Combination of Proteasome and HDAC Inhibitors for Uterine Cervical Cancer Treatment

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Abstract

Purpose: Cervical cancer cells are addicted to the expression of the human papillomavirus (HPV) oncoproteins E6 and E7. The oncogenicity of E6 is mediated in part by targeting p53 and PDZ-family tumor suppressor proteins for rapid proteasomal degradation, whereas the E7 oncoprotein acts in part by coopting histone deacetylases (HDAC) 1/2. Here, we examine the hypothesis that inhibition of proteasome function and HDAC activity would synergistically and specifically trigger cervical cancer cell death by the interruption of E6 and E7 signaling.

Experimental Design: The sensitivity and molecular responses of keratinocytes and HPV-positive and HPV-negative cervical cancer cells and xenografts to combinations of proteasome and HDAC inhibitors were tested. The expression of HDAC1/HDAC2 in situ was examined in cervical cancer, its precursors, and normal epithelium.

Results: Cervical cancer cell lines exhibit greater sensitivity to proteasome inhibitors than do HPV-negative cervical cancers or primary human keratinocytes. Treatment of cervical cancer cells with bortezomib elevated the level of p53 but not hDlG, hScribble or hMAGI. Immunohistochemical analysis revealed elevated HDAC1/HDAC2 expression in cervical dysplasia and cervical carcinoma versus normal cervical epithelium. The combination of bortezomib and HDAC inhibitor trichostatin A or vorinostat shows synergistic killing of HPV-positive, but not HPV-negative, cervical cancer cell lines. Similarly, treatment of HeLa xenografts with the combination of bortezomib and trichostatin A retarded tumor growth significantly more effectively than either agent alone.

Conclusions: A combination of proteasome and HDAC inhibitors, including bortezomib and vorinostat, respectively, warrants exploration for the treatment of cervical cancer.

Persistent infection with an oncogenic-type human papillomavirus (HPV), most commonly HPV16 and HPV18, is a necessary but insufficient cause of cervical cancer (1). HPV DNA is detected in 99.7% of cervical cancers (2), as well as a large proportion of other anogenital cancers, and also in a subset of head and neck cancers. Although cytologic screening and HPV vaccines are effective preventive measures, there are currently no virus-specific therapies for cervical cancer, and the efficacy of standard surgical and chemoradiotherapies is limited for advanced disease. Expression of two viral oncoproteins, E6 and E7, is critical for the induction and maintenance of the transformed phenotype and is lacking from normal cells (3–6). This suggests that E6 and E7 are logical targets for rational therapeutic approaches and that inhibitors should target the functions of both oncoproteins (4). Although neither has intrinsic enzymic activity, genetic and biochemical studies have defined key cellular partners through which these viral proteins transform cells (7, 8).

E6 exerts one important aspect of its oncogenic activity by binding to the HECT (homologous to the E6-AP Carboxyl Terminus) domain E3 ubiquitin ligase E6-AP (and possibly other ubiquitin ligases), and redirects its activity towards p53 and PDZ family proteins, including hDlG, hScribble, and hMAGI, for rapid proteasomal degradation (9, 10). This reduces the level of these key cellular cell cycle regulators without their mutation (11). Therefore, treatment with proteasome inhibitors might potentially recover near normal levels of wild-type p53 and PDZ tumor suppressor proteins, and thereby trigger cell death. Absent of E6, p53 protein stability is regulated by competition between E3 ligase–mediated ubiquitination and histone acetyl transferase–mediated acetylation of key lysine residues. Acetylation of lysine by histone acetyl transferases prevents ubiquitination of p53 by murine double minute (MDM2), thus stabilizing p53, whereas their deacetylation by a histone deacetylase 1 (HDAC1)-containing complex has the opposite effect. Indeed, deacetylation of p53 represses p53-dependent transcriptional activation,
Translational Relevance

There are currently no virus-specific therapies for cervical cancer, and the efficacy of standard surgical and chemoradiotherapies is limited for advanced disease. Cervical cancer cells are addicted to the expression of the human papillomavirus (HPV) oncoproteins E6 and E7. The oncogenicity of E6 is mediated in part by targeting p53 and PDZ-family tumor suppressor proteins for rapid proteasomal degradation, whereas the E7 oncoprotein acts in part by coopting histone deacetylases (HDAC1/2).

