Targeting AML1/ETO-Histone Deacetylase Repressor Complex: A Novel Mechanism for Valproic Acid-Mediated Gene Expression and Cellular Differentiation in AML1/ETO-Positive Acute Myeloid Leukemia Cells

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Received December 21, 2006; accepted March 22, 2007

ABSTRACT

In t(8;21) acute myeloid leukemia (AML), the AML1/ETO fusion protein promotes leukemogenesis by recruiting class I histone deacetylase (HDAC)-containing repressor complex to the promoter of AML1 target genes. Valproic acid (VPA), a commonly used antiseizure and mood stabilizer drug, has been shown to cause growth arrest and induce differentiation of malignant cells via HDAC inhibition. VPA causes selective proteasomal degradation of HDAC2 but not other class I HDACs (i.e., HDAC 1, 3, and 8). Therefore, we raised the question of whether this drug can effectively target the leukemogenic activity of the AML1/ETO fusion protein that also recruits HDAC1, a key regulator of normal and aberrant histone acetylation. We report here that VPA treatment disrupts the AML1/ETO-HDAC1 physical interaction, stimulates the global dissociation of AML1/ETO-HDAC1 complex from the promoter of AML1/ETO target genes, and induces relocation of both AML1/ETO and HDAC1 protein from nuclear to perinuclear region. Furthermore, we show that mechanistically these effects associate with a significant inhibition of HDAC activity, histone H3 and H4 hyperacetylation, and recruitment of RNA polymerase II, leading to transcriptional reactivation of target genes (i.e., IL-3) otherwise silenced by AML1/ETO fusion protein. Ultimately, these pharmacological effects resulted in significant antileukemic activity mediated by partial cell differentiation and caspase-dependent apoptosis. Taken together, these data support the notion that VPA might effectively target AML1/ETO-driven leukemogenesis through disruption of aberrant HDAC1 function and that VPA should be integrated in novel therapeutic approaches for AML1/ETO-positive AML.

Chromatin remodeling has been postulated to be necessary for initiation of gene transcription (Lemon and Tjian, 2000; Fry and Peterson, 2001). Histone acetyltransferases and histone deacetylases (HDACs) are competing protein modifiers that affect the dynamics of chromatin remodeling by changing the histone acetylation status. By adding acetyl groups to histone lysine residues, histone acetyltransferases promote gene transcription (Grunstein, 1997; Agalioti et al., 2002), whereas by removing acetyl groups, HDACs lead to transcriptionally silent chromatin (Laherty et al., 1997).

Emerging data support the notion that aberrant recruitment of HDAC activity contributes to disruption of gene transcription and promotes leukemogenesis in distinct cytogenetic and molecular subgroups of acute myeloid leukemia (AML) (Erickson et al., 1992). In t(8;21)(q22;q22) AML, the AML1 gene at chromosome band 21q22 fuses to the ETO gene at chromosome band 8q22 (Erickson et al., 1992; Miyoshi et al., 1993). The resulting AML1/ETO fusion gene encodes the chimeric protein AML1/ETO that silences AML1-target hematopoietic genes through directly recruiting HDAC complex to their promoters (Wang et al., 1998). Sim-
ilar mechanisms of transcriptional disruption via histone deacetylation have been described in t(15;17)-positive acute promyelocytic leukemia, where the PML/RAR, α oncogene recruits an HDAC repressor complex to the promoter of the retinoic acid-target genes (Grignani et al., 1998).

Unlike structural abnormalities (i.e., chromosome deletions or gene mutations) causing irreversible loss of gene function, genomic silencing induced by histone deacetylation can be pharmacologically reversed by HDAC inhibitors. These compounds constitute a promising class of agents that activate differentiation programs, inhibit cell cycle, and induce apoptosis in a wide range of malignant cell lines and animal models via suppression of HDAC enzymatic activity and promotion of histone hyperacetylation (Boulaire et al., 2000; Munster et al., 2001). The latter causes chromatin relaxation and uncoiling that subsequently leads to expression of genes important for normal cell growth and differentiation (Lozzio et al., 1979; Bernhard et al., 1999).

