Short Report

Critical Role of c-Myc in Acute Myeloid Leukemia Involving Direct Regulation of miR-26a and Histone Methyltransferase EZH2

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Abstract

Increased expression or aberrant activation of c-Myc plays an important role in leukemogenesis. Here, we show that in acute myeloid leukemia (AML), c-Myc directly controls the expression of EZH2, a component of the Polycomb repressive complex 2, and miR-26a. miR-26a is downregulated in primary blasts from AML patients and, during myeloid differentiation of AML cells, is induced together with a decrease in c-Myc and Ezh2 levels. Previously, EZH2 was shown to be regulated by miR-26a at the translational levels in lymphomas. However, we demonstrate that in AML, the variation of EZH2 mainly depends on c-Myc transcriptional control. We also show that enforced expression of miR-26a in AML cells is able to inhibit cell cycle progression by downregulating cyclin E2 expression. In addition, increased levels of miR-26a potentiate the antiproliferative effects of 1,25-dihydroxyvitamin D_3 (VitD) and stimulate myeloid differentiation. Our results identify new molecular targets of c-Myc in AML and highlight miR-26a attractiveness as a therapeutic target in leukemia.

Keywords
c-Myc, EZH2, miR-26a, AML

Introduction

The Myc proteins are transcription factors with essential roles in cell growth and proliferation through their ability to both positively and negatively regulate gene expression.[1,2] Mutation, amplification, or activation of the MYC oncogene family is one of the most frequent events associated with cancer.[2] c-Myc is frequently activated in acute myeloid leukemia (AML) and plays an important role in the induction of leukemogenesis.[3,4] In particular, c-Myc is upregulated by the activating mutations of *Flt3* receptor tyrosine kinase, one of the most prevalent type of mutations in AML, and by the AML-associated fusion proteins *AML1-ETO*, *PML/RARα*, and *PLZF/RARα*.[5,6] It has also been shown that c-Myc is negatively regulated by C/EBPα, a transcription factor essential for granulocytic differentiation, and that c-Myc expression is elevated in myeloid leukemias in which C/EBPα is mutated.[7] In addition, c-Myc is stabilized in AML with mutations leading to aberrant cytoplasmic localization of nucleophosmin (NPM), the most frequent genetic alteration in AML without karyotypic aberrations.[4] Finally, the *MYC* gene, located at 8q24, has been found to be one of the most commonly amplified regions in AML.[8] The importance of c-Myc in myeloid leukemogenesis has been further demonstrated by the induction of myeloid leukemia in mouse models overexpressing c-Myc in bone marrow progenitors.[9] Epigenetic defects contribute with genetic alterations in the development and progression of AML.[10] A key role in the aberrant transcriptional gene silencing that occurs in AML is played by the Polycomb repressive complex 2 (PRC2). PRC2 has histone methyltransferase activity mediated by EZH2, which methylates histone H3 on lysine (K)-27 (H3K27).[11] Inhibition of the core component Ezh2 decreased cell proliferation and stimulated myeloid differentiation of AML cells.[12,13] Although there is a large

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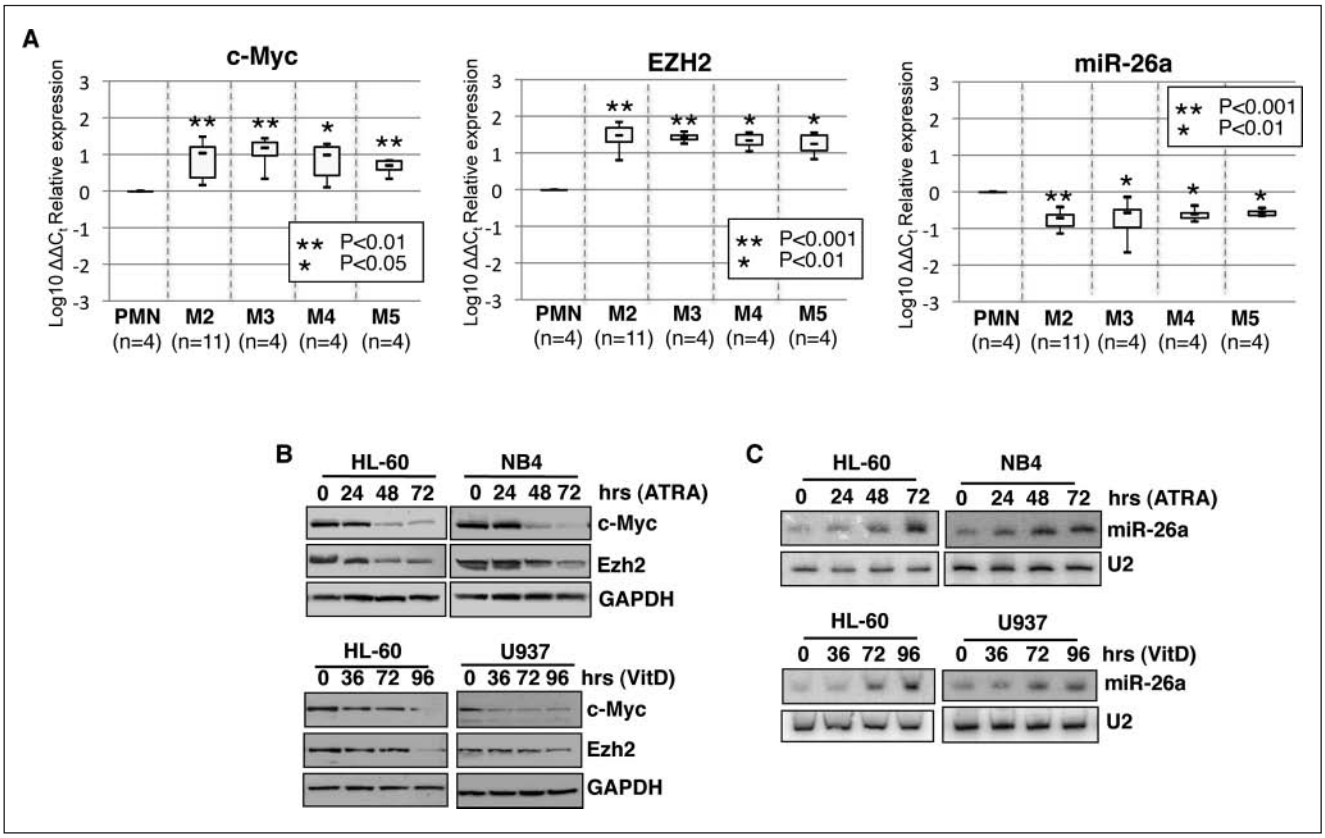

