The Histone Deacetylase Inhibitor FK228 Distinctly Sensitizes the Human Leukemia Cells to Retinoic Acid-Induced Differentiation

JURATE SAVICKIENE,[a] GRAZINA TREIGYTE,[a]
VERONIKA BORUTINSKAITE,[a] RUTA NAVAKAUSKIENE,[a]
AND KARL-ERIC MAGNUSSON[b]

[a]*Department of Developmental Biology, Institute of Biochemistry, LT-08662 Vilnius, Lithuania*

[b]*Division of Medical Microbiology, Department of Molecular and Clinical Medicine, Linköping University, SE-58185 Linköping, Sweden*

ABSTRACT: FK228 (depsipeptide) is a novel histone deacetylase inhibitor (HDACI) that has shown therapeutical efficacy in clinical trials for malignant lymphoma. In this article, we examined *in vitro* effects of FK228 on human leukemia cell lines, NB4 and HL-60. FK228 alone (0.2–1 ng/mL) inhibited leukemia cell growth in a dose-dependent manner and induced death by apoptosis. FK228 had selective differentiating effects on two cell lines when used for 6 h before induction of granulocytic differentiation by retinoic acid (RA) or in combination with RA. These effects were accompanied by a time- and dose-dependent histone H4 hyper-acetylation or histone H3 dephosphorylation and alterations in DNA binding of NF-κB in association with cell death and differentiation. Pifithrin-α (PFT), an inhibitor of p53 transcriptional activity, protected only NB4 cells with functional p53 from FK228-induced apoptosis and did not interfere with antiproliferative activity in p53-negative HL-60 cells. In NB4 cells, PFT inhibited p53 binding to the p21 (Waf1/Cip1) promotor and induced DNA binding of NF-κB leading to enhanced cell survival. Thus, beneficial effects of FK228 on human promyelocytic leukemia may be exerted through the induction of differentiation or apoptosis via histone modification and selective involvement of transcription factors, such as NF-κB and p53.

KEYWORDS: differentiation; histone deacetylase inhibitor; leukemia; NF-κB; p53

Address for correspondence: Jurate Savickiene, Department of Developmental Biology, Institute of Biochemistry, LT-08662 Vilnius, Lithuania, Voice: 370-5-272-91-87; fax: 370-5-272-91-96.
e-mail: jurate_savickiene@yahoo.com

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INTRODUCTION

Histone deacetylase inhibitors (HDACI) are a novel class of chemotherapeutic agents. They induce histone hyper-acetylation and transcription regulation through chromatin remodeling, leading to selective activation of genes associated with cell growth, differentiation, and survival.[1,2] In recent years, HDACI, like sodium butyrate, trichostatin, apicidin, trapoxin, oxamflatin, suberoylanilide hydroxamic acid (SAHA), valproic acid, and several others, have been shown to inhibit cell proliferation and induce differentiation and apoptosis in a variety of human cell lines.[2,3] FK228 (known as FR901228 or depsipeptide) is a member of the cycle peptide class of inhibitors and shows potent antitumor activity against human and murine tumors.[4–6] FK228 is used in clinical trials for acute lymphocytic leukemia, small lymphocytic lymphoma, T cell lymphoma, cutaneous T cell lymphoma, or progressive small-cell or non-small cell lung cancer.[7,8] Preclinical studies with FK228 in chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML) demonstrated effective HDAC inhibition and antitumor activity, but its use was limited by progressive constitutional symptoms and needs alternative administration schedules.[9]

Acute promyelocytic leukemia (APL) is characterized by a chromosomal translocation *t*(15; 17), which fuses the PML gene to the retinoic acid receptor α (RARα) gene, resulting in the expression of chimeric gene product PML–RARα. This oncoprotein represses the transcription of retinoic acid (RA)-responsive genes by association with a co-repressor complex containing HDAC activity.[10] The aberrant recruitment of HDAC interferes with normal cell growth and differentiation. Thus, the addition of therapeutical doses of RA causes the conformational change of co-repressor complex and recruitment of a transcriptional activator complex leading to transcriptional activation and re-initiation of differentiation. Currently, all-*trans*-RA is used successfully in differentiation therapy of APL.[10,11] A rare variant of APL, which is associated with *t*(11; 17), has failed to respond to chemotherapy and treatment with RA.[12] The combined treatment of RA and HDACI, such as sodium phenylbutyrate and FK228, can induce *in vitro* differentiation of RA-resistant APL cells.[13–15] However, HDACI efficacy and selectivity toward cancer cells remain still poorly known.

In this article, we investigated the *in vitro* activities of FK228 alone or in combination with RA using APL cell line NB4, positive by chromosomal translocation (15; 17) and functional p53 status, and negative one, HL-60. Our findings demonstrate selective efficacy of FK228 in APL cell lines, including cell survival and sensitivity to RA-induced granulocytic differentiation through mechanisms involving H3 and H4 histone modifications, and activity modulation of transcription factors, such as NF-κB and p53.