Among the different HDAC inhibitors currently undergoing clinical testing for myeloid malignancies (Byrd et al., 2005), valproic acid (VPA), an eight-carbon branched chain fatty acid with anti epileptic and mood-stabilizing activities, has been shown to inhibit growth and induce differentiation of neuroblastoma (Cinatl et al., 1996, 1997) and murine B- and human T-lymphoblastic cells (Tittle et al., 1992) via inhibition of class I HDACs (Gottlicher et al., 2001; Phiel et al., 2001). VPA seems to exert its anticancer activity by inducing proteosomal degradation of HDAC2 but not other class I HDACs (i.e., HDAC1 and HDAC3) (Kramer et al., 2003).

Göttlicher et al. (2001) recently showed that VPA has activity in AML1/ETO-positive cells, where the fusion protein associates not only with HDAC2 but also with HDAC1 to repress gene transcription (Göttlicher et al., 2001). Therefore, we hypothesized that, in addition to HDAC2 degradation, VPA must have significant pharmacological activity on HDAC1. Moreover, we show that although VPA does not directly lower HDAC1 protein expression, it disrupts the assembly of the AML1/ETO-HDAC1 repressor complex on AML1 target gene promoters, inducing histone hyperacetylation and re-expression of AML1/ETO-silenced genes. This ultimately translates into a significant antileukemic activity.

Materials and Methods

Cell Lines. Cells were incubated in RPMI 1640 medium supplemented with 15% (Kasumi-1) or 10% (ML-1, NB-4, and THP-1) fetal bovine serum (Invitrogen, Carlsbad, CA). The cells were seeded at 3–4 × 10⁵ cells/ml density for overnight and treated with 0, 0.3, 1, or 3 mM VPA (Sigma-Aldrich, St. Louis, MO).

HDAC Activity Assay. Nuclear extracts were prepared using Nuclear Extract kit (Active Motif Inc., Carlsbad, CA) according to manufacturer’s instruction. The HDAC activity was assessed using HDAC Assay kit (Upstate Biotechnology, Lake Placid, NY) according to manufacturer’s protocol.

Immunocytochemistry. Cells (4 × 10⁴) were fixed in 3.7% paraformaldehyde (Sigma-Aldrich) for 20 min at room temperature, rinsed with phosphate-buffered saline (PBS) (Invitrogen), and cyto-spun (Shandon CytoSpin 4; Thermoelectronic, Waltham, MA) at 450 rpm for 5 min onto glass slides. The slides were blocked with 10% normal goat serum (Sigma-Aldrich) in PBS for 3 h at room temperature, and then they were incubated with primary antibodies overnight at 4°C. Anti-ETO or anti-AML1 rabbit polyclonal antibodies (Calbiochem, San Diego, CA) were diluted 1:200 in 2% normal goat serum and 0.5% Triton X-100 in PBS, whereas the anti-HDAC1 rabbit polyclonal antibody (Upstate Biotechnology) was diluted 1:400. After washing in PBS, the slides were stained for 1 h with goat anti-rabbit Cy2-conjugated secondary antibody (Jackson Immunoresearch Laboratories Inc., West Grove, PA) diluted in PBS at 1:200. After PBS washing, nuclei were stained with 1 μg/ml 4,6-diamidino-2-phenylindole (Sigma-Aldrich) for 10 min. Slides were mounted with Crystal/Mount (Biomedia, Foster City, CA), and fluorescence was detected by LSM 510 confocal/multiphoton microscope (Carl Zeiss Inc., Thornwood, NY). Slides stained with secondary antibody only served as negative control.

