Induction of Cell Cycle Arrest and Apoptosis by the Proteasome Inhibitor PS-341 in Hodgkin Disease Cell Lines Is Independent of Inhibitor of Nuclear Factor-κB Mutations or Activation of the CD30, CD40, and RANK Receptors

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ABSTRACT

Purpose: The malignant Hodgkin and Reed-Sternberg cells of Hodgkin disease (HD) are known to constitutively express high levels of activated nuclear factor κB (NF-κB), which plays an important role in their survival. The proteasome inhibitor PS-341 has been recently shown to modulate tumor cell proliferation and survival by inhibiting NF-κB and modulating critical cellular regulatory proteins, but its activity in cells carrying IκBα gene mutations has not been reported previously.

Experimental Design: The activity of PS-341 in four well-characterized, HD-derived cell lines. Cell proliferation and apoptosis were determined by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfonyl)-2H-tetrazolium (MTS) and Annexin-V binding methods, respectively. Cell cycle analysis was determined by flow cytometry. Intracellular protein levels were determined by Western blot.

Results: PS-341 demonstrated a strong antiproliferative activity, which was irrespective of the status of mutations in IκBα and even the presence of CD30, CD40, or RANK receptor activation. This effect was attributable to the induction of apoptosis and cell cycle arrest at the G2-M phase. PS-341 not only inhibited nuclear localization of NF-κB but also activated the caspase cascade, increased p21 and Bax levels, and decreased Bcl-2 levels. Furthermore, PS-341 enhanced the effect of gemcitabine chemotherapy and potentiated the effect of tumor necrosis factor-related apoptosis-inducing ligand/APO2L and two agonistic antibodies to tumor necrosis factor-related apoptosis-inducing ligand death receptors R1 and R2.

Conclusions: The in vitro activity of PS-341 against HD-derived cell lines suggests that PS-341 may have a therapeutic value for the treatment of HD.

INTRODUCTION

Nuclear factor κB (NF-κB) plays a central role in regulating the expression of various genes involved in cell survival, apoptosis, carcinogenesis, and inflammation, making it a potential therapeutic target (1, 2). The NF-κB family of proteins (including p50/p105, p52/p100, p65, RelB, and c-Rel) exists in homodimers and heterodimers. In mammalian cells, the dominant responsive form is the p50/p65 heterodimer. In unstimulated cells, NF-κB is present in the cytoplasm in an inactive form, bound to inhibitors of NF-κB (IκBα, IκBβ, and IκBε). On activation, IκB is rapidly phosphorylated and ubiquinated and subsequently degraded by the proteasome pathway. Consequently, the active p50/p65 heterodimer is translocated to the nucleus and binds to a specific DNA sequence to induce gene transcription (3).

The malignant Hodgkin/Reed-Sternberg (H/RS) cells of Hodgkin disease (HD) aberrantly express the activated p50/p65 (RelA) heterodimeric form of NF-κB (4–7). This expression is observed in HD cell lines and the vast majority of primary H/RS cells in HD lymph node sections (7). Several mechanisms have been implicated in the aberrant activation of NF-κB in H/RS cells, including genetic defects in the IκBα gene, Epstein-Barr viral infection, and stimulation by cytokines (8–13). Inhibition of NF-κB activation by a dominant-negative IκBα in HD-derived cell lines has been shown to interfere with cell cycle progression and inhibit proliferation in vitro and in severe combined immunodeficient mice (7). Thus, the pharmacological inhibition of this pathway may prove to be of clinical value for the treatment of patients with HD.