MATERIALS AND METHODS

Materials

All chemicals used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO). Oligonucleotides were synthesized by MWG-Biotech AG (Ebersberg, Germany). Polyclonal anti-acetylated histone H4 antibody and polyclonal anti-phosphorylated histone H3 at serine 10 antibody were from Upstate (Lake Placid, NY). FK228 was obtained from Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan), and pifithrin α (PFT) from Calbiochem (Mannheim, Germany).

Cell Cultures

The human promyelocytic leukemia HL-60 and APL NB4 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco, Grand Island, NY) at 37°C in a humidified 5% CO_2 atmosphere and used for assays during exponential phase of growth.

Cell Viability and Growth

Cell viability was assayed by exclusion of 0.2% trypan blue. Cell number was determined by counting cells in suspension in a hemocytometer. For a dose–response determination, FK228 alone or in combination were added to a final volume of 5 mL.

Cell Differentiation and Apoptosis

The degree of differentiation was assayed by the ability of cells to reduce nitro blue tetrazolium (NBT) to insoluble blue-black formazan after stimulation by PMA.[16] Cell suspension (100 μL) was mixed with an equal volume of 0.2% NBT in phosphate-buffered saline (PBS) containing PMA (40 ng/mL) and incubated at 37°C for 30 min. NBT-positive cells were counted using a hemocytometer. At least 200 cells were scored for each determination. Apoptotic cell morphology was evaluated using fluorescence microscopy. At the end of each incubation, cells were pelleted at 500 *g* for 5 min, resuspended in 100 μL PBS (5×10^6 cells/mL) and stained with 0.01% acridine orange–0.01% ethidium bromide (AO/EtBr) mixture (1:1, v/v), 6 μL for 100 μL cell suspension.[17]

Assessment of the Early Myeloid Differentiation Marker CD11b

NB4 or HL-60 cells (5×10^5 cells/sample) were collected, washed twice with PBS, and suspended in 50 μL PBS, pH 7.4. Then 5 μL of monoclonal mouse anti-human CD11b, C3bi receptor/RPE (DakoCytomation, Glostrup, Denmark) antibody was added to the sample, gently suspended, and incubated in the dark at 4°C for 30 min. Cells were washed with PBS containing 2% bovine serum albumin, fixed in 4% para formaldehyde for 15–30 min on ice and the pellet was resuspended in PBS. Ten thousands events were analyzed for each sample by immunofluorescence using flow cytometry. Proliferating cells with and without CD11b antibodies were used as a control.

Preparation of Nuclear Extracts

Cells were harvested and pelleted at 500 *g* for 6 min, and washed twice in ice-cold PBS. Nuclei were prepared using Nuclei Isolation Kit (Sigma) according the manufacturer's recommendation. Nuclei were completely suspended in Nuclei EZ storage buffer and frozen at –70°C. Nuclear protein extracts for EMSA were prepared by lysis of nuclei in buffer, containing 20 mM Tris–HCl, pH 8,0, 200 mM EDTA, 2 mM EGTA, 20% glycerol, 400 mM NaCl, and inhibitors: 1 mM PMSF, 3 mM DTT, and protease inhibitor cocktail (Roche, Basel Switzerland). After incubation for 1 h on ice, the extracts were centrifuged at 18,000 *g* for 20 min, and were used immediately. Protein concentrations were measured using commercial RCDC Protein Assay (BioRad, Munich, Germany).

Electrophoretic Mobility Shift Assay (EMSA)

The probes used were synthetic oligonucleotides representing binding sites: (5′-AAGCCTGGGCAACATAGAAAGTCCCCATCTGTACAAAA-3′) NF-κB from the FasL promotor; (5′-AGTTGAGGGGACTTTCCCAGGC-3′) NF-κB consensus motif; (5′-ATCAGGAACATGTCCCAACATGTTGAG CTCT-3′) p53 from the p21 promoter. Complementary oligonucleotides were annealed and labeled at their 5′ ends using [γ-^{32}P-ATP] (Amersham Biosciences, Buckinghamshire, England) and T4 polynucleotide kinase (MBI Fermentas Inc., Vilnius, Lithuania). Standard DNA reactions were performed with 15 μg nuclear extracts in a 20 μL of reaction buffer (10 mM HEPES pH 7.9, 3 mM $MgCl_2$, 0,1 mM EDTA, 40 mM NaCl, 10% glycerol) containing 2 μg BSA, 1 μg poly(dI-dC), and 1 pM labeled oligonucleotide for 30 min at room temperature.[18] When desired, an unlabeled competitor oligonucleotide was added to the protein extracts at a 100-fold molar excess for a 15-min pre-incubation. DNA–protein complexes were resolved on 6% polyacrylamide gel containing 1 × Tris–borate buffer. After electrophoresis, the gels were dried and then exposed to X ray films.