A “first in class” proteasome inhibitor, bortezomib, was recently licensed for the treatment of multiple myeloma, and herein we examine its potential for therapeutic activity against cervical cancer, alone or in combination with the hydroxamate-based pan-HDAC inhibitors trichostatin A and vorinostat. Notably, vorinostat is the “first in class” HDAC inhibitor licensed in 2006 for the treatment of cutaneous T-cell lymphoma. The synergistic killing that was observed suggests the potential the combinations of these licensed proteasome and HDAC inhibitors have for the treatment of cervical cancer.

apoptosis, and growth arrest. This suggests that inhibition of p53 deacetylation may further enhance both the level of p53 and in the response cell cycle arrest and apoptosis.

The E7 oncoprotein also binds to multiple functional partners, notably pRB and the class I histone deacetylases HDAC1 and HDAC2 (12–14). E7 destabilizes pRB levels through cullin 2–mediated proteasomal degradation (15). E7 binds indirectly to both HDAC1 and HDAC2 via sequences in the zinc-finger domain of E7 (16, 17). Mutations within the zinc-finger domain of E7 do not affect binding to and degradation of pRB, but do abrogate the ability of E7 to immortalize cells, suggesting that both activities of E7 are required for immortalization (17, 18). The binding of HDAC1 and HDAC2 to E7 is mediated by association with the Mi2β protein, a component of the mammalian NuRD chromatin remodeling complex. Class I HDACs, including HDAC1, HDAC2, HDAC3, and HDAC8, are found in complexes with other transcriptional corepressors including mSin3 and SMRT/N-CoRepressor (19, 20). HDACs regulate the activity of numerous promoters including those that are E2F-dependent. E7 directs HDAC activity to the E2F2 promoter, increasing its expression (21). In addition, HDACs can directly deacetylate E2F proteins, although the function of this modification is not well understood (21). Finzer et al. showed that HDAC inhibitor treatment induces an intrinsic type of apoptosis in HPV-positive cells by disrupting the mitochondrial transmembrane potential (22, 23). This was only detected in E7, not in E6 oncogene-expressing cells. HDAC inhibition led to a time-dependent degradation of the pocket proteins pRB, p107, and p130, releasing “free” E2F1 following initial G1 arrest. Inhibition of proteasomal proteolysis, but not of caspase activity, rescued E7-directed pRB from degradation and functionally restored its inhibitory effect on the cyclin E gene, known to be suppressed by pRB-E2F1 in conjunction with HDAC1.

We hypothesized that inhibition of key E6 and E7 functions by treatment with proteasome and HDAC inhibitors might provide synergistic killing of cervical cancer cells while sparing normal cells that lack these viral oncoproteins. A “first in class” proteasome inhibitor, bortezomib (also known as PS-341 and marketed as Velcade), was recently licensed for the treatment of multiple myeloma, and herein we examine its potential for therapeutic activity against cervical cancer, alone or in combination with the hydroxamate-based pan-HDAC inhibitors trichostatin A and vorinostat. Notably, vorinostat [also known as suberoylanilide hydroxamic acid and sold as Zolinza (Merck & Co.)] is the “first in class” HDAC inhibitor licensed in 2006 for the treatment of cutaneous T-cell lymphoma.

Materials and Methods

Animals. Six-week-old female immunodeficient BNX (beige nude xid) mice were obtained from National Cancer Institute-Frederick and maintained in a pathogen-free animal facility at least 1 wk before use. All animal studies were done in accordance with institutional guidelines.

Human specimens. Studies using human tissue were done with the approval of the Johns Hopkins Institutional Review board. Fresh and archival tissues were obtained from the Department of Pathology of the Johns Hopkins Hospital and the latter assembled in tissue microarrays by a core facility.