PS-341 (Velcade, bortezomib) is a small molecule inhibitor of the ubiquitin-proteasome pathway. Proteasome plays an essential role in the degradation of most intracellular proteins, including those that regulate the cell cycle, cell survival and apoptosis, cell adhesion and trafficking, and transcription factor activation (14). By inhibiting the degradation of cytoplasmic inhibitor of nuclear factor-κB (IκBα), PS-341 inhibits the activation of NF-κB. Furthermore, PS-341 has been reported to alter the levels of p21, p27, Bcl-2, Bax, XIAP, survivin, and p53, leading to cell cycle arrest and apoptosis in several tumor types (15). PS-341 has also been shown to enhance tumor cell sensitivity to chemotherapy (16–18). Because of these favorable antitumor properties, PS-341 was rapidly developed to treat human cancer (15, 19–21).

In this study, we investigated the activity of PS-341 in...
HD-derived cell lines and determined the molecular mechanisms for its biological activity

MATERIALS AND METHODS

Cell Lines and Cell Culture. The human HD-derived cell lines HD-MYZ, HD-LM2, L-428, and KM-H2 were obtained from the German Collection of Microorganisms and Cell Cultures, Department of Human and Animal Cell Cultures (Braunschweig, Germany). Two cell lines are known to have mutations in the IkBα gene (L-428 and KM-H2). The phenotypes and genotypes of these cell lines have been published previously (22). All cell lines were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies Inc., Gaithersburg, MD), l-glutamine, and penicillin/streptomycin in a humid environment of 5% CO₂ at 37°C.

Reagents, Antibodies, and Recombinant Proteins. PS-341 was kindly provided by Millennium Pharmaceuticals, Inc. (Cambridge, MA). Recombinant human RANK ligand (RANKL, TRANCE), CD40 ligand (CD40L, CD154), and CD30 ligand (CD30L, CD153) and the pan-caspase inhibitor Z-VAD-FMK were obtained from Alexis Corp. (San Diego, CA). Recombinant human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/APO2L) trimer was kindly provided by Dr. Avi Ashkenazi (Genentech Corp., South San Francisco, CA). Agnostic monoclonal antibodies to the TRAIL death receptors R1 (ETR1) and R2 (ETR2) were provided by Human Genome Sciences, Inc. (Rockville, MD). Antibodies to extracellular signal-regulated kinase (ERK)1/2 and phosphorylated ERK1/2 (Thr202, Tyr204) and antibodies to procaspases 7, 8, 9, and 10 to cleaved caspase 3 and polyadenosine-5′-diphosphate-ribose polymerase were all from Cell Signaling Technology (Beverly, MA). A phospho p53 antibody sample kit containing antibodies to total p53 and phospho-specific antibodies to Ser6, Ser9, Ser15, Ser20, Ser37, Ser46, and Ser392 was also obtained from Cell Signaling Technology. Antibodies to Mcl-1, Bcl-x, Bcl-2, Bax, Bak, cIAP2, p14ARF, p21WAF, and p27Kip1 were obtained from R&D Systems (Minneapolis, MN); antibodies to XIAP were obtained from Alexis Corp. (San Diego, CA). Recombinant human RANK ligand (RANKL, TRANCE), CD40 ligand (CD40L, CD154), and CD30 ligand (CD30L, CD153) and the pan-caspase inhibitor Z-VAD-FMK were obtained from Alexis Corp. (San Diego, CA). Recombinant human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/APO2L) trimer was kindly provided by Dr. Avi Ashkenazi (Genentech Corp., South San Francisco, CA). Agnostic monoclonal antibodies to the TRAIL death receptors R1 (ETR1) and R2 (ETR2) were provided by Human Genome Sciences, Inc. (Rockville, MD). Antibodies to extracellular signal-regulated kinase (ERK)1/2 and phosphorylated ERK1/2 (Thr202, Tyr204) and antibodies to procaspases 7, 8, 9, and 10 to cleaved caspase 3 and polyadenosine-5′-diphosphate-ribose polymerase were all from Cell Signaling Technology (Beverly, MA). A phospho p53 antibody sample kit containing antibodies to total p53 and phospho-specific antibodies to Ser6, Ser9, Ser15, Ser20, Ser37, Ser46, and Ser392 was also obtained from Cell Signaling Technology. Antibodies to Mcl-1, Bcl-x, Bcl-2, Bax, Bak, cIAP2, p14ARF, p21WAF, and p27Kip1 were obtained from R&D Systems (Minneapolis, MN); antibodies to XIAP were obtained from Transduction Laboratories (San Diego, CA); antibodies to β-actin were obtained from Sigma Chemical Co. (St. Louis, MO); antihuman p65 antibody was obtained from Santa Cruz Biotechnology. Three NF-κB inhibitors were prepared: (a) resveratrol (Sigma Chemical) was prepared as a 100-mM stock solution in DMSO; (b) Pyrrolidine dithiocarbamate (PDTC) (Alexis) was prepared as a 25-mM stock solution in PBS; and (c) N-acetyl-L-leucinyl-L-leucinyl-norleucinal (LLnL) (Sigma Chemical) was prepared as a 25-mM stock solution in DMSO.

