Molecular Sequelae of Histone Deacetylase Inhibition in Human Retinoblastoma Cell Lines: Clinical Implications

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PURPOSE. To characterize the molecular sequelae induced in retinoblastoma (Rb) cells by histone deacetylase inhibitors (HDACIs). Hydroxamic acid-based HDACIs such as vorinostat (suberoylanilide hydroxamic acid) induce the differentiation and apoptosis of transformed cells. Vorinostat has demonstrated significant anticancer activity against hematologic and solid tumors at doses well tolerated by patients and has been approved for the treatment of patients with cutaneous T-cell lymphoma.

METHODS. The authors evaluated the effects of the HDACIs vorinostat and m-carboxycinnamic acid bis-hydroxamide on the Rb cell lines Y79 and WERI-Rb1 with the use of the MTT assay, BrdU incorporation assay, flow cytometry, immunoblotting, gene-expression profiling, quantitative RT-PCR, and NF-kB DNA-binding assay.

RESULTS. Both HDACIs were effective against both Rb cell lines, inducing growth arrest and apoptosis in vitro. Vorinostat increased p53 expression and activated caspases -8, -9 and -3, whereas caspase inhibition abrogated vorinostat-induced apoptosis. Vorinostat downregulated baseline NF-kB activity and potentiated the activity of the DNA-damaging chemotherapeutic doxorubicin. Gene expression profiling and qRT-PCR demonstrated that vorinostat modulated the mRNA levels of genes important for signal transduction, cell cycle, cellular metabolism, stress response, apoptosis, extracellular matrix synthesis, and cell differentiation. Notably, several transcripts involved in the ephrin and Notch signaling pathways were upregulated.

CONCLUSIONS. HDACIs, such as vorinostat, induce caspase-dependent apoptosis in Rb cells, downregulate baseline NF-kB activity, and potentiate the effectiveness of conventional chemotherapy. The finding that vorinostat augments the effectiveness of doxorubicin provides a rationale for future clinical studies looking at the use of vorinostat in combination with conventional chemotherapy in Rb. (Invest Ophthalmol Vis Sci. 2009;50:4072-4079) DOI:10.1167/ iovs.09-3517

TREATMENT of intraocular retinoblastoma with external beam radiation is effective, yet in recent years there has been a shift in treatment preference in favor of multimodality approaches that involve systemic multiagent chemotherapy because of fears of late complications of radiation therapy.1 However, systemic chemotherapy carries its own short- and long-term risk for toxicity, including the concern for inducing secondary malignancies.1 Selective, direct intra-arterial (ophthalmic artery) chemotherapy administration is an exciting novel approach2 that achieves drug concentrations in the orbital tissues many times higher than usually obtained with systemic administration, leading to regression of tumor mass and vitreous seeds3 while preserving retinal function,4 and the maximal systemic exposure is only a small fraction of the local concentration. Still, the incorporation of safer, targeted therapies to the multimodality approaches for intraocular tumors and the systemic management of those tumors that have escaped local control are important goals for retinoblastoma research.

Retinoblastoma is characterized by the functional inactivation of both alleles of the tumor suppressor gene Rb1, which encodes the 105-kDa nuclear phosphoprotein Rb. In its hypo-phosphorylated state, Rb binds to the activation domain of the transcription factor E2F-1 and actively represses transcription from the promoters of genes bearing E2F-1 binding sites, including many S-phase genes, leading to cell cycle arrest. Absence of the Rb protein in retinoblastoma causes the release of free, transcriptionally active E2F-1, thus permitting unrestricted cell proliferation. The “two-hit” theory of Rb1 inactivation during retinoblastoma development was proposed by Knudson in 19715 and has been widely accepted, though recent data have emphasized the importance of additional epigenetic events, genomic instability, and aneuploidy.6,7

A major mechanism of epigenetic regulation of gene expression is by control of the level of acetylation on lysine residues of the amino-terminal tails of histones through the opposing activities of histone deacetylases (HDACs) and histone acetyltransferases (HATs).8 In general, chromatin composed of nucleosomes in which the histones have low levels of acetylation is more likely to be transcriptionally silent. Importantly, dysregulated HAT or HDAC activity has been found in human cancers.9,9 Highly active HDACIs, such as the hydroxamic acid-based suberoylanilide hydroxamic acid (vorinostat)10-12 and the m-carboxycinnamic acid bis-hydroxamide (CBHA), cause an accumulation of acetylated histones in cultured cells, induce the differentiation or apoptosis of transformed cells in culture,13 and inhibit the growth of tumors in animals.10 It should be emphasized that HDACs also have nonhistone protein substrates,13 including transcription factors, signal transduction mediators, DNA repair enzymes, chaperone proteins, structural proteins, and inflammation mediators.13 In fact, HDACs may be better described as N-e-lysine deacetylases.13