Figure 1. c-Myc, EZH2, and miR-26a expression levels in AML patients and AML cell lines. (**A**) Quantitative real-time RT-PCR analysis of c-Myc, EZH2, and miR-26a in primary leukemia cells derived from AML patients and polymorphonucleated cells (PMN). Values were normalized with U6 snRNA. *P* values are indicated. (**B**) Analysis of c-Myc, EZH2, and miR-26 levels in ATRA (1 μM)– or VitD (250 ng/mL)–treated HL-60, NB4, and U937 cell lines. Total proteins were extracted from cells treated for the indicated times and analyzed by Western blot with anti–c-Myc and anti-EZH2 antibodies. Signals were normalized for GAPDH, and the values, expressed as fractions with respect to mock-treated cells, are indicated below each lane. (**C**) Analysis of miR-26 levels. Total RNA extracted from the same samples was analyzed by Northern blot with the probes indicated on the side of each image. Endogenous U2 snRNA was used as loading controls, and the values, expressed as fractions with respect to mock-treated cells, are indicated below each lane.

body of data implicating EZH2 in cancers, relatively little is known about molecular mechanisms of altered EZH2 expression in AML.

MicroRNAs (miRNAs) are emerging as important players during the execution of the hematopoietic differentiation programs, and their alteration has been associated with the establishment of leukemogenesis.[14] In MYC-induced lymphoma, EZH2 expression was reported to be controlled by miR-26a.[15] This microRNA was demonstrated to be repressed by c-Myc and induced upon downregulation of this oncoprotein.[15,16] However, other studies have shown that EZH2 levels are not controlled by miR-26a in different tumors[17] and that other microRNAs posttranscriptionally regulated EZH2 expression.[17-20] The role of miR-26a and its correlation with c-Myc and EZH2 has not yet been documented in AML.

Here, we demonstrate that EZH2 is not repressed by miR-26a in AML cell lines and that c-Myc directly binds to the promoter of EZH2 and activates its transcription, thus regulating EZH2 levels independently by miR-26a. We also confirm that c-Myc represses miR-26a expression and observe reduction of miR-26a levels in AML patients. Of note, enforced expression of miR-26a in AML cells is able to potentiate the antiproliferative effects of 1,25-dihydroxyvitamin D_3 (VitD) and to sustain myeloid differentiation. Our results identify new molecular targets of c-Myc in AML and support the role of miR-26a as a tumor suppressor in hematological malignancies.

Results and Discussion

We assessed the expression of c-Myc, EZH2, and miR-26a in human hematopoietic cells isolated from healthy donors and from diagnostic samples of AML patients (clinical data are shown in Suppl. Table S1) by qRT-PCR. An inverse correlation was observed between c-Myc, EZH2 levels, and miR-26a expression (Fig. 1A). In particular, high levels of both c-Myc and EZH2 mRNAs were detected in AML

samples compared to polymorphonucleated cells (PMN), consisting predominantly of mature granulocytes, whereas the expression of miR-26a was reduced in all AML samples.

To study the functional link between c-Myc, Ezh2, and miR-26a in AML, we utilized different human AML cell lines. The HL-60 cell line harbors *c-Myc* amplification and can be arrested in proliferation and terminally differentiated by 1,25-dihydroxyvitamin D_3 (VitD) along the monocytic/macrophage pathway and by *all-trans* retinoic acid (ATRA) along the granulocytic pathway. In agreement with previous studies,[21-23] ATRA and VitD treatments decreased c-Myc protein levels in HL-60 cells (Fig. 1B). Interestingly, we also observed similar downregulation of the Ezh2 protein. Similar results were obtained with the NB4 cell line, carrying the oncogenic fusion proteins *PML/RARα*, during ATRA-mediated granulocytic differentiation and the U937 cell line during VitD-mediated monocytic differentiation (Fig. 1B). In all AML cell lines, we observed an increase of miR-26a expression concomitantly to c-Myc downregulation (Fig. 1C).