In Vitro Proliferation Assay. Cells were cultured in 6-, 12-, and 24-well plates at a concentration of 0.5 × 10⁶ cells/ml for all cell lines and assays. Cell viability was assessed with a nonradioactive cell proliferation 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenoxy)-2-(4-sulfonyl)-2H-tetrazolium (MTS) assay using CellTiter 96 Aqueous One Solution Reagent (Promega, Madison, WI) as described previously (23). Briefly, 80 μl of cell suspension were added to 20 μl of the reagent and incubated in 96-well plates for 1 h at 37°C in 5% CO₂, and light absorbance of formazan was measured at 495 nm on a μQuant plate reader equipped with KC4 software (Biotek Instruments, Winoski, VT). Each measurement was made in triplicate, and the mean value was determined.

Flow Cytometry. Cell cycle fractions and apoptosis were determined by staining with propidium iodide (Sigma-Aldrich) and Annexin-V-Fluos (Roche Molecular Biochemicals, Indianapolis, IN), as described previously (24–26). Data were collected on a Becton Dickinson FACScan flow cytometer using CellQuest software (BD Biosciences, San Jose, CA) and analyzed using WinMDI 2.8 software (Joseph Trotter, Scripps Research Institute, La Jolla, CA).

Western Blot Analysis. Whole-cell protein extract was prepared by incubating the cells in lysis buffer (Cell Signaling Technology) for 30 min at 4°C and then centrifuging to remove cellular debris. The protein in the resulting supernatant was quantified by the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL) according to the manufacturer’s instructions, diluted 1:1 in protein SDS loading buffer (Cell Signaling Technology), and boiled for 30 min. A total of 30 μg of protein was loaded onto 10 or 15% Tris-HCl SDS-PAGE Ready Gels (Bio-Rad, Hercules, CA), transferred to a nitrocellulose transfer membrane (Pierce Chemical), and detected using SuperSignal West Dura Extended Duration Substrate (Pierce Chemical).

Immunocytochemistry for NF-κB p65 Localization. Cellular localization of the p65 subunit was performed as described previously (27). Briefly, HD cells treated with PS-341 were plated on a glass slide by centrifugation at 750 rpm using a Cytospin 4 (Thermoshandon, Pittsburgh, PA), air dried for 1 h at room temperature, and fixed with cold acetone. After brief washing in PBS, slides were blocked with 5% normal goat serum for 1 h and then incubated with rabbit polyclonal antihuman p65 antibody (dilution 1:100). After overnight incubation, the slides were washed and then incubated with goat antirabbit IgG (Alexa Fluor 594; 1:100) for 1 h and counterstained for nuclei with Hoechst 33342 (50 ng/ml) for 5 min. Stained slides were mounted with mounting medium (Sigma Chemical) and analyzed under an epifluorescence microscope (Labophot-2; Nikon, Tokyo, Japan). Pictures were captured using Photometrics CoolSnap CF color camera (Nikon, Lewisville, TX) and MetaMorph version 4.6.5 software (Universal Imaging Corp., Downingtown, PA).