Isolation of Histones and Western Blot Analysis

Histones were extracted as described previously.[19] Isolated nuclei were suspended in 5 vol of 0.4 N H_2SO_4 by stirring and incubated overnight at 0°C. The supernatant was collected by centrifugation at 15,000 *g* for 10 min at +2°C and the sediment was extracted once more. After centrifugation, both extracts were combined and histones were precipitated by adding 5 vol of ethanol at −20°C overnight. Precipitated histones were collected by centrifugation, washed several times with ethanol, and stored at −20°C until analysis. Histone electrophoresis was carried out essentially as described by Hurley.[20] Shortly, histones (5 μg) were dissolved in a buffer containing 0.9 M acetic acid, 10% glycerol, 6.25 M urea, and 5% β-mercaptoethanol, and run on a 15% polyacrylamide gel containing 6 M urea and 0.9 M acetic acid by using 0.9 M acetic acid as a buffer. After electrophoresis, gels were stained with Brilliant Blue G-colloidal (Sigma) or transferred to Immobilon™ PVDF transfer membrane (Millipore, Bedford, MA) for the evaluation of acetylated histone H4 (H4 Ac4) and phosphorylated histone H3 forms (H3 PS10) using specific antibodies. Immunoreactive bands were visualized by ECL chemiluminescence detection (Amersham Pharmacia, Uppsala, Sweden) according to the instructions of the manufacturer.

RESULTS

FK228 Induces Growth Inhibition and Death in Leukemia Cells

Different concentrations of FK228 (0.25, 0.5, 1 ng/mL) were used for the treatment of NB4 and HL-60 leukemia cells during 5 days. In both cell lines, FK228 as a single agent inhibited cell growth and induced cell death in a dose- and time-dependent manner (FIG. 1 A, B). In HL-60 cells, HDACI produced a greater growth inhibition and cell death at a shorter drug exposure time. Apoptosis was the main form of depsipeptide-induced cell death, as determined by staining with AO/Et Br. FK228 (1 ng/mL) induced massive leukemia cell apoptosis. Exposure of NB4 cells for 4–8 h to FK228 at a concentration 10 ng/mL was sufficient to induce irreversible cell death (data not shown). In both cell lines, the combined treatment with FK228 (0.25, 0.5 ng/mL) and RA (1 μM) caused the additive antiproliferative and apoptotic effects, which were more prominent in HL-60 cells (FIG. 1 C).

Distinct Effects of FK228 on RA-Induced Differentiation in Leukemia Cells

To study the effects of depsipeptide on leukemia cell differentiation, we used low doses of FK228 for co-treatment with RA or for 6-h pretreatment before the

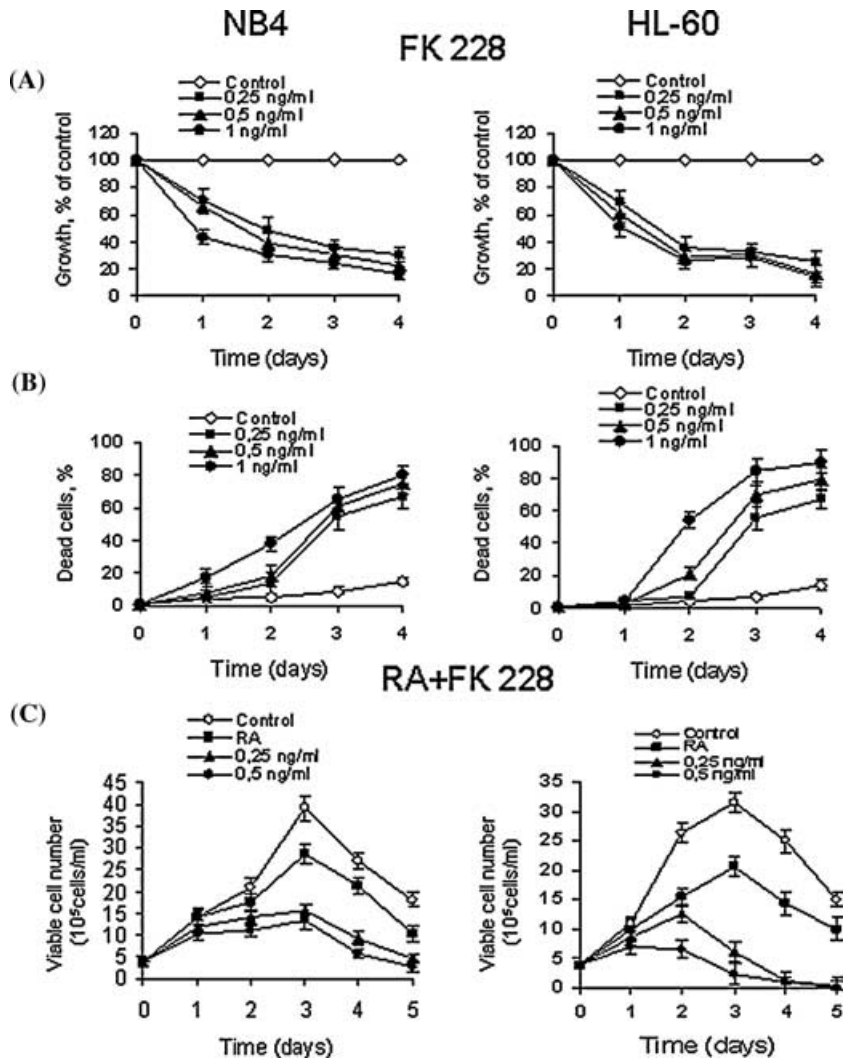

FIGURE 1. Dose-dependent effects of FK228 alone and in combination with RA on leukemia cell growth and viability. NB4 and HL-60 cells were exposed to the indicated concentrations of FK228 alone (**A, B**) and in combination with 1 μM RA (**C**) for 5 days. Aliquots of the cultures were subjected to counting following staining with 0.2% trypan blue for the determination of viable and dead cells.

