p300 and ETO and the A-E-expressing Kasumi-1 cells. We found that the promoters of the *Id1*, *p21*, and *Egr1* genes (genes activated by A-E) were co-occupied by A-E and p300 (Fig. 2D and fig. S5C). In contrast, p300 did not colocalize with A-E at the promoter (or enhancer) of the *C/EBPα* gene, which is repressed by A-E (Fig. 2D and fig. S5C). Thus, p300 can contribute to the ability of A-E to function as a transcriptional activator, and both can target similar transcriptional regulatory regions.

Given that p300 acetylates a variety of protein targets, we examined whether A-E is acetylated by p300 by overexpressing A-E and p300 in 293T cells. A-E, but not ETO, was acetylated by p300, localizing the potential acetylation sites to the AML1 portion of A-E (amino acids 1 to 177) (fig. S1E). Deletion of the Runt domain (amino acids 49 to 177) did not affect A-E acetylation (fig. S1E), leaving Lys²⁴ and Lys⁴³ as the only candidate acetylation sites (fig. S2A). We mutated Lys²⁴ and Lys⁴³ to arginine, separately and together (i.e., K24R, K43R, and K24R/K43R), and then used an antibody to acetyl lysine to confirm that both Lys²⁴ and Lys⁴³ are acetylated by p300 (Fig. 3A). As predicted, acetylation of Lys⁴³ was abrogated after deletion of the NHR1 domain (amino acids 245 to 436), the region responsible for the A-E/p300 interaction (Fig. 3B). The Lys²⁴ and Lys⁴³ residues are highly conserved in other vertebrates (fig. S2B), which suggests that their acetylation may be conserved throughout evolution.

To define how acetylation of A-E affects its functions, we compared the effects of “wild-type” A-E and the A-E K24R, K43R, and K24R/K43R mutant proteins on the in vitro behavior of transduced human HSPCs (fig. S3A). Like A-E, A-E K24R increased the number of CAFCs present at week 5, whereas A-E K43R and A-E K24R/K43R did not (fig. S3, B and C). The K43R mutation also abrogated the effect of A-E on self-renewal, whereas the A-E K24R mutant protein retained this effect (fig. S3D). Thus, acetylation of A-E at Lys⁴³ is essential for its self-renewal-promoting effects in human HSPCs [and for its effects on the self-renewal-promoting genes *Id1*, *p21*, and *Egr1* (fig. S3E)].

To investigate the role of A-E acetylation in leukemogenesis in vivo, we expressed the wild type and the K24R, K43R, and K24R/K43R mutant forms of the AE9a protein (the isoform of A-E that can induce leukemia in mouse models by itself) in fetal liver HSPCs by means of retro-viral transduction and transplantation assays (20). We examined the peripheral blood, bone marrow, and spleen of the mice 15 weeks after transplantation, and found large numbers of blast cells in the AE9a and AE9a K24R mice, but not in the AE9a K43R or AE9a K24R/K43R mice (fig. S4D). The AE9a K43R mice also had normal white blood cell counts and platelet counts and displayed less anemia than did the AE9a mice (fig. S4B), whereas the blood counts of the AE9a K24R mice were as abnormal as those of the AE9a mice. The AE9a K43R mice also had far fewer c-kit⁺ immature peripheral blood cells at 15 weeks than did the AE9a mice or the AE9a K24R mice, and the cells retained expression of CD45 (fig. S4C). All of the mice that received AE9a-transduced HSPCs developed AML; the median survival of the AE9a mice and the AE9a K24R mice was 145 and 173 days, respectively ($P < 0.001$). However, the median survival of the AE9a K43R and AE9a K24R/K43R mice was 328 days and “not reached,” respectively (Fig. 3D). Note that AE9a is also

acetylated on Lys⁴³ in vivo, as assessed in AML cells isolated from the spleens of fully leukemic mice (fig. S4E).

Because the NHR1 domain is required for the acetylation of A-E Lys⁴³, we also examined whether its deletion affected the leukemogenicity of AE9a. At 15 weeks after transplantation, the AE9a Δ NHR1 mice had normal white blood cell counts and less anemia than did the AE9a mice (fig. S1F). The AE9a Δ NHR1 mice also had far fewer GFP⁺ c-kit⁺ immature cells in the peripheral blood than did the AE9a mice at 15 weeks (0.8% versus 14%) (Fig. 3C). This in vivo result differs from what has been reported using smaller NHR1 domain deletions, perhaps reflecting the size of the deletion or the use of different model systems (21–24).