Vorinostat is an effective inhibitor of growth of a broad variety of transformed cells at doses that have relatively little toxicity.10 Ongoing clinical evaluation in patients with hematologic and solid malignancies has revealed that intravenously and orally administered vorinostat is bioavailable, biologically...
active, and well tolerated. Responses have been reported in solid tumors and hematologic malignancies. Vorinostat (Zolinza; Merck, Whitehouse Station, NJ) is approved by the US Food and Drug Administration for the treatment of cutaneous T-cell lymphoma (CTCL).17–19 Given that retinoblastoma cells lack functional Rb and are, therefore, deficient in the process of Rb-mediated HDAC recruitment to E2F-responsive promoters, we investigated their sensitivity to HDACIs. In this study, we demonstrate that both vorinostat and CBHA have antitumor activity against retinoblastoma cell lines at concentrations similar to those that are active against Rb-expressing hematologic and solid tumor cell lines and that they induce growth arrest, caspase-dependent apoptosis, and sensitization to conventional chemother- apy. These studies provide the framework for the clinical evaluation of HDACIs to overcome clinical drug resistance and improve clinical outcomes in patients with disseminated retinoblastoma.

**Materials and Methods**

**Cell Lines and Tissue Culture**

Human retinoblastoma cell lines Y79 and WERI-Rb1 were purchased from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco’s modified Eagle’s medium (DMEM; BioWhittaker, Walkersville, MD) with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% fetal calf serum (FCS; Invitrogen, Carlsbad, CA), unless stated otherwise.

**Reagents**

Suberoylanilide hydroxamic acid (vorinostat) and CBHA were dissolved in dimethyl sulfoxide. Final concentration was 1 μM, unless stated otherwise. In vitro use of vorinostat concentrations in the range of 1 to 10 μM is physiologically relevant because it is clinically achievable without prohibiting toxicity. In clinical trials, administration of vorinostat as intravenous infusion at a dose of 300 mg/m² (which was determined to be the maximum tolerated dose for the hematologic patients) resulted in average plasma vorinostat C_max of 2638 ng/mL (9.99 μM) in hematologic malignancies and C_max as high as 3298 ng/mL (12.46 μM) in solid malignancies. Intravenous vorinostat infusion at a dose of 600 mg/m² resulted in average C_max as high as 10,815 ng/mL (40.96 μM), yet some toxicity was observed at this dose. Oral administration of vorinostat at the 400 mg daily dose is approved for CTCL, resulted in C_max as high as 667 ng/mL (2.52 μM).16

**Histone Acetylation**

Y79 cells were cultured with vorinostat or CBHA or vehicle for 8 hours in medium containing 10% FBS. Histones were then extracted as previously described.22 Acetylation of core histones was determined by Western blot analysis using rabbit polyclonal antibodies against acetylated histone H3 and H4 (Upstate Biotechnology, Lake Placid, NY) and visualized with enhanced chemiluminescence.

**BrdU Incorporation Assay**

Cell proliferation in cells treated with vorinostat or CBHA was quantified by measurement of the amount of BrdU incorporated into nuclear DNA (BrdU Cell Proliferation Assay; Calbiochem, San Diego, CA) according to the instructions of the manufacturer.

**Propidium Iodide Staining**

For cell cycle analysis, 1 × 10⁶ cells were incubated with or without vorinostat or CBHA in 10% FCS for 24, 48, or 72 hours. The cells were then washed twice with PBS, permeabilized with 70% ethanol in PBS for 30 minutes at 4°C, incubated with 0.5 mL of 50 μg/mL propidium iodide (PI) solution containing 20 U/mL RNaseA (Roche Molecular Biochemicals, Indianapolis, IN) for 30 minutes, and analyzed by flow cytometry.

**MTT Colorimetric Survival Assay**

Cell survival was examined using the MTT colorimetric assay, as previously described.22 All experiments were repeated at least three times, and each experimental condition was repeated at least in quadruplicate wells in each experiment. Data reported are mean ± SD of representative experiments.

