Histone Deacetylase Inhibitors Enhance Lexatumumab-Induced Apoptosis via a p21\(^{\text{Cip1}}\)-Dependent Decrease in Survivin Levels

Steffan T. Nawrocki, Jennifer S. Carew, Leslie Douglas, John L. Cleveland, Robin Humphreys, and Janet A. Houghton

Division of Molecular Therapeutics, Departments of Oncology and Biochemistry, St. Jude Children’s Research Hospital, Memphis, Tennessee; Department of Cancer Biology, The Scripps Research Institute-Florida, Jupiter, Florida; Oncology Research Department, Human Genome Sciences, Rockville, Maryland; and Department of Cancer Biology, Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio

Abstract

Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) preferentially induces apoptosis in malignant cells by binding to the death receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5). Several agents that therapeutically exploit this phenomenon are being developed. We investigated the anticancer activity of two novel, highly specific agonistic monoclonal antibodies to TRAIL-R1 (mapatumumab, HGS-ETR1) and TRAIL-R2 (lexatumumab, HGS-ETR2) in colon cancer cell lines. Our analyses revealed that colon cancer cells display significantly higher surface expressions of TRAIL-R2 than TRAIL-R1, and are more sensitive to lexatumumab-induced apoptosis. The proapoptotic effects of lexatumumab in TRAIL-resistant HCT8 and HT29 cells were dramatically augmented by the histone deacetylase inhibitors trichostatin A or suberoylanilide hydroxamic acid. The presence of p21, but not p53, was critical for the synergy between lexatumumab and histone deacetylase inhibitors. The absence of p21 did not interfere with the formation of the death-inducing signaling complex by lexatumumab, suggesting the involvement of other apoptotic and/or cell cycle regulators. Indeed, treatment with suberoylanilide hydroxamic acid greatly reduced the expression of the inhibitor of apoptosis protein survivin and cdc2 activity in HCT116 p21\(^{+/+}\) cells but not in the HCT116 p21\(^{-/-}\) cells. Inhibition of cdc2 activity with flavopiridol decreased survivin expression and sensitized the p21-deficient cells to lexatumumab-induced apoptosis. Similarly, small interfering RNA–mediated knockdown of survivin also enhanced lexatumumab-mediated cell death. Therefore, survivin expression plays a key role in lexatumumab resistance, and reducing survivin expression by inhibiting cdc2 activity is a promising strategy to enhance the anticancer activity of lexatumumab. [Cancer Res 2007;67(14):6987–94]

Introduction

Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) selectively induces apoptosis in cancer cells by binding to two membrane-bound death receptors, TRAIL-R1 (DR4) and TRAIL-R2 (DR5; ref. 1). Following TRAIL binding, the adaptor molecule Fas-associated death domain (FADD) is recruited, which then binds to caspase-8 to form the death-inducing signaling complex (DISC), culminating in apoptosis (2). Human Genome Sciences has developed fully human agonistic monoclonal antibodies for cancer therapy that specifically bind with high affinity to either TRAIL-R1 (mapatumumab, HGS-ETR1) or TRAIL-R2 (lexatumumab, HGS-ETR2) and activate their respective receptors. These antibodies are currently being evaluated in clinical trials (3–5). Given the minimal toxicity to normal cells and the strong anticancer effects observed in many in vitro and in vivo tumor models (6), targeting death receptors to induce apoptosis in cancer cells is an attractive therapeutic strategy.

Although TRAIL has shown therapeutic potential in many cancer models, some tumor cells are insensitive to TRAIL-induced apoptosis (7, 8). Considering this, TRAIL-based therapies may be best used in combination with conventional agents. Consistent with this idea, TRAIL has been shown to synergistically enhance the anticancer activity of many frontline therapeutics (6). Histone deacetylase (HDAC) inhibitors represent one class of agent that induce synergistic levels of apoptosis when combined with TRAIL (9–13). HDAC inhibitors exhibit a multitude of cellular effects that likely contribute to their anticancer activity, including blocking cell cycle progression through the induction of the universal cyclin-dependent kinase (CDK) inhibitor p21 and the stimulation of apoptosis (14–17). Here, we investigated the ability of lexatumumab to induce apoptosis alone and in combination with HDAC inhibitors in colon cancer cells, and we determine the underlying mechanism. Our results show that colon cancer cells express higher cell surface levels of TRAIL-R2 than TRAIL-R1, and are correspondingly more sensitive to lexatumumab-induced apoptosis. Furthermore, the combination of HDAC inhibitors with lexatumumab synergistically enhances the proapoptotic effects and compromises the clonogenic survival of colon cancer cells. The synergy between these agents seems to be dependent on p21-mediated down-regulation of the inhibitor of apoptosis protein (IAP) survivin. Collectively, our findings support the use of lexatumumab for the treatment of colon cancer and identify survivin as an important factor contributing to lexatumumab resistance.