To evaluate the contribution of c-Myc activity to EZH2 and miR-26a expression, we used the c-Myc inhibitor 10058-F4. This molecule has been already successfully utilized to inhibit c-Myc activity in AML cells.[24] HL-60 and NB4 cells were treated with the inhibitor at the indicated times, and RNA and protein levels were analyzed (Fig. 2A). Treatment with 10058-F4 decreased c-Myc protein levels and produced a strong reduction in both EZH2 mRNA and protein levels. As previously shown, an increase in miR-26a levels was observed consequently to c-Myc downregulation (Fig. 2A).[15,16] As reported in other cellular systems,[15,16] chromatin immunoprecipitation (ChIP) experiments showed

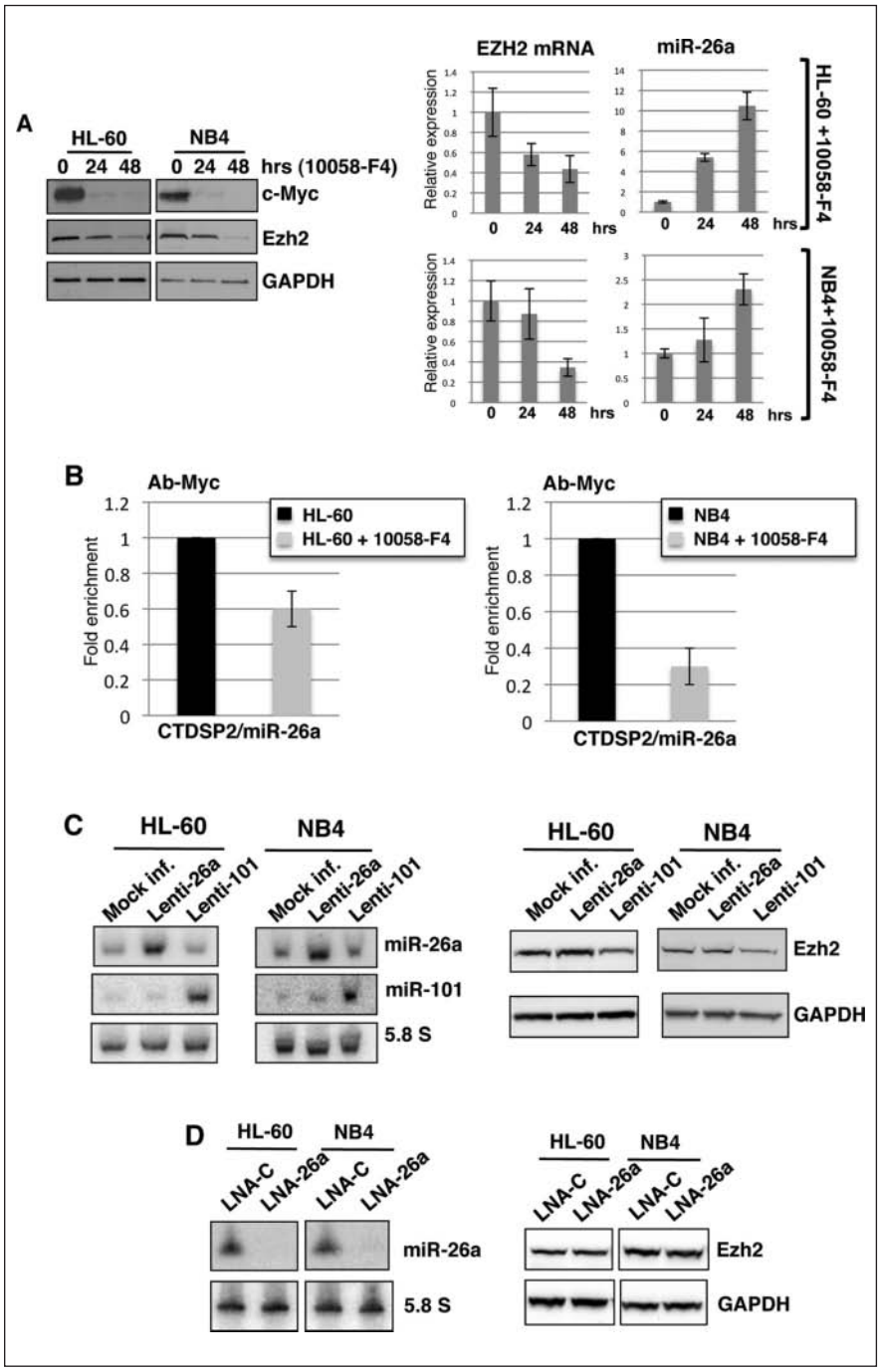

Figure 2. (**A**) HL-60 and NB4 cells were treated with 100 μM 10058-F4 for the indicated times. Total proteins were analyzed by Western blot with anti–c-Myc and anti-EZH2 antibodies. Signals were normalized for GAPDH. EZH2 and miR-26a transcripts were detected by real-time PCR. Signals were normalized for GAPDH mRNA and U6 snRNA, respectively. (**B**) Chromatin from HL-60 and NB4 cells was immunoprecipitated with anti–c-Myc antibody, and the recovered DNA was quantified by real-time PCR. Results are expressed as the relative level over control cells after correcting for differences in the amount of starting (input) chromatin materials. (**C**) Expression of miRNAs and EZH2 was analyzed by Northern and Western blots, respectively, from AML cell lines infected with the empty vector (mock infected) or lentivirus expressing miRNAs (Lenti-26a or Lenti-101) and incubated for 7 days. (**D**) Expression of miR-26a and EZH2 was analyzed by Northern and Western blots, respectively, from AML cell lines transfected with the LNA oligonucleotides against miR-26a (LNA-26a) or scrambled LNA (LNA-C) and incubated for 48 hours.

the binding of c-Myc on the miR-26a promoter region also in AML cell lines (Fig. 2B). Binding that is reduced after treatment with the c-Myc inhibitor 10058-F4 indicated that coherently to other cancers,[15,16] c-Myc inhibits miR-26a expression in AML.