Cell culture. Cervical cancer cell lines HeLa, SiHa, CaSkI, ME180, HT3, and C33A were obtained from the American Type Culture Collection and cultured in DMEM supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin at 5% CO2. Keratinocytes were obtained from Invitrogen and cultured in defined Keratinocyte-SFM.

Drugs. The proteasome inhibitor bortezomib (Millennium Pharmaceuticals, Inc.) was dissolved in 0.9% NaCl before each injection. The stock solutions of the HDAC inhibitors trichostatin A and vorinostat (Sigma) were dissolved in DMSO and then diluted into PBS before each injection.

Xenograft murine model. Mice were inoculated s.c. in their right flank with 5×106 HeLa cells in 0.1 mL DMEM. When the tumor was measurable, the mice were randomly assigned into four groups receiving bortezomib only, trichostatin A only, both bortezomib and trichostatin A, and PBS only. Treatment was given i.v. twice weekly via tail vein at 1 mg/kg bortezomib, and s.c. twice weekly at left flank at 1 mg/kg trichostatin A. The control group received the vehicle alone at the same schedule. Caliper measurements of the longest perpendicular tumor diameters were done every 2 d to estimate the tumor volume (mean ± SE; mm3), using the following formula: 4/3 × (width/2)2 × (length/2), representing the three-dimensional volume of an ellipse. Animals were sacrificed when their tumors reached 2 cm.

Cell viability assay. Cell viability was determined by 2,3-bis-[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT) assay (Roche Diagnostics GmbH). Cells seeded at the concentration of 1,000 per well in 100 μL medium in a 96-well plate were treated with proteasome inhibitors at specified concentrations. After the indicated periods, the cells were incubated according to the manufacturer’s protocol with the XTT labeling mixture for 4 h. Formazan dye was quantified using a spectrophotometric plate reader to measure the absorbance at 450 nm (ELISA reader 190; Molecular Devices). All experiments were done in triplicate.

Antibodies and Western blot analysis. The following antibodies were used for detection with standard Western blot analysis techniques at the concentration recommended by the manufacturer: anti-p53, anti-hDlg-1 and anti-hScribble (Santa Cruz); anti-hSMAC, monoclonal anti-histone deacetylase-1, 2, 6, anti-p-α-tumor (Sigma); and peroxidase-linked and peroxidase-linked antirabbit or antimouse IgG (Amersham).
**Determination of apoptotic cells by flow cytometry.** Induction of apoptosis was determined by annexin-V/7-AAD staining and active caspase-3 staining. Annexin-V/7-AAD staining was done using Annexin V-PE Apoptosis Detection Kit I (BD Pharmingen) according to the manufacturer’s protocol. Briefly, $1 \times 10^5$ cells were resuspended in Binding Buffer, 5 μL of annexin V-PE and 5 μL of 7-AAD were then added into the cells which were then incubated at room temperature for 15 min, and the cells were analyzed by flow cytometry. Active caspase-3 staining was done using phycoerythrin-conjugated rabbit anti-active caspase-3 monoclonal antibody (BD Pharmingen) according to the manufacturer’s protocol. Briefly, cells were fixed and permeabilized using the Cytotox/Cytoperm kit (BD Pharmingen) for 20 min at 4°C. After washing, the cells were stained with phycoerythrin-conjugated rabbit antiactive caspase-3 monoclonal antibody using 20 μL per 1 × 10^6 cells for 30 min at room temperature. Following incubation with the antibodies, the cells were washed, resuspended, and analyzed by flow cytometry on a Becton Dickinson FACSCalibur. Data analysis was done with CellQuest software (Becton Dickinson Immunocytometry System).

**Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay.** Paraffin-embedded tissue sections were processed for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) using an established method to assay for cell death-associated DNA double-strand breaks (24).