**LDH Release Assay**

Y79 cells were preincubated with the pan-caspase inhibitor ZVAD-FMK, the caspase-9 inhibitor LEHD-FMK, the caspase-8 inhibitor IETD-FMK, or the caspase-3 inhibitor DEVD-FMK (all used at 20 μM and all from Calbiochem) for 1 hour before exposure to vorinostat (for 36 hours). Quantification of cell death was performed by measuring the activity of lactate dehydrogenase (LDH) released from the cytosol of damaged cells into the culture supernatant with a cytotoxicity detection kit (Roche Molecular Biochemicals) according to the instructions of the manufacturer.

**Immunoblotting Analysis**

Immunoblotting analysis was performed as previously described.22 Reagents used were mouse monoclonal antibodies for Bcl-2, Bax, and tubulin; polyclonal antibodies for caspases-3 and -9 (Santa Cruz Bio-technology, Santa Cruz, CA); monoclonal antibody for PARP (Biomol, Plymouth Meeting, PA); monoclonal antibody for p53 (Calbiochem); monoclonal antibody for caspase-8 and polyclonal antisera against Bid (Cell Signaling, Beverly, MA); protease inhibitor mixture (Complete; Roche Molecular Biochemicals) and SDS (Invitrogen); and an enhanced chemiluminescence kit, which includes the peroxidase-labeled anti-mouse and anti-rabbit secondary antibodies (ECL; Amer sham, Arlington Heights, IL).

**Caspase Activity Assay**

Y79 and WER-Rb1 cells (5 × 10⁶) were treated with vorinostat for 0 to 24 hours, washed in PBS, and harvested by centrifugation at 800g for 10 minutes at 4°C. Caspase-8 and -3 enzymatic activity was measured with respective colorimetric assay kits (ApoAlert; Clontech, Palo Alto, CA), as previously described,23 normalized for protein content and expressed in arbitrary units.

**Quantification of NF-κB Activity**

The DNA binding activity of NF-κB in retinoblastoma cells was quantified by enzyme linked immunosorbent assay (ELISA) using an NF-κB p65 transcription factor assay kit (Trans-AM; Active Motif North Amer ica, Carlsbad, CA), according to the instructions of the manufacturer, as previously described.24

**RNA Extraction, Global Gene Expression Profiling, and Confirmatory Relative Quantification of Selected Transcripts**

Y79 and WER-Rb1 cells were treated with vorinostat for 0 to 24 hours. Total RNA was extracted and purified with an RNAeasy kit (Qiagen, San Diego, CA) and was analyzed by chip hybridization (GeneChip Human Genome U133 Plus 2.0 Array; GeneChip; Affymetrix, Santa Clara, CA), as previously described.25 Confirmation of the microarray results was performed for selected genes, chosen based on putative function, by RT-PCR. Y79 and WER-Rb1 cells were independently treated with
Acetylation of Histones in Retinoblastoma Cells

Vorinostat and CBHA Induced Accumulation of Acetylated Histones in Retinoblastoma Cells

We first investigated the effect of vorinostat and CBHA on histone acetylation status in Y79 retinoblastoma cells. Y79 cells treated with vorinostat or CBHA for 8 hours exhibited significantly increased acetylation of histones H3 and H4 than of controls (Fig. 1). Equal loading of histones was confirmed by Coomasie Blue staining.

Vorinostat- and CBHA-Induced Growth Arrest and Apoptosis in Human Retinoblastoma Cells

We then investigated the effect of vorinostat and CBHA on the growth and survival of retinoblastoma cells. Treatment of Y79 and WERI-Rb1 cells with vorinostat or CBHA for 48 hours potently suppressed cellular proliferation, as quantified by BrdU incorporation (Figs. 1B–E). Cell cycle analysis by PI fluorescence that corresponded to the G0/G1 peak of the control cells, indicating the inhibition of proliferation. In addition, there was an accumulation of cells in the sub-G1 region with an intensity of PI fluorescence lower than the level of fluorescence that corresponded to the G0/G1 peak of the control cells, indicating the induction of apoptosis in Y79 (Fig. 2) and WERI-Rb1 (Fig. 3) cells treated with vorinostat or CBHA.