induction of granulocytic differentiation by RA. In the HL-60 cell population, at 0.1 ng/mL FK228, a dose that does not induce apoptosis in combination with RA, the NBT-positive cell number increased more than twice on days 1 and 2 compared to RA alone (FIG. 2 A). In NB4 cells, the potentiating effect

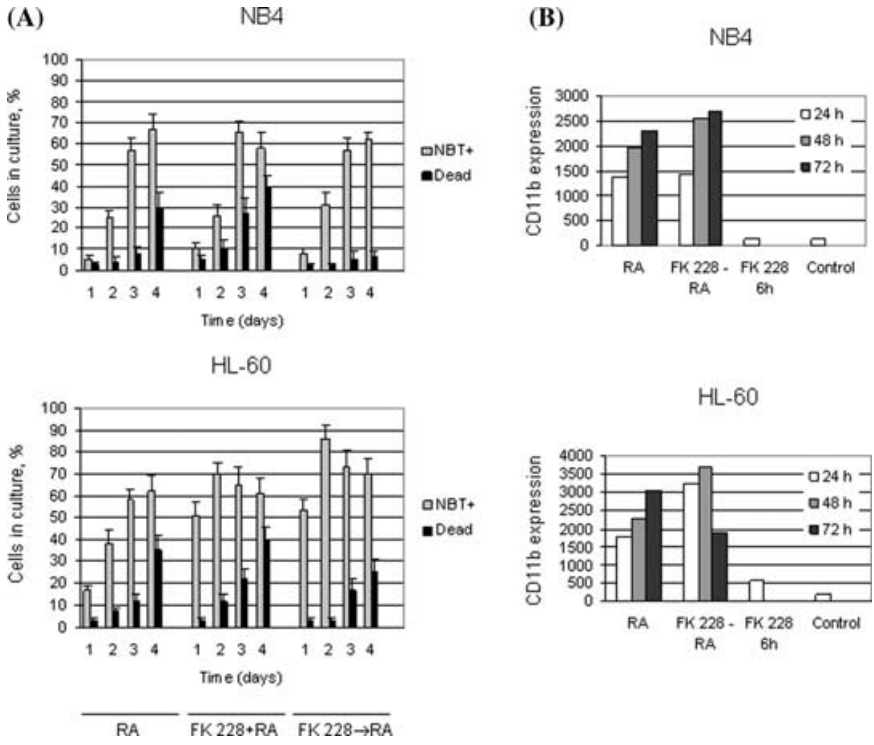

FIGURE 2. Time-dependent effects of FK228 on leukemia cell differentiation. HL-60 and NB4 cells were co-treated with RA (1 μM) and FK228 (0.1 and 0.25 ng/mL, respectively) for 4–5 days or pretreated with FK228 (0.25 ng/mL) for 6 h before the induction of differentiation by RA. Granulocytic differentiation (**A**) was determined by the ability of cells to reduce NBT. Results are mean ± SEM; $n = 3$. Expression of the differentiation marker CD11b, indicated as mean channel FL2-H fluorescence (**B**), was determined by a flow cytometry analysis. Data are representative of three independent experiments that gave comparable results.

was observed using RA with 0.25 ng/mL FK228 (an optimal dose for cell survival [FIG. 1] in such a combination) on day 3 only. FK228 alone failed to induce differentiation of NB4 cells and partially (to about 20%) induced HL-60 cell differentiation (data not shown). However, after 24-h exposure to RA following 6-h pretreatment with 0.25 ng/mL FK228 (an optimal dose and time for histone H4 acetylation in FIG. 3), HL-60 cell differentiation increased threefold compared to control (RA) and reached a maximum (about 90%) on day 2 with minimal cytotoxicity (FIG. 2 A). The same pretreatment caused substantial increase in the early differentiation marker CD11b expression in HL-60 cells and no marked changes in NB4 cells during a commitment stage of granulocytic differentiation (FIG. 2 B).