**Immunohistochemistry of tissue microarrays.** Immunohistochemical analysis of paraffin-embedded tissues was done as previously described (25). Briefly, 5-μm tissue microarray sections were deparaffinized and rehydrated. Antigen retrieval was done and slides were incubated for 5 min with 3% hydrogen peroxide, then washed and incubated in antibody dilution 1:250 for 60 min at room temperature. The avidin-biotin-peroxidase complex method of DAKO was used to visualize antibody binding, and slides were subsequently counterstained with hematoxylin. The staining was scored by three observers blind to specimen identity to obtain a consensus. Staining intensity was scored as 0 for negative, 1 for weak, 2 for intermediate, or 3 for strong.

**Statistical analysis.** Results are reported as mean ± SD. Unless otherwise indicated, the statistical significance of difference was assessed by two-tailed Student’s t test using Prism (V.4 Graphpad). The level of significance was set at $P < 0.05$. The combination index (CI) of bortezomib and trichostatin A or vorinostat was calculated by the method of Chou and Talalay (26). The minimum CI was determined by fitting a response surface to the data (27), and then calculating the CI using the fitted values. Synergy was also depicted by isobologram, in which the drug combinations leading to a 50% loss in cell viability are plotted, or a bar chart if one (or both) of the agents fails to achieve this level.

**Results**

**Sensitivity of cervical cancer cell lines to proteasomal inhibition with bortezomib is accompanied by increases in p53 but not PDZ protein levels.** We examined the impact of bortezomib treatment on the viability of cultured cervical cancer cell lines. Bortezomib produced a dramatic drop in the viability of CaSki, SiHa, ME180, or HeLa cells after 48 hours of treatment at nanomolar concentrations (Fig. 1A). In contrast to the HPV16-transformed CaSki and HPV18-transformed HeLa cells, the effect of bortezomib upon the viability of primary human keratinocytes or the HPV-negative cervical cancer line C33A was limited (Fig. 1A). Likewise, ME180 cells, which contain HPV68, exhibit far greater susceptibility to bortezomib treatment than does the HPV-negative cervical cancer line HT-3. These findings imply that increased susceptibility to bortezomib is associated with transformation by HPV, regardless of the oncogenic type.

Prior studies with other proteasome inhibitors have suggested that the HPV oncogene E6 exerts its effect by triggering the ubiquitination and subsequent proteasomal degradation of cellular tumor suppressor genes, notably p53 and PDZ family members. To identify potential mechanisms by which bortezomib triggers the death of cervical cancer cells, we examined the levels of p53 in treated and untreated cells (Fig. 1B). Bortezomib treatment triggered a dramatic increase in wild-type p53 levels in HeLa cells, and to a lesser extent in CaSki cells. A similar phenomenon was observed in primary keratinocytes, presumably by blockade of mdm2-dependent p53 degradation, whereas the levels of mutant p53 present in C33A cells remained similar despite bortezomib treatment. Surprisingly, bortezomib treatment exhibited little impact upon the levels of PDZ family members hMAGI, hScribble, or hDlg in any of the cell types tested regardless of HPV status (Fig. 1B). This may reflect inhibition of a different spectrum of proteasomal activities by bortezomib, as compared with the proteasomal inhibitors used in previous studies of E6 regulation of PDZ proteins (28–30). Furthermore, bortezomib treatment of HeLa cells did not significantly alter pRB levels (not shown).