Chromatin Immunoprecipitation. ChiP was performed using the commercially available Chromatin Immunoprecipitation Assay kit (Upstate Biotechnology) according to the manufacturer’s protocol. In brief, Kasumi-1 cells, untreated or treated with VPA for 6 h, were cross-linked for 20 min at room temperature by adding 270 μl of formaldehyde directly to 10 ml of culture medium. Formaldehyde was then quenched with glycine at 0.125 M final concentration. Cells were washed twice with ice-cold PBS containing protease inhibitors (Protease Inhibitor Cocktail Set III; Calbiochem), and then they were harvested in SDS lysis buffer with protease inhibitors and left on ice for 15 min. Chromatin was then fragmented to an average size of 0.2 to 1 kilobases by sonication. After centrifugation, the supernatant was equally divided and 10-fold diluted in ChIP dilution buffer. Approximately 5 to 10% of the solution was then saved for control (input DNA). After preclearing with 80 μl of salmon sperm DNA/protein A/agrose-50% slurry (Upstate Biotechnology) for 30 min at 4°C, antibodies (see below) were added and incubated overnight with gentle rotation at 4°C. Another 80-μl aliquot of salmon sperm DNA/protein A/agrose-50% slurry was then added to the solution, which was incubated for an additional 4 h at 4°C with agitation. The beads were then washed one time with low salt immune complex buffer, high salt immune complex buffer, LiCl immune complex buffer and two times with Tris-EDTA buffer (Upstate Biotechnology). The immunoprecipitated protein-DNA complex was eluted with elution buffer (1% SDS and 0.1 M NaHCO₃). The input and immunoprecipitated chromatin were then incubated at 65°C for 6 h to reverse the formaldehyde cross-links and digested with proteinase K (RNA grade, 20 μg/ml; Invitrogen) for 4 h at 50°C to remove proteins. The DNA was extracted with pheno-chloroform, precipitated with ethanol, and dissolved in water. The antibodies used for immunoprecipitation were anti-acetyl-histone H4, anti-acetyl-histone H3, anti-HDAC1 (Upstate Biotechnology); anti-ETO (Calbiochem); and anti-RNA pol II (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Immunoprecipitated chromatin was analyzed by PCR with primers specific for the IL-3 gene promoter: forward, 5′-CCAGGTGATG-3′ and reverse, 5′-GGGGCAGGCGTCGGAAGGAT-3′. The cycle number and the amount of template were optimized to ensure that results were within the linear range of PCR amplification.

Immunoprecipitation and Western Blot. Whole cellular lysates were prepared in 1× cell lysis buffer [20 mM Tris-HCl (Fisher Scientific, Fairlawn, NJ), pH 7.5, 150 mM NaCl (Fisher Scientific), 1 mM Na₃EDTA (Invitrogen), 0.2% Nonidet P-40 (Roche Diagnostics, Indianapolis, IN), 1 mM β-glycerophosphate (Sigma-Aldrich), and 1 mM Na₂VO₄ (Sigma-Aldrich)] supplemented with protease inhibitors (Protease Inhibitor Cocktail Set III) and 2 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich). Anti-β-actin, anti-Ku70, pro-caspase 3, pro-caspase 9, and glyceraldehyde-3-phosphate dehydrogenase antibodies were purchased from Santa Cruz Biotechnology, Inc.; anti-ETO and anti-AML1 were purchased from Calbiochem; and anti-acetyl-histone H4, anti-acetyl-histone H3, anti-HDAC1, anti-HDAC2, and anti-methylated histone were purchased from Upstate Biotechnology. Equivalent gel loading was confirmed by probing with antibodies against β-actin for whole cell lysates or Ku70 for nuclear extract. For immunoprecipitations, the cell lysates were immunoprecipitated with the primary antibodies overnight at 4°C, and the immunocomplexes were collected by washing four times with lysis buffer and boiling in 1× Laemmli buffer for 10 min. After being
separated by SDS polyacrylamide gel (Bio-Rad, Hercules, CA) electrophoresis, the proteins were transferred onto polyvinylidene difluoride membranes (GE Healthcare, Piscataway, NJ), and the blots were incubated with indicated antibodies in Tris-buffered saline/ Tween 20 buffer [10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.1% Tween 20 (GE Healthcare)] containing 5% nonfat dried milk powder. Proteins recognized by the antibodies were detected using the Chemiluminescent Detection kit (Pierce Chemical, Rockford, IL, and GE Healthcare).