FK228 Induces Histone H4 and H3 Modifications

We analyzed where a short-time (2–8 h) exposure to FK228 at different concentrations (0.25–10 ng/mL) could promote histone H4 acetylation. Analysis of changes in histone acetylation by staining with brilliant blue G-colloidal (FIG. 3 A) or by immunoblotting (FIG. 3 B) demonstrated that FK228 induced a dose- and time-dependent increase in histone H4 acetylation in NB4 and HL-60 cells. In untreated cells, histones were predominantly un-acetylated and mono-acetylated (FIG. 3 A). Maximum histone H4 hyper-acetylation at FK228 concentrations of 5–10 ng/mL was achieved after 4 h; however, those doses caused high cytotoxic effect and marked cell death. Data in FIGURE 3 B clearly showed the moving of histone H4 into the highly acetylated isoforms at HDACI doses higher than 1 ng/mL. In HL-60 cells, the level of acetylated histone H4 to tri- and tetra-isoforms occurred at lower HDACI concentration (0.25 ng/mL) than in NB4 cell line during 4 h of treatment.

Phosphorylation is another important histone modification that is often associated with chromatin condensation during mitosis and induction of apoptosis or DNA damage.[21] Next, we examined histone H3 phosphorylation at serine 10 in leukemia cell response to FK228. In HL-60 cells, increasing doses of FK228 resulted in a decrease of histone H3 phosphorylation in parallel with

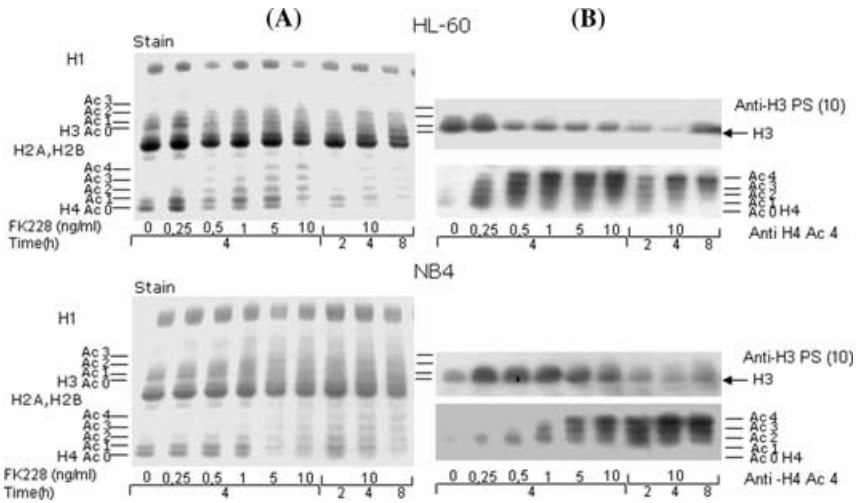

FIGURE 3. FK228 induces dose- and time-dependent histone H4 and H3 modifications. Histones from nuclear proteins of untreated or HDACI-treated NB4 and HL-60 cells were subjected to AU (15% polyacrylamide, acetic acid, urea) electrophoresis, gel staining with brilliant blue G-colloidal (**A**) and immunoblotting (**B**) with anti-acetylated histone H4 and anti-phosphorylated histone H3 at serine 10 antibodies. The five acetylation states of histone are indicated, representing un-acetylated (Ac 0), mono- (Ac 1), di- (Ac 2), tri- (Ac 3), and tetra- (Ac 4) acetylated forms.

an increase of histone H4 hyper-acetylation (FIG. 3 B), indicating a tight link between the aceylation/phosphorylation status of histone H3 and H4. The accumulation of histone H3 in phosphorylated form was noticed after 8 h treatment with 10 ng/mL FK228 (an apoptogenic dose). In less apoptosis-sensitive NB4 cells, the changes in histone H3 phosphorylation were similar, but occurred more slowly.

Modulation of Transcription Factor NF-κB Binding Activity by FK228

The nuclear transcription factor NF-κB acts as a survival factor and is required for the proliferation and differentiation of different cancer cells.[22] Therefore, we performed EMSA to study the NF-κB binding activity in response to FK228. As shown in FIGURE 4 A, 4-h exposure to different doses of FK228