Electrophoretic Mobility Shift Assay. Electrophoretic mobility shift assay was performed to determine the activation and nuclear translocation of NF-κB as described previously but using minor modifications (28). Briefly, 4 μg of nuclear protein extract were incubated with 16 fmol of a 32P-labeled, 45-mer double-stranded DNA oligonucleotide derived from the human immunodeficiency virus long terminal repeat (5′-TTTGTACAAAG-GACTTTCGCCGTGGGACTTTCAGGGAGCGTGG-3′; italicized areas indicate NF-κB-binding sites) for 30 min at 37°C. The resulting complex was resolved from free oligonucleotide using electrophoresis on 6.6% native polyacrylamide gels.

RESULTS

PS-341 Inhibits HD Cell Proliferation in Vitro Irrespective of IkBα Gene Mutation. It is well established that H/RS cells constitutively express activated NF-κB (4, 6). On the basis
of the antiproliferative activity of PS-341 in other NF-κB-expressing lymphoid malignancies, including multiple myeloma (16–18), we first investigated whether PS-341 had a similar antiproliferative effect on HD-derived cell lines. Results were compared between cell lines that are known to have mutations in the \( \text{IkB}\alpha \) gene (L-428 and KMH-2) and cell lines that do not have such mutations (HD-MYZ and HD-LM2; Ref. 10). For these experiments, HD cells were incubated with increasing concentrations of PS-341 (0–50 nM) for 48 h, and the viable cell number was determined using an MTS assay. As shown in Fig. 1A, PS-341 had antiproliferative activity in all four HD cell lines in a dose-dependent manner. The most sensitive cell line was the HD-LM2 line, with an IC\(_{50}\) between 2.5 and 5 nM. The other three cell lines had an IC\(_{50}\) ranging between 10 and 20 nM. Thus, a PS-341 concentration of 20 nM was used, unless otherwise specified. Using this concentration, PS-341 inhibited HD cell proliferation in a time-dependent manner, with significant antiproliferative activity being observed as early as 24 h after treatment and lasting for \( \geq 72 \) h (Fig. 1B). Thus, PS-341 had antiproliferative activity in HD-derived cell lines irrespective of \( \text{IkB}\alpha \) mutations.

**PS-341 Activity Is Maintained in the Presence of CD30, CD40, and RANK Receptor Activation.** We and others have reported previously that H/RS cells express CD30, CD40, and RANK receptors, all of which can activate NF-κB (28–30). Because H/RS cells are frequently exposed to the ligands of these receptors in vivo, we examined whether PS-341 can maintain its antiproliferative activity in HD cells in the presence of activating CD30L, CD40L, and RANKL. For these experiments, HD cells were incubated with one of these ligands with or without PS-341 for 48 h, and viable cell numbers were determined using an MTS assay. This is a representative of two independent experiments performed in triplicate. Similar results were observed in the other three cell lines.
maintained its antiproliferative effect in the presence of these activating ligands.

**PS-341 Induces Cell Cycle Arrest and Apoptosis in HD-Derived Cell Lines Irrespective of Ik-B Gene Mutation.**

To determine whether the antiproliferative activity induced by PS-341 was caused by cell cycle arrest or induction of apoptosis, we examined the effect of PS-341 on cell cycle fractions. For these experiments, HD-derived cells were incubated with increasing concentrations of PS-341 (2.5–50 nM) for 48 h before cell cycle fractions were determined using the propidium iodide staining method. In the HD-LM2 cells, PS-341 induced cell death (sub-G0 fraction) without G2-M arrest. In the other three cell lines, cell cycle arrest at the G2-M phase was followed by cell death. Percentages in the left top quadrant, dead cells; percentage in the right top quadrant, cells in the G2-M phase. In B, HD cells were incubated for 24 and 48 h with medium or 20 nM PS-341. Cell cycle fraction was determined using the propidium iodide staining method. PS-341 induced accumulation of the cells in the G2-M phase in the L-428 and KM-H2 cells as early as 24 h after treatment.