Reverse Transcription-PCR. Treated or untreated cells were harvested in TRIzol Reagent (Invitrogen), and RNA was extracted according to the manufacturer’s recommended protocol. The RT step and nested RT-PCR for amplification of IL-3 transcript were carried out using 2 μl of amplification products from the first-round PCR, as described previously (Klisovic et al., 2003).

Band Depletion Assay for Transcriptional Complexes. Kasumi-1 cells treated with VPA for 6 and 12 h were cross-linked with 1% formaldehyde (Fisher Scientific) at room temperature for 20 min, pelleted, and washed in ice-cold PBS supplemented with protease inhibitors (Protease Inhibitor Cocktail Set III). Cells were subjected to sonication (output 10 W; 5 times/10 s each) and centrifuged at 16,000 g for 5 min at 4°C. Lysates were denatured at 100°C for 5 min in 1× Laemmli buffer, and Western blot was performed as described under Immunoprecipitation and Western Blot.

Apoptosis Analysis. Apoptosis was evaluated by propidium iodide (PI) (KPL, Inc., Gaithersburg, MD) staining. Kasumi-1, NB-4, THP-1, and ML-1 cells were treated with 0, 1, and 3 mM VPA for 24 or 48 h. Approximately 10⁶ cells were harvested and fixed in ice-cold 70% ethanol for 15 min, pelleted by centrifugation, and stained at room temperature with PI staining solution [40 μg/ml PI and 100 μg/ml RNase A (Invitrogen) in PBS] for 30 min at room temperature. DNA content was then analyzed using a FACSCalibur (BD Biosciences, San Jose, CA).

Statistical Analysis. Descriptive statistics to include means and standard deviations of apoptosis rates of different cell lines before and after VPA treatment were computed and compared using paired t tests.

Results

VPA Inhibited HDAC Activity and Induced Hyperacetylation of Histones. Because AML1/ETO promotes leukemogenesis in part by recruiting class I HDACs (Wang et al., 1998), we tested the pharmacological activity of the HDAC inhibitor VPA in AML1/ETO-positive Kasumi-1 cells. Treatment of Kasumi-1 cells with VPA, at concentrations (0.3–3 mM) similar to levels attainable in blood in treated patients (Chavez-Blanco et al., 2005), resulted in a dose- and time-dependent increase in histone H3 and H4 hyperacetylation compared with baseline untreated controls (Fig. 1A). VPA-induced histone H3 hyperacetylation was further confirmed by immunocytochemistry (Fig. 1B). These results were consistent with the dose-dependent decrease of HDAC enzymatic activity in VPA-treated Kasumi-1 cells (Fig. 1C) and confirmed the HDAC inhibitory activity of VPA in our in vitro model.

VPA Decreased AML1/ETO, HDAC1, and HDAC2 Protein Levels in Nucleus. Because HDAC1 has been shown to be a crucial component in the control of acetylation and it is recruited by AML1/ETO onto the promoter of AML1 target
genes, we postulated that to induce a significant antileukemic effects in t(8;21) AML, VPA needs to exert its pharmacological activity on HDAC1 in addition to the reported ability to modulate negatively HDAC2 protein expression (Kramer et al., 2003). To prove our hypothesis, we treated Kasumi-1 cells with 1 mM VPA for 24 h, and we subjected nuclear extracts to immunoblotting. We observed a marked decline of both HDAC1 and HDAC2 protein levels (Fig. 2A). The levels of these proteins in the whole cell lysate, however, remained unchanged (Fig. 2B), supporting the possibility of relocation of these proteins from the nucleus to other cellular compartments (i.e., cytoplasm). At 48 h, consistent with previously reported results (Kramer et al., 2003), HDAC2 but not HDAC1 levels decreased in the whole-cell lysate (data not shown). Interestingly, AML1/ETO protein in nucleus was also diminished by exposure to 1 mM VPA (Fig. 2A), whereas no obvious change was observed in total cell lysates (Fig. 2B).