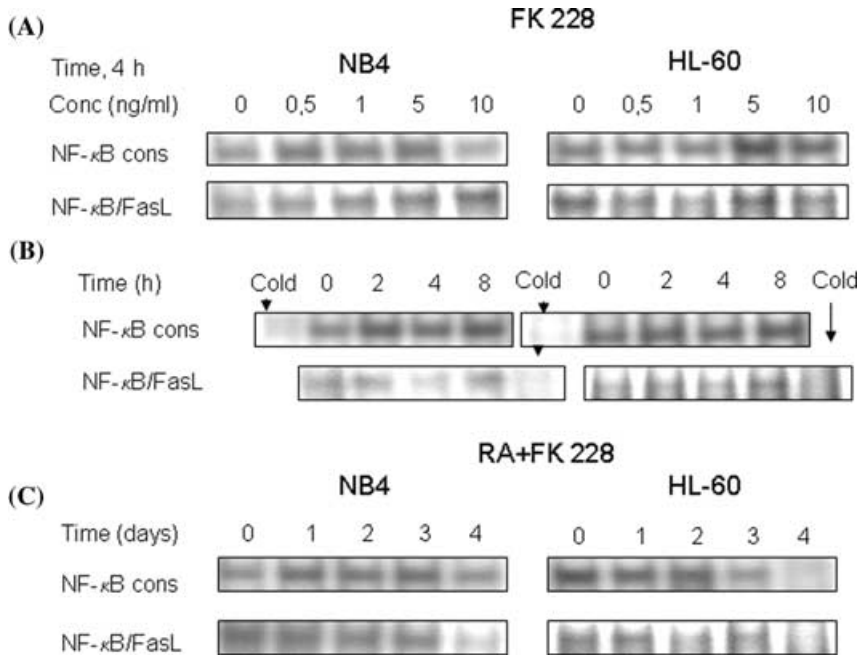

FIGURE 4. Alterations in NF-κB binding activity in response to FK228 alone or in combination with RA. Nuclear extracts were prepared from control cells and treated with different doses of FK228 during 4 h (**A**) or treated with 5 ng/mL FK228 for a different time (**B**), and in combination with RA (1 μM) and FK228 (0.25 ng/mL) during 4 days (**C**). EMSA was performed using a total of 15 μg protein from each nuclear extract and oligonucleotides, containing NF-κB consensus motif or NF-κB binding site from the FasL promotor. Specific DNA complexes with NF-κB were eliminated competitively by the addition of 100-fold molar excess of unlabeled oligonucleotide (cold).

caused cell line-specific increase in the intensity of the NF-κB–DNA complex formation at concentrations of 0.5–5 ng/mL and decrease at the apoptotic dose 10 ng/mL in both cell lines. HDACI caused a dose-dependent increase in NF-κB binding to the FasL promotor. In both cell lines, DNA binding to NF-κB was altered in response to 5 ng/mL FK228 (FIG. 4 A) and more prominent at 8 h exposure (FIG. 4 B), which is consistent with histone H4 hyper-acetylation occurring at 4–8 h (FIG. 3). The combined treatment with FK228 (0.25 ng/mL) caused the maintenance of elevated NF-κB binding activity during cell differentiation and suppression during differentiation-leading apoptosis on day 3 or 4 in HL-60 and NB4 cells, respectively (FIGS. 2 and 4 C). However, in this case, no upregulation of NF-κB binding to the FasL promotor was found in cells undergoing apoptosis.

PFT Protects p53-Positive NB4 Cells from Apoptosis

To determine whether HDACI-mediated apoptosis depends on p53 function, we used PFT, a transcriptional inhibitor of p53, and FK228 at doses that cause a similar level of NB4 and HL-60 cell death (FIG. 1). PFT (30 μM) itself inhibited p53-negative HL-60 cell growth at the same level as in combination with FK228 (0.25 ng/mL). PFT failed to inhibit p53-positive NB4 cell growth and increased cell survival in combination with 0.5 ng/mL FK228 (FIG. 5 A). Next, we exposed HL-60 and NB4 cells to PFT for 4 h, and after drug washing the cells were treated without or with low (0.2 ng/mL) and high (1 ng/mL) concentrations of FK228 for 4 days. As shown in FIGURE 5 B, PFT increased the survival of NB4 cells much better than of HL-60, and did not attenuate HL-60 cell death induced by a high dose of depsipeptide.

Induction of DNA damage by apoptogenic agents is known to activate p53, which in turn acts as a transcriptional regulator of several target genes, including CDK inhibitor p21 (Waf1/Cip1).[23] EMSA revealed that in NB4 cells with functional p53, a high dose (10 ng/mL) of FK228 caused maximal induction of p53 binding to the p21 promotor at 8 h of incubation (FIG. 6 A). Long-term treatment for 4 days with a moderate dose of FK228 (0.5 ng/mL) showed constitutive p53 binding activity, which was upregulated in NB4 cells on days 2–4. As shown in FIGURE 6 B, p53 binding to the p21 promotor was maintained during 4 h of FK228 (1 ng/mL) treatment and suppressed following 4 h treatment with PFT itself and in combination with HDACI. The same treatment decreased NF-κB binding activity in HL-60 cells, but enhanced it in NB4 cells.

DISCUSSION

Here, we have examined the *in vitro* activities of FK228 for APL cell lines, NB4 and HL-60, which exhibit promyelocytic phenotype and respond to RA