The ability of PS-341 to induce cell death and apoptosis in the HD cells was further confirmed by Annexin-V staining. For these experiments, cells were incubated with either medium or PS-341 (20 nM) for 48 h. Cells were then double stained with propidium iodide and Annexin-V before they were analyzed using two-color flow cytometry. As shown in Fig. 3, PS-341 increased Annexin binding in all four HD-derived cell lines, indicative of the induction of apoptosis.

**PS-341 Inhibits NF-κB Activation in HD-Derived Cell Lines.**

PS-341 has been reported to inhibit NF-κB activation in several tumor types. Whether PS-341 could also inhibit NF-κB activation in HD cell lines with normal or mutated IkBα had not been determined. For these experiments, we examined the effect of PS-341 on the nuclear localization of the NF-κB p65 subunit in HD cells. HD cells were incubated with medium or PS-341 (20 nM) for 2–8 h. Cells were then immunostained with p65-specific immunofluorescent antibodies as described in “Materials and Methods.” Nuclei were counterstained with Hoechst staining. Results were compared between cell lines that have IkBα mutations and those that do not. As shown in Fig. 4, unstimulated L-428 cells (IkBα mutations) and HD-MYZ cells (no IkBα mutations) constitutively expressed active NF-κB.
with localization of the p65 subunit in the nuclei and cytoplasm. When the L-428 and HD-MYZ cells were incubated with PS-341 (20 nM), the active p65 subunit remained in the cytoplasm without translocating into the nuclei, indicative of NF-κB inactivation. This effect was observed as early as 2 h after treatment and was maintained for ≥8 h. Thus, PS-341 inhibited NF-κB activation even in cells that harbored IκBα mutations.

The constitutive expression of NF-κB was inhibited in the HDLM-2 cell line by the antioxidant PDTC (50 μM) but not by the proteasome inhibitor LLnL (6.25 μM) or antioxidant resveratrol (50 μM; Fig. 4C). Although PDTC inhibited NF-κB, it had only a modest killing effect on HDLM-2 cells (20% cell death within 48 h; Fig. 4C). In contrast, PS-341 was very effective in killing HD-LM2 cells (Fig. 4C). As expected, neither resveratrol nor LLnL, which did not inhibit NF-κB, was effective (data not shown). Collectively, these data suggest that, under normal culture conditions, inhibition of NF-κB activation is not the predominant mechanism of PS341-induced cell death.

**PS-341 Activates the Caspase Cascade and Alters the Levels of Cell Cycle Regulatory Proteins.** On the basis of the ability of PS-341 to induce apoptosis of HD cells, we investigated whether this process was caspase dependent. For these experiments, HD-derived cell lines were incubated with medium or PS-341 (20 nM) for 48 h before the percentage of dead cells was determined using dual staining with Annexin-V and propidium iodide. PS-341 induced apoptosis in all four HD cell lines to a variable degree. Left bottom quadrant, viable cells. Percentages of dead cells in each quadrant are shown.

![Fig. 3 Induction of apoptosis by PS-341. Hodgkin disease (HD) cells were incubated with medium or PS-341 (20 nM) for 48 h before the percentage of dead cells was determined using dual staining with Annexin-V and propidium iodide. PS-341 induced apoptosis in all four HD cell lines to a variable degree. Left bottom quadrant, viable cells. Percentages of dead cells in each quadrant are shown.](image)

PS-341 has been reported previously to regulate the level of several proteins that control the cell cycle. Here, we found that PS-341 increased p21\(^{WAF}\) levels in all four HD cell lines without a significant effect on p27\(^{Kip}\) (Fig. 6A). In addition, PS-341 increased cyclin D2 levels in the L-428 cell line and cyclin D1 levels in the HD-MYZ and L-428 cells (Fig. 6A). Furthermore, PS-341 did not change the total cellular content of p53 but increased its phosphorylation status at the serine 15 site in one cell line (KM-H2) and decreased the MDM2 protein level in two cell lines (HD-LM2 and KM-H2; Fig. 6A).