Materials and Methods

Cell lines and antibodies. GC3/c1 human colon cancer cells were established as previously described (18). The human cell lines HCT116, HCT78, HT29, HFF, and human umbilical vascular endothelial cell (HUVEC) were obtained from American Type Cell Culture Collection. p21\(^{1/-}\), p21\(^{-/-}\), p53\(^{1/-}\), and p53\(^{-/-}\) isogenic HCT116 cell lines were kindly provided by Dr. Bert Vogelstein (John Hopkins, Baltimore, MD). Cell lines were maintained in RPMI supplemented with 10% fetal bovine serum and 1-glutamine under conditions of 5% CO\(_2\) at 37°C. Antibodies were obtained from the following commercial sources: anti-FADD (BD Transduction Laboratories),

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Steffan T. Nawrocki, Division of Molecular Therapeutics, Department of Oncology, St. Jude Children’s Research Hospital, 332 North Lauderdale, Memphis, TN 38105. Phone: 901-233-4687; E-mail: Steffan_Nawrocki@yahoo.com.

©2007 American Association for Cancer Research.
anti-p53, TRAIL-R2, caspase-8, XIAP, cIAP1, and cIAP2 (M BI, International), anti-survivin and cdc2 (Santa Cruz Biotechnology), anti-actin (Sigma Chemical), and anti-p21 (Upstate). Mapatumumab and lexatumumab, human monoclonal TRAIL-R1 and TRAIL-R2 antibodies, respectively, were kindly provided by Human Genome Sciences. Trichostatin A (TSA) and propidium iodide (PI) were purchased from Sigma. Suberoylanilide hydroxamic acid (SAHA) was synthesized as previously described (19). Flavopiridol was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD).

Analysis of TRAIL receptor surface expression. Cells were collected and incubated with phycoerythrin-conjugated antibodies for the TRAIL receptors 1, 2, 3, or 4 (eBioscience) for 1 h. After washing twice with fluorescence-activated cell sorting (FACS) buffer (PBS, 5% fetal bovine serum, and 0.01% NaN3), cells were resuspended in 500 μL of FACS buffer and analyzed using a FACSCalibur flow cytometer (BD Biosciences).

Immunoblotting. Cells (1 × 106) were incubated with 2.5 μmol/L of SAHA, 100 ng/mL of lexatumumab, 500 nmol/L of flavopiridol, or the indicated drug combinations. Cells were harvested and lysed as previously described (20). Approximately 50 μg of total cellular protein from each sample was subjected to SDS-PAGE, proteins were transferred to nitrocellulose membranes, and the membranes were blocked with 5% milk in a TBS solution containing 0.1% Tween 20 for 1 h. The blots were then probed overnight with the indicated primary antibodies, washed, and probed with species-specific secondary antibodies coupled to horseradish peroxidase. Immunoreactive material was detected by Western Lightning chemiluminescence (Perkin-Elmer).

Quantification of DNA fragmentation. DNA fragmentation was measured by PI staining and FACS analysis as described previously (21). Briefly, cells were plated in six-well plates and following incubation with the indicated drugs, cells were harvested, pelleted by centrifugation, and resuspended in PBS containing 25 μg/mL of PI, 0.1% Triton X-100, and 0.1% sodium citrate. Cells were incubated in PI solution for 1 h followed by flow cytometric analysis of stained cells with FACSCalibur (BD Biosciences).

Active caspase-3 assay. Cells were plated and treated as described above. Following drug treatment, cells were washed in PBS and resuspended in appropriate buffers as described in the FITC-conjugated monoclonal active caspase-3 antibody apoptosis kit (PharMingen). Cells were fixed, permeabilized, and stained with a FITC-conjugated anti-active caspase-3 antibody as described previously (22). Flow cytometric analysis of stained cells was done with FACSCalibur (BD Biosciences).