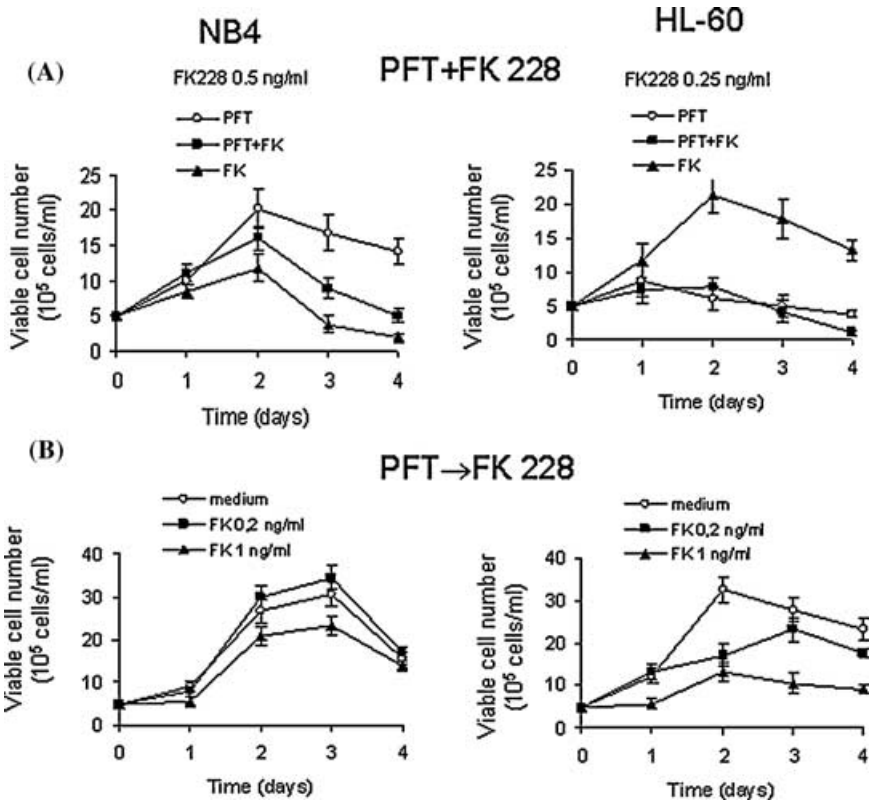

FIGURE 5. PFT protects p53-positive NB4 cells from apoptosis. (**A**) NB4 and HL-60 cells were incubated with 30 μM PFT itself or with 0.5 ng/mL and 0.25 ng/mL FK228, respectively, without or with PFT for 4 days or (**B**) exposed to PFT for 4 h and then transferred onto a fresh medium without or with different concentrations of FK228 for 4 days. Aliquots of the cultures were subjected to counting following staining with 0.2% trypan blue for the determination of the total number of viable cells. Results are mean ± SEM ($n = 3$).

that induces a terminal differentiation, followed by natural apoptosis of malignant cells.[24] We demonstrated that FK228 exhibits distinct antiproliferative and cytotoxic effects on both cell lines. This may be due to a difference in a set of genes that are independently controlled in each cell line.[25] For instance, the apoptotic process in differentiating NB4 cells is temporarily inhibited by the upregulation of the apoptosis inhibitor, survivin.[24] In human cancer cells, the protective role of p53 against apoptosis was shown in the upregulation of its downstream gene, p21,[25,26] which controls cell cycle progression.[27,28] HDACI is known to upregulate the $p21^{Waf1/Cip1}$ gene inducing the accumulation of acetylated histones in the p21 gene promotor in many cancer cells.[29,30] Furthermore, upregulation of p21, Fas, FasL,

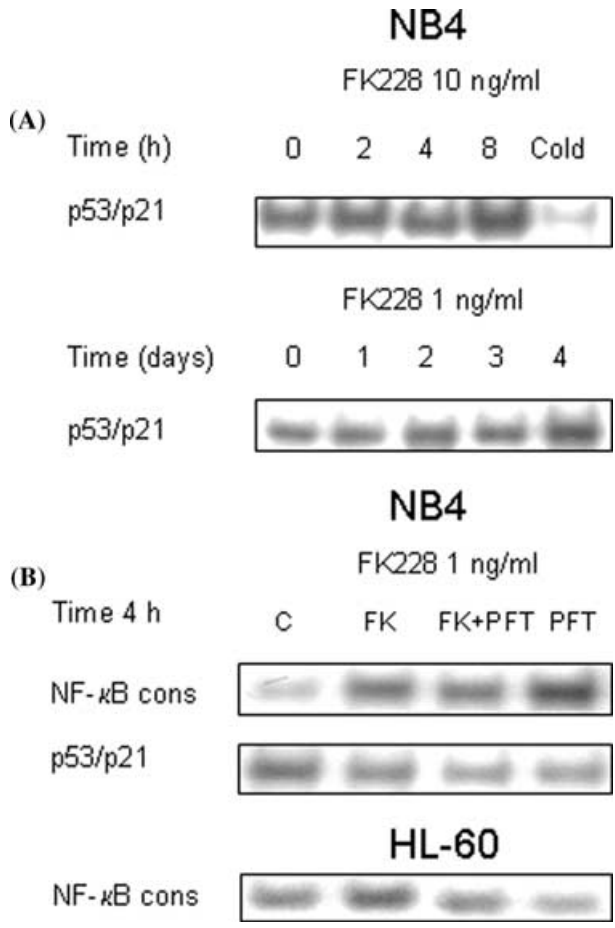

FIGURE 6. PFT affects NF-κB and p53 binding activity in leukemia cells with different functional statuses of p53. (**A**) Nuclear extracts were prepared from control NB4 cells and treated with FK228 (10 ng/mL) for 2–8 h, or with FK228 (1 ng/mL) for 4 days; and (**B**) NB4 and HL-60 cells were treated with PFT (30 μM) alone and co-treated with PFT and FK228 (1 ng/mL) for 4 h. EMSA was performed using a total 15-μg protein from each nuclear extract and oligonucleotides, containing NF-κB consensus motif and p53 binding site from the p21 promotor.