VPA Disrupted AML1/ETO-HDAC1 Complex and Induced Its Dissociation from Target Gene Promoters.

To determine whether VPA functionally inactivates the AML1/ETO-HDAC1 complex, we first conducted immunoprecipitation assays of these proteins. Kasumi-1 cells were treated with 1 mM VPA for 6 and 12 h, and the cell lysates were immunoprecipitated with HDAC1 antibody and subjected to immunoblotting with anti-ETO. We found that AML1/ETO coimmunoprecipitated with HDAC1 in untreated cells but not in VPA-treated cells (12 h), suggesting that the physical interaction of AML1/ETO with HDAC1 was disrupted by drug exposure (Fig. 3).

Disruption of AML1/ETO/HDAC1 physical interaction by VPA was further assessed by band depletion assays similar to those used to detect formation of TOP1 cleavable complexes (Desai et al., 2001; Li et al., 2003). The results of these assays indicated that VPA released AML1/ETO-HDAC1 complex from its DNA binding. In brief, Kasumi-1 cells untreated or treated with VPA were fixed with 1% formaldehyde that covalently binds proteins to DNA. These newly formed high-molecular-weight complexes lack mobility, and they cannot be resolved by SDS-polyacrylamide gel electrophoresis. We found that, following exposure to VPA, the immunoblotting bands corresponding to HDAC1 and AML1/ETO became more intense compared with untreated cells (Fig. 4A, top left, and B, top right), suggesting an increased level of DNA-free HDAC1 and AML1/ETO proteins. Interestingly, HDAC1 was dissociated from DNA/chromatin at an earlier time (detectable at 6 h) than AML1/ETO (detectable at 24 h). Importantly, when immunoblotting was applied to the samples not subjected to formaldehyde cross-linking, AML1/ETO and HDAC1 protein levels remained unchanged (Fig. 4A, bottom left, and B, bottom right). Similar results were obtained in
Kasumi-1 and NB-4 treated with increasing concentrations of the HDAC inhibitor depsipeptide (FK228) (data not shown).

**Perinuclear Relocation of AML1/ETO-HDAC1 Complex Was Observed upon VPA Treatment.** We have shown above that the physical interaction and DNA binding capability of the AML1/ETO-HDAC complex were disrupted by VPA with altered levels of these proteins in the nuclear extracts but not in the whole cell lysates. To investigate whether the inactivated proteins were relocated into different cellular compartments, immunocytochemistry was performed in Kasumi-1 cells treated with 1 mM VPA for 12 and 24 h using antibodies against HDAC1, AML1, or ETO. Consistent with previous reports (Erickson et al., 1996; Wang et al., 1999; Bakin and Jung, 2004), we observed that both AML1/ETO and HDAC1 were present in diffuse patterns in the nuclei of the untreated cells. However, these patterns changed to a perinuclear distribution following exposure to VPA for 12 to 24 h (Fig. 5, A–C), supporting that drug exposure interfered with aberrant binding of AML1/ETO-HDAC1 to DNA.

**VPA Restored the Transcription of AML1/ETO-HDAC1-Repressed Genes through Local Chromatin Remodeling.** To assess whether the observed global changes of AML1/ETO-HDAC1 following VPA treatment reflected those occurring at promoter of AML1/ETO-target genes, we used ChIP assays. In Kasumi-1 cells, IL-3 is silenced (Fig. 6A, bottom) via AML1/ETO that is enriched on the IL-3 promoter (Fig. 6A, top). In contrast, in NB-4 cells that do not harbor AML1/ETO, IL-3 is expressed (Fig. 6A, bottom) via AML1 wild type (Fig. 6A, top).

To investigate the dynamics of transcriptional repression on the gene promoter mediated by VPA, Kasumi-1 cells were treated with 1 mM VPA for 6 h, and then they were subjected to the ChIP assay. We found that AML1/ETO and HDAC1 were released from the IL-3 gene promoter with concurrent recruitment of RNA pol II after 6 h of exposure to VPA (Fig. 6B, top). Furthermore, we observed that VPA treatment induced the accumulation of hyperacetylated histones H3 and H4 on the IL-3 gene promoter (Fig. 6B, top) and restoration of IL-3 gene transcription (Fig. 6B, bottom).