The effect of PS-341 on members of the Bcl-2 family was also examined (Fig. 6B). In the HD-LM2 cells, the Bax level significantly increased within 24 h and remained elevated for ≥48 h. In contrast, the Bcl-2 level decreased in the L-428 cells without significant changes in the Bax protein levels. PS-341 had no significant effect on Bcl-XL or Mcl-1 levels in any of the cell lines.

Finally, we examined the effect of PS-341 on several members of the IAP family (Fig. 6C). All cell lines expressed detectable levels of XIAP, c-IAP1, and c-IAP2. PS-341 slightly decreased c-IAP1 in one cell line (L-428) but had no significant effect on the level of other IAP family members in any of the cell lines.

**PS-341 Enhances the Activity of Chemotherapy, TRAIL/APO2L, and Antagonistic Antibodies to TRAIL Death Receptors R1 and R2.** PS-341 has been reported to have synergy with chemotherapy in several tumor models. To explore whether a similar synergistic effect could be achieved in HD cells, we incubated HD cells with submaximal concentrations of PS-341 (5 nM), with or without submaximal concentrations of gemcitabine (0.05 μM), for 72 h. The viable cells were counted using the MTS assay. A synergistic effect was observed only in the HD-LM2 cells (Fig. 7A), whereas an additive effect was observed in the remaining cell lines (data not shown).

We have reported previously that HD cell lines express the TRAIL receptors R1 and R2 and that TRAIL/APO2L is modestly effective in inducing apoptosis in the cells (31). The activity of two agonistic antibodies to TRAIL receptors R1 (ETR1) and R2 (ETR2) has not been studied previously in HD cell lines. At equal concentrations of TRAIL/APO2L, ETR1,
and ETR2 (0.1 μg/ml), 40–50% of the HD-LM2 cells were killed within 24–48 h. When HD-LM2 cells were incubated with a minimally toxic concentration of PS-341 (5 nM) with 0.1 μg/ml TRAIL/APO2L, ETR1, or ETR2, a synergistic killing effect was observed within 24–48 h (Fig. 7B). This synergistic effect was less prominent in the remaining HD cell lines (data not shown).

**DISCUSSION**

This is the first study demonstrating the activity of PS-341 in cell lines known to have IκBα mutation. In this study, we found that PS-341 was effective as an antiproliferative agent in all four HD-derived cell lines that were tested. Two of these cell lines are known to have mutations in the IκBα gene, resulting in a truncated, nonfunctional IκBα protein (10). Thus, it would seem that proteasome inhibition might not be effective in these cell lines. However, we found that IκBα mutation had no effect on PS-341 activity, which suggests that IκBβ or other unknown inhibitors can substitute for the IκBα protein. Furthermore, some PS-341 activities may be mediated by NF-κB-independent mechanisms. In any case, the presence of IκBα mutations was therapeutically irrelevant.

An additional concern was whether PS-341 could maintain its activity in the presence of activating cytokines (13). It is well established that malignant H/Rs cells express several receptors that belong to the tumor necrosis factor family, including CD30, CD40, and RANK (28, 32, 33). Activating these receptors by their ligands activates several shared signaling pathways, in-