### Table 1. Relative Expression of 21 Selected Transcripts in Y79 and WERI-Rb1 Cells

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Y79 + Vorinostat Treatment (hours)</th>
<th>WERI-Rb1 + Vorinostat Treatment (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4-Aminobutyrate aminotransferase</td>
<td>0.84</td>
<td>1.23</td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td>1.17</td>
<td>1.79</td>
</tr>
<tr>
<td>Angiopoietin-like 4</td>
<td>1.23</td>
<td>1.16</td>
</tr>
<tr>
<td>BCL2-interacting killer (BIK)</td>
<td>0.81</td>
<td>1.87</td>
</tr>
<tr>
<td>(p130)</td>
<td>0.95</td>
<td>0.58</td>
</tr>
<tr>
<td>(p107)</td>
<td>0.73</td>
<td>0.46</td>
</tr>
<tr>
<td>RBL2</td>
<td>0.95</td>
<td>0.58</td>
</tr>
</tbody>
</table>
| Cells treated with vorinostat (1 μM) for 0, 1, 2, 6, 8, 12, or 24 hours (control cells were arbitrarily assigned a value of 1) were quantified by real-time RT-PCR and confirmed the results obtained by Affymetrix arrays. Selected targets included transcripts involved in cellular metabolism, stress response, apoptosis, extracellular matrix synthesis, and the ephrin and Notch signaling pathways. mRNA levels of Rb, p107, and p130 are also reported. As previously demonstrated, WERI-Rb1 cells did not express RBl mRNA (Und, undetected), but Y79 cells expressed RBl mRNA, which was truncated and nonfunctional.

### Statistical Analysis

To evaluate the differences among various experimental conditions, one-way analysis of variance was performed, and post hoc tests (Duncan and Dunnett T3 tests) served to evaluate differences between individual pairs of experimental conditions. In all analyses, *P < 0.05* was considered statistically significant.

### RESULTS

**Vorinostat- and CBHA-Induced Accumulation of Acetylated Histones in Retinoblastoma Cells**

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**Involvement of p53 and Bcl-2 Family Members in Vorinostat-Induced Apoptosis in Retinoblastoma Cells**

We evaluated the effect of vorinostat on p53 expression in retinoblastoma cells. We found that vorinostat increased p53 protein expression in retinoblastoma cells, suggesting that it could contribute to vorinostat-induced growth arrest and apoptosis in this model (Fig. 4). Subsequently, we investigated the involvement of members of the Bcl-2 family in vorinostat-induced apoptosis. Vorinostat treatment promoted cleavage of the Bcl-2 family member BH3-interacting domain death agonist (Bid; Fig. 4). Cleavage of Bid results in a truncated form (tBid), which translocates to the mitochondria and results in an allosteric activation of Bak and Bax, inducing their intramembranous oligomerization, leading to mitochondrial dysfunction. These events are counteracted by the antiapoptotic members of the Bcl-2 family, such as Bcl-2.
We also found that vorinostat downregulated the expression of Bcl-2, thus shifting the balance toward the proapoptotic members of the Bcl-2 family (Fig. 4). In addition, vorinostat upregulated the expression of the proapoptotic Bax. These data support the pivotal role of mitochondria and the Bcl-2 family members in vorinostat-induced apoptotic signaling.

Involvement of Caspases in Vorinostat-Induced Apoptosis in Retinoblastoma Cells

The involvement of caspases in vorinostat-induced apoptosis was evaluated in lysates of cells treated with vorinostat for 0 to 24 hours. We found by immunoblotting that vorinostat induced the cleavage of caspases-9 and -8, and then the cleavage of caspase-3, in our model (Fig. 5A). We also found cleavage of PARP, a protein well known to be enzymatically cleaved during apoptosis, resulting in the classical 85-kDa PARP fragment.

Next, we evaluated the activation of caspase-8 and -3 after treatment with vorinostat in our Rb model. We found strong activation of both caspases in Y79 and WERI-Rb1 cells (Figs. 5B, C, E). These data support the activation of caspases in HDACI-treated Rb cells.

We found that the pan-caspase inhibitor ZVAD-FMK completely abrogated vorinostat-induced apoptosis in Y79 cells (Fig. 6A), establishing the functional role for caspases in this model. Moreover, the caspase-9 inhibitor LEHD-FMK, the caspase-8 inhibitor IETD-FMK, and the caspase-3 inhibitor DEVD-FMK also exerted protective effects (Fig. 6A).