**VPA Had a Pronounced Effect on HDAC-Associated Cell Lines.** Because we showed that VPA treatment resulted in the dysfunction of AML1/ETO-HDAC complex and restored the transcription of AML1/ETO silenced gene, we next analyzed whether these events would also bring significant antileukemia effects. In addition to AML1/ETO-positive Kasumi-1 cells, we analyzed NB-4 cells that harbor PML/RARα.
reported to physically interact with HDACs (Grignani et al., 1998; Wang et al., 1998) as well as THP-1 and ML-1 that lack the aberrant recruitment of HDACs. After 48-h treatment with 1 and 3 mM VPA, we showed a dose-dependent increase in the apoptosis rate (i.e., percentage of sub-G₁) only in HDAC-associated cell lines (i.e., Kasumi-1 and NB-4), whereas MLL-associated cell lines (THP-1 and ML-1) seemed to be resistant (Fig. 7). Apoptosis in Kasumi-1 (and NB-4; data not shown) cells was caspase-mediated as demonstrated by dose-dependent cleavage of PARP, caspase 3, and caspase 9 following exposure to VPA (Fig. 8A) as well as 2-fold decrease in the apoptosis rate following treatment with the pan-caspase inhibitor zVAD-FMK (Fig. 8B). Notably, VPA-treated Kasumi-1 cells were induced to partial differentiation as shown by increased expression of CD11b (Fig. 8C).

**Discussion**

VPA is a well known antiepileptic and mood stabilizer drug recently proven to have significant activity against malignant cells (Gottlicher, 2004). Although recent studies have shown that VPA inhibits HDAC activity (Gottlicher et al., 2001; Gurvich et al., 2004), stimulates cell differentiation, induces apoptosis (Gottlicher et al., 2001; Kawagoe et al., 2002; Tang et al., 2004), and alters gene expression (Gottlicher et al., 2001; Blaheta and Cinatl, 2002), the molecular mechanism underlying these actions remains to be elucidated. Kramer et al. (2003) have recently shown that, different from other HDAC inhibitors (i.e., trichostatin and MS-275), VPA induces selective proteasomal degradation of HDAC2, but not other class I HDACs (i.e., HDAC1 and HDAC3). These authors hypothesized that HDAC2 degradation is one of the main mechanisms leading to persistence of HDAC inhibition, in spite of short exposure to the drug. Because several groups have reported that in addition to HDAC2, AML1/ETO associates with other class I HDACs, we hypothesized that VPA treatment needs to exert a significant activity also on HDAC1 to produce a significant antileukemic effect.

Consistent with this hypothesis, the current study revealed that VPA induced a functional disruption of the AML1/ETO-HDAC1 complex, in turn leading to caspase-dependent apoptosis. We showed that 1) VPA caused HDAC inhibition and global core histone H3/H4 post-translational...
modifications; 2) VPA decreased AML1/ETO, HDAC1, and HDAC2 protein levels in the nucleus; 3) VPA directly targeted the AML1/ETO-HDAC1 complex by disrupting its physical interaction and breaking its DNA/chromatin binding capability; and 4) VPA restored IL-3 gene transcription by inducing dynamic changes of transcriptional factors on the IL-3 gene promoter, including the accumulation of hyperacetylated histone H3 and H4, the dissociation of general transcriptional repressors (i.e., AML1/ETO and HDAC1), and the recruitment of general transcriptional activators (i.e., RNA pol II). Finally, the functional and anti-leukemic relevance of these pharmacological effects was demonstrated by the fact that VPA stimulated partial cell differentiation and caspase-mediated apoptosis. These data elucidate the molecular mechanism of gene regulation and cell apoptosis induced by VPA.