To determine whether the induction of miR-26a is responsible for the EZH2 decrease, different AML cell lines (HL-60 and NB4) were infected with a lentivirus containing a miR-26a expression cassette (Lenti-26a) and with a control lentivirus expressing miR-101, a miRNA that regulates EZH2 in different tumors.[17,18] miR-101 is expressed at very low levels in different AML cell lines and does not increase during myeloid differentiation (data not shown). Enforced expression by lentiviral transduction significantly increased miR-26a and miR-101 levels (Fig. 2C). However, conversely to miR-101, EZH2 protein levels were not decreased by miR-26a overexpression. To investigate if the difference in EZH2 regulation was due to altered 3′-UTR length, 3′ RACE was performed on EZH2 mRNA in NB4, HL-60, and U937 cells. The 3′-UTR identified corresponded to the NCBI reference sequences and contained the reported miR-26a binding site (data not shown). EZH2 expression was also shown to be repressed by miR-214 and miR-137.[19,20] However, these miRNAs are not significantly expressed in the AML cell lines utilized in this study (data not shown) and were not further analyzed. To better analyze the correlation between miR-26a and Ezh2 levels, FITC-labeled locked nucleic acid (LNA) oligonucleotides complementary to miR-26a (LNA-26a) or an unrelated miRNA (LNA-C) were utilized in order to inactivate the endogenous miRNA. Transfection efficiency was evaluated by fluorescence microscopy (Suppl. Fig. S1), and