Together, these results indicate that acetylation of AE9a at Lys⁴³ is required for AE9a-induced leukemogenesis in mice. Note that A-E, A-E K24R, A-E K43R, and A-E K24R/K43R are expressed at similar levels in the 293T cells (fig. S5A) and they have similar DNA binding activity in electrophoretic mobility shift assays (fig. S5B) (25). To determine whether A-E is acetylated in t(8;21) leukemia patient samples, we analyzed two A-E–positive patient samples and found detectable A-E Lys²⁴ and A-E Lys⁴³ acetylation in both (Fig. 3E and fig. S3G).

To define whether acetylation of A-E is required to inhibit differentiation, we examined the level of glycoporphin A and CD11b expression on transduced cells grown in differentiation-driving cytokines. Both A-E and A-E K24R/K43R triggered a similar decrease in the expression of these differentiation markers (fig. S3F). Thus, the absence of A-E Lys⁴³ acetylation affects the self-renewal signals provided by A-E, but not the delay or block of differentiation, which is consistent with its effects on the myeloid differentiation genes *C/EBP α* and *PU.1* (fig. S3E). We performed mass spectrometry and found that a Lys⁴³-acetylated A-E peptide (but not the non-acetylated peptide) preferentially bound a variety of proteins, including several components of the transcriptional pre-initiation complex (TAF). We performed peptide pull-down assays and found binding of both TAF7 and TAF_{II}250 (Fig. 4C). This suggests that the acetylation of A-E Lys⁴³, which is critical for A-E–induced transcriptional activation, may work at least in part by promoting the recruitment of bromodomain-containing TAF proteins. Of course, histone acetyltransferases other than p300 may also contribute to the acetylation of A-E, and the Lys⁴³-containing region may recruit transcription factors or other coactivators in addition to bromodomain-containing proteins.

Given the importance of the interaction of A-E with p300, and its subsequent acetylation, we assessed whether inhibiting p300 function by means of RNA interference or chemical inhibitors would alter leukemia cell growth. Knockdown of p300 decreased the level of A-E acetylation (fig. S6A) and decreased the expression of *Id1*, *p21*, and *Egr1* (Fig. 2C). We also treated primary t(8;21)⁺ leukemia cells isolated from patients, as well as t(8;21)⁺ Kasumi-1 cells with the p300 inhibitor Lys-CoA-Tat and a second p300 inhibitor C646 (26, 27); both inhibitors decreased the levels of *Id1*, *p21*, and *Egr1* mRNA as well as the levels of acetylated A-E Lys⁴³ and histone H3 (Fig. 4B and fig. S6A). Lys-CoA-Tat also inhibited the growth of the t(8;21) positive primary patient leukemia cells and Kasumi-1 cells (as did C646), with little effect on the growth of normal human HSPCs (Fig. 4A and fig. S6C). Furthermore, both Lys-CoA-Tat and C646 inhibited the growth of MO-91, U937, and HEL cells, with minimal effect on THP-1, Mono-Mac-1, or HL60 cells (fig. S6C). Thus, other acetylated proteins—perhaps the nonhistone substrates of p300—may regulate the sensitivity of cells to p300 inhibition, which suggests that inhibition of p300 function could have broader therapeutic potential in AML.

We next used two different mouse leukemia models to examine the effect of p300 inhibitors on leukemia cell growth in vivo. We treated 3×10^6 AE9a-expressing or MLL-AF9-expressing leukemia cells ex vivo with Lys-CoA-Tat or C646 for 12 hours before injecting the cells into sub-lethally irradiated C57Bl/6 mice (day 0) (28). Both p300 inhibitors reduced the number of immature GFP⁺ c-kit⁺ cells in the AE9a mice (fig. S7A), leading to lower white blood cell counts, less anemia, and less thrombocytopenia relative to the recipients of DDDD-Tat-treated or C37-treated AE9a cells (fig. S7B). These p300 inhibitors significantly increased the median survival from 36 days to 54 days for Lys-CoA-Tat and from 32 days to 43 days for C646 ($P < 0.0001$; fig. S7C). In contrast, Lys-CoA-Tat did not affect the survival of the MLL-AF9 leukemia mice, and C646 had only a minimal effect on MLL-AF9-driven leukemia, increasing the median survival from 23 days to 28 days (fig. S7C). Thus, p300 inhibitors can block the transcriptional activating function of A-E and decrease the growth of A-E-expressing leukemia cells, providing a potentially therapeutic approach to t(8;21) AML (and possibly other AML subtypes as well).