Upregulation of Caspase-8 mRNA in HDACI-Treated Retinoblastoma Cells

We have previously reported that human retinoblastoma cells at baseline lack significant procaspase-8 mRNA and protein expression because of epigenetic gene silencing, resulting in apoptosis resistance. As mentioned, in the present study, we detected the presence of cleaved caspase-8 protein by immunoblot in vorinostat-treated Y79 cells, and the caspase-8 inhibitor IETD-FMK partially attenuated the proapoptotic effect of vorinostat. We thus investigated whether vorinostat induced reexpression of the silenced caspase-8 gene. We found a significant increase in caspase-8 mRNA levels in vorinostat-treated Y79 (Fig. 6B) and WERI-Rb1 (Fig. 6C) cells, but caspase-3 or caspase-6 mRNAs did not increase to the same degree.
Transcriptional Profile of Vorinostat-Treated Retinoblastoma Cells

To define molecular pathways regulating HDACI-induced apoptosis, we characterized the gene expression profiles of Y79 and WERI-Rb1 cells treated with vorinostat or CBHA in 2% FCS for 24 (B, C, respectively), 48 (D, E, respectively), and 72 (F, G, respectively) hours or were left untreated (A) for 72 hours. Vorinostat and CBHA induced declines in the percentages of cells in the S and G2 phases compared with control cells, indicating the inhibition of proliferation. In addition, there was an accumulation of cells in the sub-G1 region, with intensity of PI fluorescence lower than the level of fluorescence that corresponded to the G0/G1 peak of the control cells, indicating the induction of apoptosis in cells treated with vorinostat or CBHA.

Vorinostat-Induced Downregulation of Constitutive NF-κB Activity in Retinoblastoma Cells

NF-κB is constitutively active in retinoblastoma cells and is crucial for their viability and resistance to apoptosis. We found that vorinostat rapidly and profoundly suppressed the baseline activity of NF-κB in retinoblastoma cells (Fig. 7A).

Vorinostat-Induced Sensitization of Retinoblastoma Cells to DNA-Damaging Chemotherapy

Finally, we studied the effect of vorinostat on the sensitivity of retinoblastoma cells to DNA damage, using as a model the chemotherapeutic agent doxorubicin. We found that vorinostat (0.5 μM) strongly sensitized Y79 and WERI-Rb1 cells to low concentrations of doxorubicin (0.25 μg/mL for 48 hours; Figs. 7B, C).

DISCUSSION

We have evaluated the effects of HDAC inhibition on two Rb-deficient retinoblastoma cell lines in vitro and found that it potently suppresses proliferation, induces caspase-dependent apoptosis, suppresses baseline NF-κB activity, and increases sensitivity to DNA-damaging chemotherapy. These results were obtained with vorinostat concentrations similar to those that are active against Rb-expressing hematologic and solid

Transcriptional Profile of Vorinostat-Treated Retinoblastoma Cells

To define molecular pathways regulating HDACI-induced apoptosis, we characterized the gene expression profiles of Y79 and WERI-Rb1 cells treated with vorinostat for 0 to 24 hours compared with vehicle-treated controls harvested at the same time (GeneChip Human Genome U133 Plus 2.0 Array; Affymetrix). Analyses of these gene expression profiles showed that vorinostat modulated the mRNA levels for genes important for signal transduction (Fos, FosB, calmodulin 1, glutamate receptor AMPA 2, STAT3, adrenomedullin, nerve growth factor receptor, fibroblast growth factor receptor 4, GABA receptor), cell cycle (thymidylate kinase, galectin-1, aurora kinase B), cellular metabolism (4-aminobutyrate aminotransferase, carnitine palmitoyltransferase 1A), stress response (GADD45B), apoptosis (STK17B, BIK), extracellular matrix function (fibronectin 1, collagen type I, type VI and type XVIII, spondin 1, decorin), and control of cell differentiation. Notably, several transcripts involved in the ephrin (ephrin-A1, A3, A4, B2, and B3, ephrin receptors A3 and A4) and Notch (Notch2, Notch3, and the Notch effector HEY1) signaling pathways were upregulated. A list of known genes whose transcripts demonstrated the most prominent change on treatment with vorinostat is presented in Supplementary Table S1, online at http://www.iovs.org/cgi/content/full/50/9/4072/DC1. For validation, selected transcripts were quantified by real-time RT-PCR in RNA obtained from a separate experiment (Table 1). Additionally, we examined the mRNA levels of RB1, RBL1 (p107), and RBL2 (p130) by real-time RT-PCR (Table 1).

FIGURE 3. Vorinostat and CBHA induce growth arrest and apoptosis in WERI-Rb1 cells. PI analysis of WERI-Rb1 cells treated with vorinostat or CBHA in 2% FCS for 24 (B, C, respectively), 48 (D, E, respectively), and 72 (F, G, respectively) hours or were left untreated (A) for 72 hours. Vorinostat and CBHA induced declines in the percentages of cells in the S and G2 phases compared with control cells, indicating the inhibition of proliferation. In addition, there was an accumulation of cells in the sub-G1 region, with intensity of PI fluorescence lower than the level of fluorescence that corresponded to the G0/G1 peak of the control cells, indicating the induction of apoptosis in cells treated with vorinostat or CBHA.