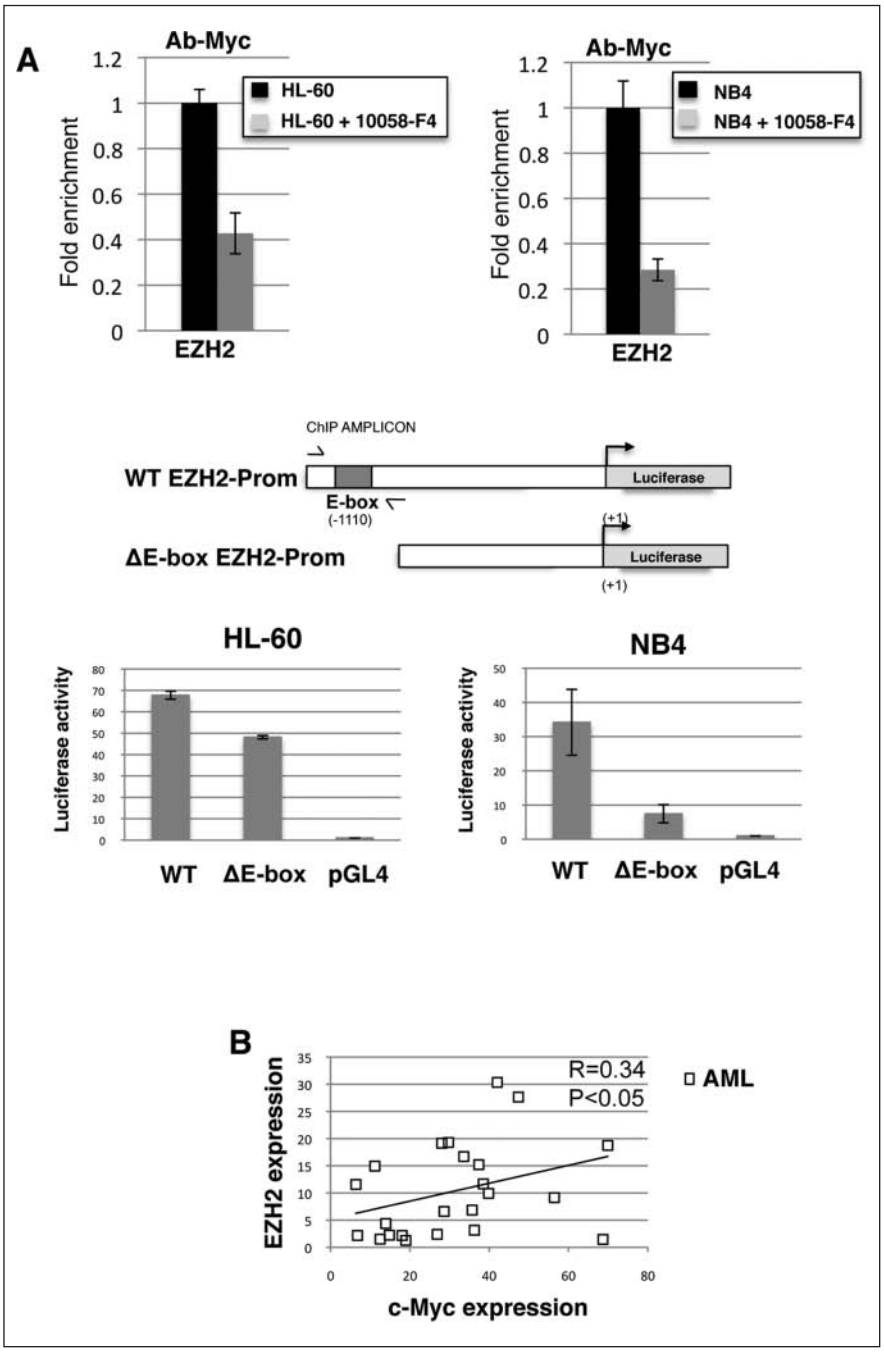

Figure 3. c-Myc regulates the expression of EZH2 in AML. (**A**) Chromatin from HL-60 and NB4 cells was immunoprecipitated with anti–c-Myc antibody, and the recovered DNA was quantified by real-time PCR. Results are expressed as the relative level over control cells after correcting for differences in the amount of starting (input) chromatin materials. Binding sites for c-Myc and position of the qRT-PCR amplicon are indicated in the schematic representation of the FF-luciferase constructs containing the wild-type (WT) or mutant promoter in the binding sites for c-Myc (ΔE-Box). HL-60 and NB4 cells were electroporated with WT or ΔE-Box reporters. FF luciferase values were normalized to RL luciferase reading and then normalized again to empty pGL4 vector to obtain the transcription efficiency. The histograms represent the means ± SEMs from 3 independent experiments. (**B**) Direct correlation between c-Myc and EZH2 mRNA levels determined by qRT-PCR in 23 AML samples and Pearson correlation and linear regression analysis. R = regression coefficient.

only experiments with a percentage of transfected cells over 70% were considered for further analysis. The LNA-miRNA hybrids are thermally stable; therefore, miRNA inhibition can be judged by the decrease in hybridization signal in Northern blots. HL-60 and NB4 cells transfected with LNA-26a showed a substantial decrease in miR-26a levels (Fig. 2D). However, Ezh2 protein levels were not affected by miR-26a inactivation (Fig. 2D). Altogether, these data indicated that EZH2 is not a direct target of miR-26a in AML.

Because there was a strong correlation between c-Myc and EZH2 expressions during AML cell differentiation, we next determined whether the expression of EZH2 was directly controlled by c-Myc. The c-Myc/Max complex recognizes the E-box sequence (5′-CANNTG-3′) in the promoters of specific target genes and stimulates their transcription.[1] By analyzing the EZH2 promoter region, a canonical E-box was identified (data from mapper.chip .org). To clarify whether c-Myc binds the EZH2 promoter *in vivo*, we carried out ChIP assays with a c-Myc antibody in HL-60 and NB4 cells (Fig. 3). To assess the specific binding of c-Myc, ChIP experiments were also performed on cells treated with the c-Myc inhibitor, 10058-F4. DNA from the c-Myc immunoprecipitates was amplified with a couple of PCR primers located in the EZH2 promoter region surrounding the putative E-box. A specific enrichment of the EZH2 promoter regions in c-Myc–immunoprecipitated samples from untreated cells was observed. After 48 hours of c-Myc inhibitor treatment, the immunoprecipitations were strongly reduced, accordingly to decreased c-Myc protein levels (Fig. 3A). In addition, DNA protein complex analysis performed with AML cell line nuclear extracts showed specific c-Myc–containing complexes assembled on the E-box in the EZH2 promoters (Suppl. Fig. S2). These results indicated that c-Myc could bind to the EZH2 promoter. To assess the contribution of c-Myc in EZH2 transcription, luciferase fusion constructs containing the wild-type EZH2 promoter (WT) and a mutant deleted of the E-box (Myc-Mut) were produced and analyzed by transfection into HL-60 and NB4 cells (Fig. 3A). As expected, deletion of the E-box decreased EZH2 promoter activity.

We further correlated the mRNA levels of EZH2 with c-Myc expression in primary AML samples (Fig. 3B). A statistically significant direct correlation between the 2 mRNAs was observed.

It was previously shown that EZH2 levels are much higher in proliferating cells as compared to nonproliferating and differentiated cells.[25] This has been proven to be controlled, at least in part, at the level of transcription by the pRB-E2F pathway.[25] E2F1 and c-Myc reciprocally increase the amounts during cell cycle progression, and similarly to E2F1, c-Myc plays a central role in G_1/S transition. A major conclusion of our results is that in myeloid leukemia cells, c-Myc can participate in the transcriptional regulation of EZH2, thus enforcing E2F1 activity and contributing to increased EZH2 levels in leukemia cells. These data confirmed that the inhibition of c-Myc transcriptional activity is a useful strategy to arrest proliferation of leukemic cells.

We next examined the role of miR-26a in AML. Indeed, this miRNA can function either as a tumor suppressor gene or as an oncogene in different tumors.[15,16,26-28] The AML cell line U937, expressing low levels of miR-26a, was infected with Lenti-26a and then treated with VitD (Fig. 4). Cells infected with Lenti-26a showed higher levels of miR-26a than control cells but almost identical levels of Ezh2 (Suppl. Fig. S3). Cell cycle analysis of infected cells in the absence of VitD showed that high levels of miR-26a increased the percentage of cells in G_1 arrest and decreased the number of cells in S phase (Fig. 4A). The effects of miR-26a on cell growth appeared to be mediated, at least in part, by the downregulation of cyclin E2, direct targets of this miRNA that play a relevant role in transition through the G_1-S checkpoint (Fig. 4B).[26] In addition, enforced expression of miR-26a increased the expression levels of the tumor suppressor $p21^{CIP1}$ (Fig. 4B) and potentiated the antiproliferative effects of VitD by increasing cells arrested in G_1 after VitD treatment (Fig. 4A).