Our results show that acetylation of A-E (and AE9a) by p300 is required for their leukemogenic effects in a human preleukemia model and a mouse AML model. The NHR1 domain of A-E provides a docking site for p300, allowing A-E and p300 to colocalize at the regulatory regions of many A-E up-regulated genes, including those involved in self-renewal (e.g., *Id1*, *p21*, and *Egr1*). The critical consequence of this interaction is that A-E is acetylated—an event essential for its self-renewal-promoting effects and its ability to activate gene expression. Thus, even though A-E can bind p300 and presumably bring it to chromatin where it can acetylate histone residues, it is the acetylation of A-E itself that is the key step, perhaps at least in part by recruiting bromodomain-containing proteins such as TAF_{II}250 or TAF7 (see our model for A-E-induced leukemogenesis in Fig. 4D). The discovery that site-specific lysine acetylation of the AML1-ETO oncogenic fusion protein contributes potently to leukemogenesis suggests that inhibition of its acetylation merits exploration as a possible therapeutic strategy for t(8;21)⁺ leukemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank members of the Nimer lab for thoughtful suggestions and comments; D. J. Meyers for the HAT inhibitors; E. Chuang for help with manuscript preparation; E. Dolezal for help with patient samples; K. Debeer for help with graphic design; M. E. Figueroa for help with data analysis; S. Pereira Mendez for technical assistance; and the MSKCC Animal Core Facility, Flow Cytometry Core Facility, and Anti-tumor Assessment Core Facility for their help. P.A.C. is a cofounder, paid consultant, and shareholder of Acylin Therapeutics Inc., which is developing p300 HAT inhibitors. P.A.C. and Johns Hopkins University hold a patent on HAT inhibitors that has been licensed to Acylin Therapeutics Inc. Supported by a Leukemia Lymphoma Society SCOR grant (S.D.N., R.G.R.), a Leukemia Lymphoma Society fellowship (L.W.), an Empire State Stem Cell Scholar award (L.W.), a Clinical Scholars award (F.L.), a Starr Foundation grant (X.-J.S., R.G.R., S.D.N.), NIH grant GM62437 (P.A.C.), and the Gabrielle's Angel Foundation. The microarray data have been submitted to the National Center for Biotechnology Information Gene Expression Omnibus (GEO) GSE28317.

References and Notes

1. Choudhary C, et al. *Science*. 2009; 325:834.10.1126/science.1175371 [PubMed: 19608861]
2. Gu W, Roeder RG. *Cell*. 1997; 90:595. [PubMed: 9288740]
3. Wang L, et al. *Blood Cells Mol Dis*. 2009; 43:30. [PubMed: 19386523]
4. Yamaguchi Y, et al. *J Biol Chem*. 2004; 279:15630. [PubMed: 14752096]
5. Kitabayashi I, Yokoyama A, Shimizu K, Ohki M. *EMBO J*. 1998; 17:2994. [PubMed: 9606182]
6. Nimer SD, Moore MA. *Oncogene*. 2004; 23:4249. [PubMed: 15156180]

7. Wildonger J, Mann RS. *Development*. 2005; 132:2263. [PubMed: 15829516]
8. Minucci S, Nervi C, Lo Coco F, Pelicci PG. *Oncogene*. 2001; 20:3110. [PubMed: 11420727]
9. Klampfer L, Zhang J, Zelenetz AO, Uchida H, Nimer SD. *Proc Natl Acad Sci USA*. 1996; 93:14059. [PubMed: 8943060]
10. Liu Y, et al. *Cancer Cell*. 2006; 9:249. [PubMed: 16616331]
11. Zhang J, Kalkum M, Yamamura S, Chait BT, Roeder RG. *Science*. 2004; 305:1286. [PubMed: 15333839]
12. Breems DA, Blockland EA, Neben S, Ploemacher RE. *Leukemia*. 1994; 8:1095. [PubMed: 8035601]
13. Mulloy JC, et al. *Blood*. 2002; 99:15. [PubMed: 11756147]
14. Jankovic V, et al. *Proc Natl Acad Sci USA*. 2007; 104:1260. [PubMed: 17227850]
15. Viale A, et al. *Nature*. 2009; 457:51. [PubMed: 19122635]
16. Wilson A, Laurenti E, Trumpp A. *Curr Opin Genet Dev*. 2009; 19:461. [PubMed: 19811902]
17. Rhoades KL, et al. *Proc Natl Acad Sci USA*. 1996; 93:11895. [PubMed: 8876234]
18. Pabst T, et al. *Nat Med*. 2001; 7:444. [PubMed: 11283671]
19. Vangala RK, et al. *Blood*. 2003; 101:270. [PubMed: 12393465]
20. Yan M, et al. *Nat Med*. 2006; 12:945. [PubMed: 16892037]
21. Yan M, Ahn EY, Hiebert SW, Zhang DE. *Blood*. 2009; 113:883. [PubMed: 19036704]
22. Kwok C, Zeisig BB, Qiu J, Dong S, So CW. *Proc Natl Acad Sci USA*. 2009; 106:2853. [PubMed: 19202074]
23. Mannari D, Gascoyne D, Dunne J, Chaplin T, Young B. *Leukemia*. 2010; 24:891. [PubMed: 20111069]
24. Park S, et al. *Blood*. 2009; 113:3558. [PubMed: 19204326]
25. Okumura AJ, Peterson LF, Okumura F, Boyapati A, Zhang DE. *Blood*. 2008; 112:1392. [PubMed: 18511808]
26. Zheng Y, et al. *J Am Chem Soc*. 2005; 127:17182. [PubMed: 16332055]
27. Bowers EM, et al. *Chem Biol*. 2010; 17:471. [PubMed: 20534345]
28. Wang L, et al. *Cell Death Differ*. 2007; 14:306. [PubMed: 16778832]
29. Zhao X, et al. *Genes Dev*. 2008; 22:640. [PubMed: 18316480]

Fig. 1.