**Overexpression of HDAC1, HDAC2, and HDAC6 in cervical cancer cells in vivo and in vitro.** Because bortezomib treatment was only partially effective in triggering the death of cervical cancer cells, we examined the possibility of inhibiting a transforming activity of HPV that was independent
of proteasomal activity. E7 is known to interact with both HDAC1 and HDAC2, and mutational analysis of E7 suggests that this interaction may contribute to transformation. It is unclear, however, how frequently these HDACs are expressed in cervical cancer, and therefore we sought to address their expression pattern by immunohistochemistry. Commercial antisera to the nuclear class I histone deacetylases HDAC1 and HDAC2, as well as the cytoplasmic class II histone deacetylase HDAC6, are available and we confirmed their specificity by Western blot analysis (Fig. 2). Therefore, to examine the potential of targeting these HDACs for treatment of cervical cancer we examined their expression level in squamous cell carcinoma (n = 141 cases) and adenocarcinoma (n = 24 cases) of the uterine cervix, as well as cervical dysplasia (n = 50) and normal cervical epithelium (n = 8). Robust and consistent nuclear expression was observed for HDAC1 and HDAC2 in squamous cell carcinoma, adenocarcinoma, and even cervical dysplasia (Fig. 2). Interestingly, expression of HDAC1 and HDAC2 was apparent on the basal layer of normal cervical epithelium, but was rapidly lost at higher strata, consistent with an earlier study (31). The expression of HDAC6 was cytoplasmic, as expected for this class II HDAC (32), but was otherwise consistent with HDAC1 and HDAC2 (Fig. 2).

Synergistic effect of proteasome and pan-HDAC inhibitors in inducing caspase-mediated apoptosis in cervical cancer cell lines. Trichostatin A, a potent and broad spectrum HDAC inhibitor, was previously shown to trigger cell cycle arrest and apoptosis in HeLa cells. Therefore, we tested the combination of bortezomib and trichostatin A for possible synergistic killing of HeLa cells (Fig. 3). Isobologram analysis indicates that rather than simple additive killing, the combination of bortezomib and trichostatin A is highly synergistic, consistent with inhibition of complementary transforming activities. Like trichostatin A, vorinostat is a hydroxamate-based pan-HDAC inhibitor. Potent synergistic killing of HeLa cells treated with vorinostat and bortezomib was also observed. This phenomenon was extended to HPV16-transformed SiHa and CaSki cells and HPV68-transformed ME180 cells. The optimal CI for each cell line was achieved at the following concentrations: SiHa (CI = 0.42) bortezomib 6 nmol/L/vorinostat 50 μmol/L;

**Fig. 2.** Expression levels of HDAC1, HDAC2, and HDAC6 in normal cervical epithelium versus cervical cancer and its precursors. A, representative micrographs showing immunohistochemical staining of HDAC1, HDAC2, and HDAC6 in normal cervical epithelium, cervical dysplasia (CIN), squamous cell carcinoma (SCC), and adenocarcinoma. HDAC1 and HDAC2 are nuclear class I HDACs, whereas HDAC6 is a cytoplasmic class II HDAC. B, the mean and SD of the staining intensity score (arbitrary units) for HDAC1, HDAC2, and HDAC6 were plotted for normal cervical epithelium (n = 8), CIN (n = 50), SCC (n = 141), and adenocarcinoma (n = 24). C, Western blot analysis of HDAC1, HDAC2, and HDAC6 protein levels in two independent pools of primary human keratinocyte cultures, and C33A, HeLa, and CaSki cells. β-actin is provided as a loading control.
HeLa (CI = 0.52) bortezomib 3.12 nmol/L/trichostatin A 150 nmol/L; HeLa (CI = 0.42) bortezomib 0.8 nmol/L/vorinostat 0.8 μmol/L; ME180 (CI = 0.6) bortezomib 25 nmol/L/vorinostat 12.5 μmol/L; CaSki (CI = 0.1) bortezomib 50 nmol/L/vorinostat 3.12 μmol/L. This synergistic killing was not observed in the HPV-negative cervical cancer lines C33A and HT-3, which express mutant p53.