FIGURE 4. Involvement of p53 and Bcl-2 family members in vorinostat-induced apoptosis in retinoblastoma cells. Immunoblotting analysis of Y79 cells treated with vorinostat (0–24 hours) revealed the induction of p53 protein expression. Vorinostat also induced the cleavage of Bid in Y79 cells, a member of the Bcl-2 family, which, on cleavage, translocates to the mitochondria to promote apoptosis. Moreover, vorinostat downregulated the expression of the antiapoptotic Bcl-2 and upregulated the expression of the proapoptotic Bcl-2 family member Bax.
tumor cell lines.22,27,28 These findings provide the preclinical rationale for clinical studies of HDACIs, alone and in combination with other therapies, to benefit patients with metastatic retinoblastoma.

Attempts to identify biological therapies for retinoblastoma have focused on agents, such as retinoids and butyrates, inducing a genetic program of growth arrest, differentiation, and apoptosis in vitro. Sodium butyrate inhibits the growth of retinoblastoma cell lines,29,30 induces differentiation,30 decreases Bcl-2 levels, increases Bax levels, stimulates the release of cytochrome c from the mitochondria, and induces caspase-dependent cleavage of PARP and apoptosis.31,32 Butyrates potentiate the apoptosis-inducing effect of vincristine and cisplatin on retinoblastoma cells in vitro.33 Butyrates are known to act as HDACIs,34 but only at high (millimolar) concentrations,34 which limits their clinical efficacy but simultaneously raises the hypothesis that more potent HDACIs could be effective agents for the treatment of retinoblastoma.

Several other compounds have been shown to exert HDACi activity,13,55–58 among them the anticonvulsant valproic acid,59 the antifungal agent trichostatin A (TSA),60 MS-275,13 and depsipeptide (FR901228).13,40 Dalgard et al.41 recently reported a growth-suppressive effect of trichostatin A, vorinostat, and MS-275 on human retinoblastoma cells in vitro. MS-275 significantly reduced tumor burden in mouse and rat models of retinoblastoma.41

In the present study, we found that vorinostat and CBHA induced the accumulation of acetylated histones, followed by growth arrest and apoptosis. Vorinostat induces p53 expression, cleavage of Bid, downregulation of Bcl-2, and upregulation of Bax, followed by caspase-mediated apoptosis in human retinoblastoma cells, and it potentiates the anticancer activity of doxorubicin. Interestingly, caspase-8 was one of the caspases activated by vorinostat. We have previously reported that human retinoblastoma cells have a deficiency in caspase-8 expression secondary to epigenetic gene silencing by overmethylation, which leads to resistance to apoptosis.23 In this study, vorinostat induced caspase-8 and caspase-3 in Rb cells.
study, we found that the expression of caspase-8 mRNA was restored in our model by vorinostat, which indicates that HDACIs may reverse gene silencing and restore sensitivity to apoptosis.

The transcription factor NF-κB is constitutively active in retinoblastoma cells and is crucial for their viability and resistance to apoptosis. Vorinostat rapidly and profoundly suppressed the baseline activity of NF-κB in retinoblastoma cells. This difference can be attributed to the fact that sodium butyrate activates the 26S proteasome, which degrades short-lived proteins including the NF-κB inhibitor IκB in retinoblastoma cells, whereas vorinostat has been found to suppress the activity of the 26S proteasome.

Because NF-κB inhibition strongly sensitizes retinoblastoma cells to DNA-damaging chemotherapeutic agents, we next investigated the effect of vorinostat on apoptosis induced by such agents. We found that vorinostat potentiated the effect of doxorubicin on retinoblastoma cells. This finding suggests that novel therapies combining vorinostat with conventional chemotherapy could improve outcomes in patients with aggressive retinoblastoma.

In conclusion, we have reported growth-suppressive and proapoptotic effects of HDAC inhibition on human retinoblastoma cell lines. Vorinostat is an effective HDACI. It has good availability after oral or intravenous administration, is generally well tolerated, and is active against a broad variety of transformed cells. Clinical activity has been documented in CTCL, and clinical evaluation in other malignancies is ongoing. Our data provide the framework for clinical evaluation of vorinostat in patients with aggressive retinoblastomas, alone and in combination with conventional chemotherapeutic agents, to improve patient outcomes.

References