The enhanced self-renewal capacity and transcriptional activation induced by A-E in human HSPCs requires the NHR1 domain. **(A)** The NHR1 domain (amino acids 245 to 436) is required for A-E to increase CAFC formation by human CD34⁺ cells transduced with the indicated retroviral vector. The number of cobblestone areas at week 5 is shown ($\pm$ SD; $n = 3$). **(B)** Deletion of NHR1 affects the ability of A-E to promote self-renewal in liquid culture. CD34 expression was examined weekly in MIGR1-, A-E-, and A-E Δ NHR1-transduced human CD34⁺ cells ($\pm$ SD; $n = 3$). **(C)** *M-CSFR*, *GM-CSF*, and *IL-3* promoter activity was examined in cells transfected with MIGR1, A-E, or A-E Δ NHR1 ($\pm$ SD; $n = 3$). All values were standardized to the level of *Renilla* luciferase activity.

Fig. 2.

The NHR1 domain of A-E interacts with p300, which potentiates its transcriptional activating properties. **(A)** The NHR1 domain is required for the interaction of A-E with p300. An antibody to Flag was used for immunoprecipitation (IP); antibodies to p300 or Flag were used for Western blotting (WB). **(B)** AE9a also interacts with p300. An antibody to hemagglutinin (HA) was used for immunoprecipitation; antibodies to p300 or HA were used for Western blotting. **(C)** Knockdown of p300 decreased the expression of A-E-activated target genes. Kasumi-1 cells were transduced with shRNAs against p300 or a control shRNA. Quantitative polymerase chain reaction (qPCR) was performed to quantify the level of target gene expression ($\pm$ SD; $n = 3$). **(D)** A-E and p300 colocalize on some A-E up-regulated genes, as shown by ChIP-seq assays. Representative examples of A-E and p300 co-occupancy are shown as custom tracks in the UCSC genome browser. The locations of the Runx1 consensus binding sites are shown by green lines on the x axis. DNA binding was also analyzed by qPCR amplification of the regions indicated by the red and green arrows on the x axis ($\pm$ SD; $n = 3$).

Fig. 3. The acetylation of A-E Lys⁴³ by p300 is required for A-E-induced leukemogenesis. **(A)** Acetylation of Flag-tagged A-E by p300. Antibodies to Flag or to acetyl lysine were used for immunoprecipitation or Western blotting. **(B)** The ability of A-E to bind p300 is required for its lysine acetylation. Antibodies to Flag or to acetyl A-E Lys²⁴ or Lys⁴³ were used for immunoprecipitation or Western blotting. **(C)** Lethally irradiated recipient mice were injected with HSPCs transduced with AE9a or AE9a ΔNHR1. The number of GFP⁺ c-kit⁺ cells in the peripheral blood is shown 15 weeks after transplant. **(D)** Survival of mice receiving AE9a-transduced or AE9a mutant-transduced HSPCs ($P < 0.0001$, $n = 15$ per group). **(E)** Acetylation of A-E Lys⁴³ was detected in blast cells from t(8;21)⁺ leukemia patients. Antibodies to ETO, acetyl A-E Lys⁴³, or AML1 were used for immunoprecipitation or Western blotting (29).

Fig. 4.

Inhibition of p300 or lysine acetylation blocks leukemogenesis by inhibiting cell growth of A-E-activated target genes. **(A)** Lys-CoA-Tat (50 μ M) inhibited the growth of primary t(8;21)⁺ leukemia cells and Kasumi-1 cells ($\pm$ SD; $n = 3$) but not HL-60 cells or normal human CD34⁺ HSPCs. **(B)** Lys-CoA-Tat (50 μ M) blocks A-E Lys⁴³ and histone H3 acetylation in Kasumi-1 cells, as detected by antibodies to acetyl A-E Lys⁴³ or to histone H3. **(C)** The binding of TAF_{II}250 and TAF7 to A-E peptides that contain acetylated or unacetylated Lys²⁴ or Lys⁴³ was studied using a peptide pull-down assay. Antibodies to TAF_{II}250 or TAF7 were used for Western blotting. **(D)** A hypothetical model showing how p300, and acetylation of A-E by p300, might cooperate to induce leukemia.