Although E7 interacts with the class I histone deacetylases HDAC1 and HDAC2, trichostatin A, and vorinostat broadly inhibit class I and class II HDACs with nmol/L Kis. Furthermore, we have shown that like HDAC1 and HDAC2, the class II histone deacetylase HDAC6 is also highly expressed in cervical cancer (Fig. 2). Therefore, it is possible that the synergy with bortezomib occurs through inhibition of HDAC6 activity (32–34), rather than HDAC1 and HDAC2. Indeed, there is strong precedent for this possibility from studies in multiple myeloma (33) and ovarian cancer (32). Combination therapy with bortezomib and the HDAC6-specific inhibitor tubacin exhibited synergistic killing of multiple myeloma by triggering massive accumulation of polyubiquitinated protein (33). However, we found that the bortezomib and tubacin combination did not provide synergistic killing of HeLa cells, suggesting that inhibition of HDAC6 does not contribute to the synergy of bortezomib and trichostatin A (Fig. 3B). In support of this, HDAC6 is not up-regulated in CaSki cells (Fig. 2B), although synergistic killing by bortezomib and trichostatin A is observed.

Although combination treatment with bortezomib and HDAC inhibitors kills HeLa cells, the pathway of cell death is not clear. We therefore examined the effect of bortezomib and...
trichostatin A upon apoptosis in HeLa cells by annexin V and 7-AAD double staining and flow cytometric analysis. Evidence of synergy was observed in the massive apoptosis triggered by treatment with bortezomib and trichostatin A in combination (Fig. 4A). Cell death by apoptosis and the synergy of these two agents were further confirmed by a high percentage of cells staining positive for active caspase-3 (Fig. 4B).

**In vivo activity of proteasome and pan-HDAC inhibitors against a cervical cancer xenograft.** We then examined whether bortezomib and trichostatin A could be used in combination to treat xenograft tumors in immunodeficient mice. Immunodeficient mice (15/group female BNX mice) were inoculated with HeLa cells, and when the tumor became palpable, the mice were randomly assigned to four treatment arms: vehicle alone, 1 mg/kg bortezomib, 1 mg/kg trichostatin A, or both 1 mg/kg bortezomib and trichostatin A. Treatment with either the proteasome inhibitor alone or the HDAC inhibitor alone slowed the rate of tumor growth and significantly prolonged survival of the mice (Fig. 5A). The effect of treatment with these agents together was to further slow tumor growth and prolong the survival of the mice (Fig. 5B). This was associated with marked apoptosis, as evidenced by TUNEL staining, in the tumors harvested upon reaching 2 cm³ from the group treated with both agents as compared with the tumors treated with vehicle alone (Fig. 5C). Likewise, immunoblot analysis of tumors treated with either agent alone showed some increase in poly(ADP-ribose)polymerase cleavage as compared with vehicle-treated tumors, whereas poly(ADP-ribose)polymerase cleavage was more extensive in tumors harvested from animals treated with both agents (Fig. 5D).

**Discussion**

Efforts at directly targeting E6 and E7 with small molecules have had limited success, probably because this approach is best suited to inhibition of enzymic activity rather than protein-protein interactions. Typically, such protein-protein interactions are interrupted using peptides, but their efficacy as *in vivo* reagents is often compromised by their inability to maintain secondary structure, their susceptibility to proteolytic degradation, and difficulties in penetrating cells. As an alternative, we have attempted to inhibit cellular enzymic activities, namely proteasomal proteolysis and histone deacetylation, that are both coopted by and, as indicated by genetic analysis, essential to HPV oncoproteins during cellular transformation. Although this approach loses some of the specificity of directly targeting the HPV oncoproteins, the possibility of a therapeutic index remains given the requirement for these cellular processes in E6/E7-mediated oncogenesis. This hypothesis is borne out in the preferential ability of proteasome and HDAC inhibitors, alone but most particularly in combination, to trigger apoptosis in HPV-transformed, as compared with HPV-negative cervical cancers cells or keratinocytes.