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**Fig. 4** Effect of PS-341 on nuclear localization of nuclear factor κB (NF-κB) subunit p65. In A, L-428 cells, which are known to carry inhibitor of nuclear factor-κB mutations, were incubated with 20 nM PS-341 for 2–8 h. P65 was visualized under a fluorescence microscope using Alexa-Fluor 594 secondary antibody (top panel). The figure shows that PS-341 inhibited nuclear localization of p65 in L-428 cells within 2 h of incubation. Hoechst staining (bottom panel) shows that the nuclei were intact. In B, PS-341 shows a similar effect on the HD-MYZ cells, which carry wild-type IκBα. In C, inhibition of NF-κB activation in the HD-LM2 cells was achieved by the antioxidant PDTC but not resveratrol or LLnL (left panel). NF-κB activity was determined after 48 h of incubation using electrophoretic mobility shift analysis as described in “Materials and Methods.” However, PDTC remained relatively ineffective compared with PS-341 (right panel).
including ERK and NF-κB (4, 31). We found that although PS-341 did not alter ERK levels or phosphorylation status, it maintained its activity in the presence of these ligands, suggesting that PS-341 would be active in vivo. This also suggests that PS-341 alters protein levels downstream from p-ERK that may be crucial for its function.

In this study, PS-341 induced apoptosis and cell cycle arrest at the G2-M phase (16, 17, 34–36). Apoptosis was me-

**Fig. 5** PS-341-induced apoptosis in Hodgkin disease (HD) cells is caspase mediated. In A, HD cells were incubated with medium or 20 nM PS-341 for 6–48 h. Whole-cell lysates were examined by Western blot for changes in intracellular proteins. PS-341 cleaved caspases 8, 9, and 3 and poly(ADP-ribose) polymerase (PARP). Furthermore, PS-341 down-regulated c-FLIP in the HDL-M2 cells. In two cell lines that expressed BID (HD-MyZ and HD-LM2), PS-341 also decreased BID levels, suggesting its cleavage. In B, PS-341-induced cell death of HD cells was either partially or completely blocked by the pan-caspase inhibitor Z-VAD-FMK. HD cells were incubated with medium, Z-VAD-FMK (20 μM), PS-341 (20 nM), or a combination of PS-341 and Z-VAD-FMK. After 48 h in culture, the viable cells were counted using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfonyl)-2H-tetrazolium (MTS) assay. Each value represents a mean of three independent experiments done in triplicate.

**Fig. 6** Effect of PS-341 on Bcl-2 family, IAPs, and cell cycle proteins. In A, PS-341 up-regulated p21 and increased p53 phosphorylation (serine 15) without inducing changes in total p53. Cyclins D2 and D1 also were each up-regulated in two cell lines. In B, PS-341 up-regulated Bax in the HD-LM2 cells and down-regulated Bcl-2 in the L-428 cells. PS-341 had little or no effect on Bcl-x or Mcl-1. In C, PS-341 had little or no effect on the IAP family.
mediated by cleavage and activation of apical caspases, including caspases 8 and 9 and the excisionary caspase 3, with subsequent cleavage of polyadenosine-5′-diphosphate-ribose polymerase. It is not clear how PS-341 can activate caspase 8, which is normally activated through death receptor activation. It is also not clear whether caspase 9 activation in HD cell lines is independent of caspase 8 activation or linked through BID cleavage or an alternate caspase. Consequently, these data show that PS-341 has significant antiproliferative activity against HD-derived cell lines in vitro, irrespective of IKKα gene mutation and in the presence of activating cytokines. Evaluating PS-341 activity in vivo in patients with relapsed HD is warranted.

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3215

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Correction: Induction of Cell Cycle Arrest and Apoptosis by the Proteasome Inhibitor PS-341 in Hodgkin Disease Cell Lines Is Independent of Inhibitor of Nuclear Factor-κB Mutations or Activation of the CD30, CD40, and RANK Receptors

In this article (Clin Cancer Res 2004;10:3207–15), which was published in the May 1, 2004, issue of Clinical Cancer Research (1), Figure 5A contained duplicate panels for cleaved caspase-3 and cleaved PARP in the L-428 cells. Figure 6A contained duplicate panels for phospho-p53 and total p53 in the HD-Myz and HD-LM2 cells. These errors occurred during the assembly of the figures and have no bearing on the results or conclusions of the study. The corrected figures are shown below; the figure legends remain the same. Because this is an old study, some of the original blots could not be retrieved to generate complete, corrected figures. The authors regret these errors.

Reference


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