downregulation of antiapoptotic Bcl-X_L, and activation of caspase-3 were reported in adult T cell lymphoma, uveal melanoma, and neuroblastoma cells treated with HDACI.[7,31–33] Our EMSA results demonstrate the activation of NF-κB binding to the FasL promotor by FK228, suggesting the importance of the Fas/FasL system in depsipeptide-induced cell death. However, this activity decreases in the presence of RA, supporting the idea that the Fas/FasL

pathway is likely not involved in the apoptosis of terminally differentiated leukemia cells.[18,34,35]

FK228 was effective in inducing apoptosis in cell lines that are different by p53 status. Recently, a synthetic compound PFT has been reported to be a specific inhibitor of p53 transactivation and was proposed in cancer treatment.[36] However, PFT shows different effects; for instance, the inhibition of cell growth and induction or enhancement of p53-dependent apoptosis in mouse epidermal cells JB6[37]or, in contrast, the protection of neurons and cardiomyocytes against apoptosis induced by DNA-damaging agents and doxorubicin, respectively.[38,39] In our study, we demonstrate that p53-defective HL-60 cells displayed enhanced apoptotic potential compared to NB4 upon treatment with 30 μM PFT alone. PFT-pretreated NB4 cells with functional p53 exhibited increased cell survival after subsequent treatment with FK228. One of the postulated mechanisms of PFT activity is an inhibition of p53 translocation from the cytoplasm to the nuclei and prevention of its binding to specific DNA sites.[38] The latter suggestion is illustrated in our study by the reduced p53 binding activity to the p21 promotor in NB4 cells treated with PFT alone or together with FK228. PFT was shown also to reduce the activation of p53-regulated genes, including bax, cyclin G, and mdm2 that control the degradation rate of p53.[36,39] Furthermore, PFT-induced apoptosis may be mediated through a p53-independent pathway by the involvement of p38 and extracellular kinase activities of ERKs, or by the upregulation of proapoptotic bax and activation of caspases.[37,40] In our study, PFT induces apoptosis in p53-negative HL-60 cells and does not interfere with the antiproliferative action of FK228.

Another important fact is that FK228 enhanced and accelerated differentiation in HL-60 cells more effectively than in NB4 cells even at lower doses. Moreover, 6-h treatment with depsipeptide was sufficient to potentiate RA-mediated granulocytic differentiation in HL-60 cells. These effects were accompanied by different intensities of histone H4 acetylation and histone H3 dephosphorylation. Recent data point to a molecular link between both histone modifications. In general, increased histone acetylation (hyper-acetylation) triggers chromatin remodeling and transcriptional activation, while histone phosphorylation is often associated with chromatin condensation that includes mitosis and apoptosis.[21,41] Our results also reveal a coordinated correlation between the dynamic of histone H4 acetylation/histone H3 dephosphorylation (at serine 10) or H4 deacetylation/H3 phosphorylation upon treatment with FK228. The responses to HDACI, as a consequence of histone acetylation, may influence the pattern of gene expression. A finite subset of genes (about 9% of the genome) was found to be regulated in HL-60 cells upon treatment with the HDACI, trichostatin, over 50% of which were transcription factors or transcription augmenters. A number of genes were altered in expression, whereas others had opposite regulatory transcription profiles.[42] HDACI and RA alone induce distinct subsets of genes (about 3%), and as was shown in NB4 cells, the majority of genes were induced by the combination of RA and

HDACI, SAHA.[43] The cellular responses to HDACI, such as differentiation or apoptosis, often depend on the intrinsic characteristics of cancer cells.[44] The selective differentiation effects in HL-60 cells, obtained by 6-h treatment with FK228 followed by RA, may be explained by intensive histone H4 acetylation and, possibly, a rapid downregulation of transcription of primary response genes, such as myb and c-myc, that are amplified in HL-60 cells.[4,42] In certain circumstances the transcriptional activity of NF-κB activity could explain the effectiveness of HDACI to trigger differentiation or apoptosis. In certain cell types, HDACI can activate NF-κB–dependent gene expression resulting in the upregulation of anti-apoptotic genes, such as Bcl-X_L,[44,45] and in others it can suppress NF-κB activation.[7,46] Our EMSA results demonstrate time- and dose-dependent differences in the transcription factor NF-κB binding activity between HL-60 and NB4 cell lines in association with cell survival and maturation to granulocytes. FK228 reduced NF-κB binding activity during apoptosis in both the leukemia cell lines that have been demonstrated in ATL cells too.[47] Thus, this transcription factor could be considered as one of the general targets of FK228.

In summary, our data have provided evidence for myeloid cell line-specific, differential activity of FK228 in the enhancement of RA-mediated differentiation that is associated with the regulation of gene expression mediated through chromatin remodeling.

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