Following observation of miR-26a–mediated inhibition of proliferation, we analyzed miR-26a contribution to AML differentiation. Monocytic differentiation of VitD-treated U937 cells was evaluated by analyzing the induction of specific markers for monocytic cell differentiation such as the presence of CD14 surface antigen, GM-CSF receptor mRNA expression, and staining in nitro blue tetrazolium (NBT) reduction assay (Fig. 4C and 4D). Notably, all the analyzed differentiation markers indicated that miR-26a–overexpressing cells presented a potentiation of VitD-mediated myeloid differentiation capability compared to the mock-infected counterpart. Similar results were also obtained during the differentiation of the HL-60 cell line, where the enforced expression of miR-26a was able to potentiate the VitD-mediated monocytic and ATRA-mediated granulocytic differentiation compared to the mock-infected counterpart (Suppl. Fig. S4). Thus, the induction of miR-26a expression following c-Myc downregulation contributes to arrest leukemia cell proliferation and ultimately sustains myeloid differentiation of AML cells.

All together, these data indicate that miR-26a acts as a tumor suppressor in AML and, in the future, suggests its potential use as a therapeutic tool for promoting proliferation arrest of leukemic cells.

Materials and Methods

Reagents. All-trans retinoic acid (ATRA) and 1,25-dihydroxyvitamin D_3 (VitD) were purchased from Sigma-Aldrich (St. Louis, MO) and utilized at a concentration of

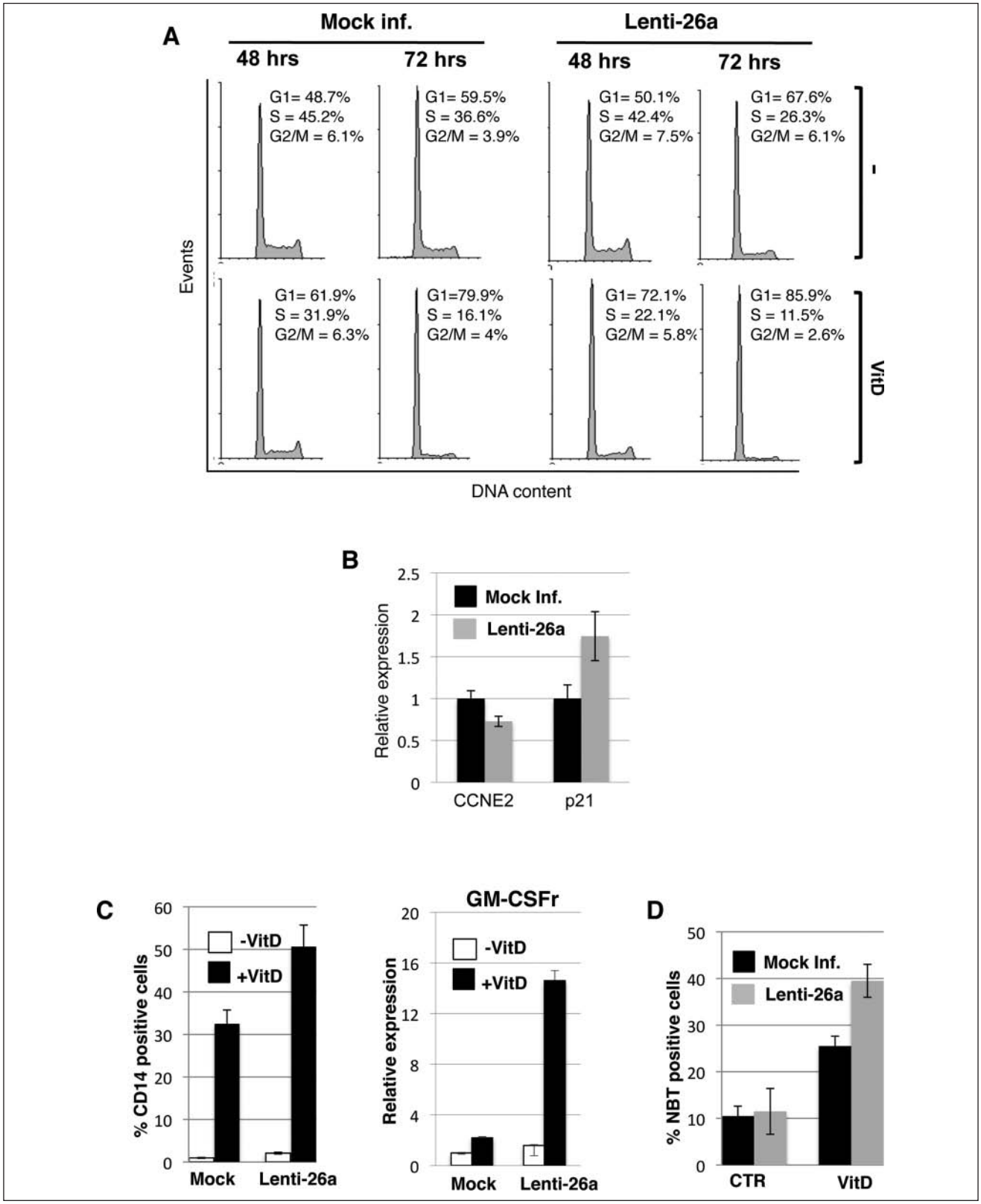

Figure 4. miR-26a inhibits proliferation and stimulates myeloid differentiation of AML cells. (**A**) Representative cell cycle analysis of U937 cells infected with Lenti-26a and treated with VitD. (**B**) Quantitative real-time RT-PCR analysis of cyclin E2 and $p21^{CIP1}$ in U937 cells with enforced expression of miR-26a (Lenti-26a) and control (mock infected). (**C**) Analysis of myeloid-specific surface markers of U937 cells treated with VitD for 72 hours. The histograms indicate the percentage of $CD14^+$ cells on GFP^+ population and expression levels of GM-CSFr mRNA measured by qRT-PCR. Data were normalized for GAPDH mRNA. (**D**) NBT assay of infected cells treated with VitD for 4 days. The histogram indicates the percentage of NBT-positive cells.