The ability of bortezomib to trigger death of the cervical cancer cell lines is associated with a recovery in the levels of wild-type p53 but no change in the levels of PDZ proteins known to be targeted by E6 for proteasomal degradation, or pRB. The failure of bortezomib to detectably enhance the levels of PDZ protein or pRB in the cervical cancer lines tested implies that they are degraded via proteasomal activities distinct from those targeted by this inhibitor; notably, bortezomib effectively inhibits the chymotryptic activity of the proteasomal machinery, but does not impact their caspase activity, and actually enhances the trypic activity (35). It is therefore possible that other proteasomal inhibitors targeting a broader spectrum of activities could be more effective against cervical cancer by recovering both p53 and PDZ proteins. The natural product NPI-0052 irreversibly inhibits all three proteasomal activities, and like vorinostat, is orally bioavailable, suggesting the potential of this combination (36).
The relative insensitivity of the HPV-negative cervical cancer lines HT-3 and C33A, which both carry p53 mutations, suggests that recovery of wild-type p53 in HPV-positive cervical cancers is relevant to the latter’s sensitivity to bortezomib. It is important to recognize, however, that recovery of wild-type p53 levels in cervical cancer by bortezomib treatment is not likely to be its only antitumor mechanism. Indeed, overexpression of p53 in cervical cancer cells only marginally slowed their proliferation (37), and bortezomib is cytotoxic for cancer cells of diverse origin that do not express p53 (25, 38). Bortezomib will trigger the accumulation of potentially toxic levels of polyubiquitinated proteins. In addition, bortezomib has been shown to inhibit neoangiogenesis in multiple tumor models and to block hypoxic responses (39–41).

Trichostatin A and vorinostat are chemically related inhibitors of the HDAC family, including both the nuclear and cytoplasmic HDACs including HDAC6. However, the half maximal effective concentration (EC50) of vorinostat inhibition of HeLa cell growth is 3 ± 1 μmol/L as compared with 0.18 ± 0.1 μmol/L for trichostatin A, reflecting its lower potency against purified HDAC protein (42). Accumulating evidence suggests that a lysosomal pathway can compensate for intracellular polyubiquitinated protein degradation when proteasome activity is insufficient (33, 43–45). A critical component of the lysosomal protein degradation pathway is the microtubule-associated deacetylase HDAC6 that directly interacts with misfolded and/or polyubiquitinated proteins to target them for lysosome-mediated protein degradation via aggresome formation/autophagy (46–48). Because misfolded and ubiquitinated proteins are degraded via both proteasomes and HDAC6-dependent autophagy, simultaneous inhibition of proteasome and HDAC6 has been proposed as a new strategy to synergistically induce cell death in multiple myeloma and pancreatic cancer settings (33, 34). However, here we show that inhibition of HDAC6 does not account for the synergy between bortezomib and vorinostat or trichostatin A for killing cervical cancer cells. Rather, it suggests that inhibition of other HDACs is likely to be important, most likely HDAC1 and HDAC2 because they are known to interact indirectly with E7, and genetic evidence suggests that these interactions are critical for E7-mediated transformation. Furthermore, knockdown of HDAC1 or HDAC2 expression in HeLa cells suppresses proliferation and promotes autophagy and apoptosis of HeLa cells, respectively, but knockdown of HDAC4 and HDAC7 had no discernable effect on proliferation (31, 49, 50). Nevertheless, further studies are needed to validate the central role of HDAC1 and HDAC2 inhibition in the synergistic killing of cervical cancer cells by trichostatin A or vorinostat and bortezomib.

The combination of bortezomib and trichostatin A proved more effective in slowing the growth of HeLa xenografts and prolonging the survival of mice than either treatment alone. However, we did not observe synergy, but rather an additive effect. This may reflect the use of maximal, rather than...
submaximal doses, and suggests that additional titrations may be required to show synergy. Furthermore, vorinostat, or possibly newer HDAC1/HDAC2-specific inhibitors, may be more effective in vivo than trichostatin A, and clinical trials using bortezomib and vorinostat are ongoing in other cancer types. Finally, bortezomib acts via several mechanisms to retard tumor growth in vivo, including blockade of neoangiogenesis and hypoxic responses, that may potentially make synergy with trichostatin A treatment in vivo less apparent than in vitro.

Disclosure of Potential Conflicts of Interest

Vorinostat, used in the study, is manufactured by Merck. R. Roden is a paid consultant for Merck. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

References


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