Histone deacetylase is a common molecular target for differentiation treatment of AML (Minucci et al., 2001). Remodeling of the chromatin template by inhibition of HDAC activities also represents a major goal for transcriptional therapy in other neoplastic diseases (Wu et al., 2001). In general, histone acetylation mediates gene activation, whereas deacetylation favors repression (Kornberg and Lorch, 1999). We used Kasumi-1 cells as a relevant model given that the encoded AML1/ETO chimeric protein seems to contribute to leukemogenesis by aberrantly recruiting HDAC activity and silencing AML1-target genes important for hematopoiesis. Our study showed that AML1/ETO-HDAC1 was bound to AML1 target gene (IL-3) promoter in Kasumi-1 cells, in which IL-3 gene is silenced, whereas only AML1 was accumulated on the IL-3 gene promoter in NB-4 cells, in which the IL-3 gene is active. VPA treatment disrupted AML1/ETO-HDAC1 physical interaction and released from the IL-3 gene promoter, eventually leading to the accumulation of hyperacetylated histones and the recruitment of RNA pol II. Importantly, band depletion assays for transcriptional factors showed that the dissociation of transcriptional repressors AML1/ETO-HDAC1 was a global event, highlighting AML1/ETO-HDAC1 as a promising VPA target in AML. This was further supported by the observation that VPA had a significant cytotoxic effect on HDAC-associated cell lines (Kasumi-1 and NB-4), compared with MLL-associated cell lines (THP-1 and ML-1), suggesting that VPA played a critical role in cell differentiation and apoptosis by targeting HDAC-repressor complexes.

To detect dynamic changes induced by VPA on AML1/ETO and HDAC1 DNA binding, we adapted a band depletion assay used previously to detect covalent protein-DNA complexes (i.e., topoisomerase-DNA) induced by camptothecin (Desai et al., 2001; Li et al., 2003). After camptothecin exposure, topoisomerase I is trapped on DNA covalently. This high-molecular-weight complex cannot be resolved on the SDS-polyacrylamide gel electrophoresis gel. Disappearance of the corresponding band indicates a decrease in topoisomerase I levels in the DNA-free protein pool. We speculated that the same assay could be used to assess pharmacologically induced dynamic changes in the trans-interaction of transcriptional factors with gene promoters, using formaldehyde for DNA-protein cross-linking. We showed that, following VPA treatment, HDAC1 and AML1/ETO gel bands increased in intensity, suggesting increased levels in the DNA-free protein pool. This was further confirmed via ChIP assay by showing that HDAC1 and AML1/ETO were released from the IL-3 gene promoter. Similar results were observed in Kasumi-1 cells treated with the HDAC inhibitor depsipeptide (G. Marcucci, unpublished data). Consistent with these results, Western blot for nonformaldehyde-treated samples did not show changes in AML1/ETO and HDAC1 protein levels, suggesting that AML1/ETO and HDAC1 increase in the free protein pool results from the dissociation of these two repressors from AML1 target gene promoters, rather than from changes in their gene expression levels.

Notably, despite being considered a “favorable” prognostic group, only approximately 50% of AML patients with t(8;21) achieve long-term remission (Marcucci et al., 2005). We have recently reported that mutations of the KIT gene encoding a 145-kDa transmembrane glycoprotein, a member of the type III receptor tyrosine kinase family, are detectable in t(8;21) AML and confer a higher relapse risk (Paschka et al., 2006). Furthermore, we reported that AML1/ETO recruits DNMT1 (Liu et al., 2005), thereby suggesting that DNA methylation mediated by the fusion protein may contribute to target gene silencing, and, in turn, leukemogenesis. These studies have uncovered a variety of therapeutic targets in t(8;21) AML, and they suggested novel treatment strategies to improve the currently disappointing clinical results. These strategies should integrate tyrosine kinase inhibitors targeting KIT mutations, hypomethylating agents targeting aberrant DNMT activity (AML1/ETO-DNMT1), and VPA and/or other inhibitors targeting the HDAC1-AML1/ETO complex.

Acknowledgments

We thank Drs. Tiansheng Shen and Marko I. Klisovic for valuable insight and technical expertise.

References


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