1 μM and 250 ng/mL, respectively, unless differently specified. The c-Myc inhibitor, 10058-F4, was purchased from Calbiochem (Darmstadt, Germany) and utilized at a concentration of 100 μM, unless differently specified.

Cell cultures. HL-60, NB4, and U937 cell lines were maintained in RPMI 1640 medium supplemented with 1x penicillin/streptomicin solution, 1x L-glutamine, and 10% fetal bovine serum. Normal polymorphonuclear cells were isolated from the peripheral blood (PB) of informed healthy donors as reported.[29,30] PB/BM cells were obtained from 23 informed, newly diagnosed AML patients. Cases were classified according to the French-American-British classification and showed an initial percentage of circulating blasts more than 80%.

Cell proliferation and differentiation. For cell cycle analysis, 2×10^5 cells were resuspended in 50% FCS, fixed in 70% ethanol for 24 hours, incubated with 50 Ag/mL propidium iodide (Sigma-Aldrich) and 50 U/mL DNase free RNase A (Sigma-Aldrich), and analyzed after 3 hours (10,000 events) using an Epics XL Cytometer (Beckman Coulter, Brea, CA). Differentiation was assessed by NBT dye reduction assay and direct immunofluorescence staining of cells using an Allophycocyanin (APC) anti-human CD11b (Becton Dickinson, Franklin Lakes, NJ), APC anti-human PerCP-Cy5.5 anti-human CD14 (Becton Dickinson), and PE-IgG1 isotype control (eBioscience, San Diego, CA) as previously described.[30] A minimum of 10,000 events were collected for each sample with a flow cytometer (CyAn ADP, Dako, Glostrup, Denmark) by using Summit 4.3 software (CyAn ADP, Beckman Coulter, Brea, CA) for data acquisition and analysis.

miRNA expression constructs. The miRNA expression cassettes were generated by cloning a fragment of the pri–miR-26 (from –142 to +123 bp relative to the 5′-end of mature miR-26a) and pri–miR-101 (from –101 to +102 bp relative to the 5′-end of mature miR-101) into the pSP65-U1 cassette plasmid and then subcloned into the EcoRV site of the lentiviral vector pRRLcPPT.hPGK.EGFP.WPRE. Infective particles were produced and utilized as previously described.[31]

RNA extraction and analysis. Total RNA was prepared using Qiazol reagent (Qiagen, Venlo, the Netherlands). Northern blot analysis of miRNA was performed as previously described.[31] Specific oligonucleotides were utilized as probes. Hsa–miR-26a analysis by real-time PCR was performed using miScript System (Qiagen), and Δ-Δ Ct values were normalized with those obtained from the amplification of the endogenous U6 snRNA (Qiagen). mRNA analysis was performed using oligonucleotides reported in the oligo table, and relative quantification was performed using as endogenous control, hs-HPRT1 (Qiagen). G-CSFr, GM-CSFr, and M-CSFr were detected with TaqMan oligonucleotides Hs00167918_m1, Hs00166144_m1, and Hs00234622_m1, respectively, and normalized by the amplification of GAPDH Hs99999905_m1 (Applied Biosystems, Foster City, CA). All reactions were performed in triplicate. To assess correlation between c-Myc and EZH2 expression, we used Pearson correlation and linear regression analysis. These functions examine each pair of measurements to determine whether the 2 variables tend to move together or in the opposite direction.

Immunoblot analysis. There was 80 μg of whole cell extract that was separated by 10% SDS-PAGE and electroblotted to nitrocellulose membrane (Protran, Schleicher & Schuell, Dassel, Germany). Immunoblots were incubated with antibodies to c-Myc (sc-40, Santa Cruz Biotechnology, Santa Cruz, CA), EZH2 (AC22, Cell Signaling Technology, Danvers, MA), anti-lamin A/C antibody (sc-7292, Santa Cruz Biotechnology), and GAPDH (Abcam, Cambridge, UK).

Chromatin immunoprecipitation assay. DNA/protein cross-linking was obtained by incubating the cells for 10 minutes at 37°C in 1% formaldehyde. After sonication, chromatin was immunoprecipitated overnight with 10 μL of anti–c-Myc antibody (sc-764, Santa Cruz Biotechnology) as previously described.[28] Immunoprecipitation without specific antibody was performed as negative control. Negative control amplifications were performed on the intergenic region. The relative occupancy of the immunoprecipitated factor at a locus was estimated by using the comparative threshold method.[31] Oligonucleotides for miR-26a promoter and intergenic region detection were previously described.[16] ChIP-EZH2_PROM oligonucleotides were utilized for EZH2 amplification.

Promoter analysis. The regions placed 1110 bp from the transcription start site of the EZH2 gene were amplified and cloned upstream of the *luc2* reporter gene in pGL4.10 vector (Promega, Fitchburg, WI), giving rise to WT Prom plasmid. Deletion of the E-box–containing region was performed to obtain the Myc-Mut plasmid. There was 9 μg of each pGL4 derivative plasmid and 1 μg of control pRL-TK vector that were individually transfected by electroporation into HL-60 and NB4 cells. After 48 hours, cells were harvested and assayed with Dual Luciferase Assay (Promega) according to the manufacturer's instructions.

Additional methods are described in the online Supplementary Materials and Methods.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol. 2005;6:635-45.
2. Eilers M, Eisenman RN. Myc's broad reach. Genes Dev. 2008;22: 2755-66.
3. Hoffman B, Amanullah A, Shafarenko M, Liebermann DA. The proto-oncogene c-myc in hematopoietic development and leukemogenesis. Oncogene. 2002;21:3414-21.
4. Renneville A, Roumier C, Biggio V*, et al*. Cooperating gene mutations in acute myeloid leukemia: a review of the literature. Leukemia. 2008;22:915-31.
5. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood. 2002;100:1532-42.
6. Muellar-Tidow C, Steffen B, Cauvet T*, et al*. Translocation products in acute myeloid leukemia activate the Wnt signaling pathway in hematopoietic cells. Mol Cell Biol. 2004;24:2890-904.
7. Johansen LM, Iwama A, Lodie TA*, et al*. c-Myc is a critical target for C/EBPalpha in granulopoiesis. Mol Cell Biol. 2001;21:3789-806.
8. Slovak ML, Ho JP, Pettenati MJ*, et al*. Localization of amplified MYC gene sequences to double minute chromosomes in acute myelogenous leukemia. Genes Chromosomes Cancer. 1994;9:62-7.
9. Luo H, O'Neal J, Kreisel F, Le Beau MM, Tomasson MH. c-Myc rapidly induces acute myeloid leukemia in mice without evidence of lymphoma-associated antiapoptotic mutations. Blood. 2005;106:2542-61.
10. Chen J, Odenike O, Rowley JD. Leukaemogenesis: more than mutant genes. Nat Rev Cancer. 2010;10:23-6.
11. Cao R, Zhang Y. The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Curr Opin Genet Dev. 2004;14:155-64.
12. Villa R, Pasini D, Gutierrez A*, et al*. Role of the polycomb repressive complex 2 in acute promyelocytic leukemia. Cancer Cell. 2007;11:513-25.
13. Fiskus W, Wang Y, Sreekumar A*, et al*. Combined epigenetic therapy with the histone methyltransferase EZH2 inhibitor 3-deazaneplanocin A and the histone deacetylase inhibitor panobinostat against human AML cells. Blood. 2009;114:2733-43.
14. Fatica A, Bozzoni I. Role of microRNAs in hematological malignancies. Expert Rev Hematol. 2009;4:415-23.
15. Sander S, Bullinger L, Klapproth K*, et al*. MYC stimulates EZH2 expression by repression of its negative regulator miR-26a. Blood. 2008;112:4202-12.
16. Chang TC, Yu D, Lee YS*, et al*. Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet. 2008;40:43-50.
17. Varambally S, Cao Q, Mani RS*, et al*. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science. 2008;322:1695-9.
18. Friedman JM, Liang G, Liu CC*, et al*. The putatative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. Cancer Res. 2009;69:2623-9.
19. Juan AH, Kumar RM, Marx JG, Young RA, Sartorelli V. Mir-214-dependent regulation of the polycomb protein Ezh2 in skeletal muscle and embryonic stem cells. Mol Cell. 2009;36:61-74.
20. Szulwach KE, Li X, Smrt RD*, et al*. Cross talk between microRNA and epigenetic regulation in adult neurogenesis. J Cell Biol. 2010; 189:127-41.
21. Jiang G, Albihn A, Tang T, Tian Z, Henriksson M. Role of Myc in differentiation and apoptosis in HL60 cells after exposure to arsenic trioxide or all-trans retinoic acid. Leuk Res. 2008;32:297-307.
22. Xu B, Liu P, Li J, Lu H. All-trans retinoic acid induces thrombospondin-1 expression in acute promyelocytic leukemia cells though down-regulation of itstranscription repressor, c-MYC oncoprotein. Biochem Biophys Res Commun. 2009;382:790-4.
23. Eckhardt SG, Dai A, Davidson KK, Forseth BJ, Wahl GM, Von Hoff DD. Induction of differentiation in HL60 cells by the reduction of extrachromosomally amplified c-myc. Proc Natl Acad Sci U S A. 1994;91:6674-8.
24. Huang MJ, Cheng YC, Liu CR, Lin S, Liu HE. A small-molecule c-Myc inhibitor, 10058-F4, induces cell-cycle arrest, apoptosis, and myeloid differentiation of human acute myeloid leukemia. Exp Hematol. 2006;34:1480-9.
25. Bracken AP, Pasini D, Capra M, Prosperini E, Colli E, Helin K. EZH2 is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer. EMBO J. 2003;22:5323-35.
26. Kota J, Chivukula RR, O'Donnell KA*, et al*. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell. 2009;137:1005-17.
27. Lu J, He ML, Wang L*, et al*. MiR-26a inhibits cell growth and tumorigenesis of nasopharyngeal carcinoma through repression of EZH2. Cancer Res. 2011;71:225-33.
28. Huse JT, Brennan C, Hambardzumyan D*, et al*. The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. Genes Dev. 2009;23:1327-37.
29. Gabert J, Beillard E, van der Velden VH*, et al*. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia: a Europe Against Cancer program. Leukemia. 2003;17:2318-57.
30. Cimino G, Lo-Coco F, Fenu S*, et al*. Sequential valproic acid/all-trans retinoic acid treatment reprograms differentiation in refractory and high-risk acute myeloid leukemia. Cancer Res. 2006;66: 8903-11.
31. De Marchis ML, Ballarino M, Salvatori B, Puzzolo MC, Bozzoni I, Fatica A. A new molecular network comprising PU.1, interferon regulatory factor proteins and miR-342 stimulates ATRA-mediated granulocytic differentiation of acute promyelocitic leukemia cells. Leukemia. 2009;23:856-62.