Clibonogenic survival assay. Cells were plated at a density of 500 cells/well in six-well plates and allowed to attach overnight. Cells were treated in triplicate with 50 ng/mL of lexatumumab, 500 nmol/L of SAHA, or both drugs for 24 h. After removal of drug-containing medium, cells were allowed to form colonies for 10 days. The colonies were washed with PBS, fixed with methanol, and stained with crystal violet. Colonies were scored using a gel documentation system (AlphaInnotech). The surviving fraction was determined by dividing the number of surviving colonies in the drug-treated wells by the number of colonies in the untreated control groups.

DISC formation. Lexatumumab and anti-human IgG (FC-specific; Sigma) were premixed on ice for 15 min. Cells were then stimulated in a cold PBS. Cell lysis and immunoprecipitation of receptor complexes were determined by immunoblotting.

Cdc2 activity assay. Cells were incubated in 10 cm plates with 2.5 μmol/L of SAHA for 24 h and then lysed by 1% Triton X lysis buffer as previously described (21). Lysates were incubated with an anti-cdc2 antibody for 1 h followed by incubation with protein A/G-Sepharose beads for 12 h at 4°C. Immunocomplexes were washed with lysis buffer and then incubated with 1 μg of histone H1, 150 μmol/L of ATP, and 20 μCi of [γ-32P]ATP in 50 μL of reaction buffer [25 mmol/L Tris (pH 7.2), 10 mmol/L MgCl2] for 15 min at 30°C. Adding SDS sample buffer stopped the reaction and samples were loaded onto a 12% SDS-PAGE gel after boiling for 5 min. The gel was stained with Coomassie blue, dried, and autoradiographed. Band intensity was quantified using a gel documentation system (AlphaInnotech).

Preparation and transfection of small interfering RNAs. Cells were transfected with SMARTpool survivin small interfering RNA (siRNA) or siRNA directed against firefly luciferase (nontarget) from Dharmacon as described previously (19). Briefly, cells were transfected with 100 nmol/L of the above siRNA using OligofectAMINE (Invitrogen) for 4 h according to the manufacturer’s protocol. Following silencing, cells were treated for 24 h with 100 ng/mL of lexatumumab, 2.5 μmol/L of SAHA, or its combination. Apoptosis was measured by DNA fragmentation and active caspase-3 assay as described above. The efficiency of RNAi was determined by immunoblotting.

Figure 1. Colon cancer cells express high surface levels of TRAIL-R2 and are more sensitive to lexatumumab than mapatumumab. A, cell surface expression of TRAIL receptors. The indicated colon carcinoma cells were collected and stained for TRAIL receptors using phycoerythrin-conjugated antibodies. Surface expression was determined by flow cytometry as described in Materials and Methods. Columns, mean; bars, SD (n = 3). B, lexatumumab induces caspase-3 activation in colon cancer cell lines. Cells were treated with 100 ng/mL of lexatumumab or mapatumumab for 24 h. Levels of active caspase-3 were determined using a FITC-conjugated active caspase-3 kit followed by flow cytometry. Columns, mean; bars, SD (n = 3). C, lexatumumab induces apoptosis in colon cancer cell lines. Cells were treated for 24 h with 100 ng/mL of lexatumumab or mapatumumab and apoptosis was determined by PI-FACS analysis as described in Materials and Methods. Columns, mean; bars, SD (n = 3).
Results
Colon cancer cells preferentially express high levels of TRAIL-R2 on the cell surface and are sensitive to lexatumumab-induced apoptosis. To investigate the anticancer activity of mapatumumab and lexatumumab, we first evaluated the surface expression of TRAIL receptors in four human colon carcinoma cell lines. All of the cell lines analyzed had more detectable cell surface expression of TRAIL-R2 than TRAIL-R1 (Fig. 1A). The TRAIL decoy receptors TRAIL-R3 and TRAIL-R4 were expressed at very low levels in all cells (Fig. 1A). The colon cancer cell lines were significantly more sensitive to lexatumumab-induced apoptosis than to mapatumumab (Fig. 1B and C). Cells were then incubated in fresh medium for 10 d and colonies were stained and scored as described in Materials and Methods. Columns, mean; bars, SD (n = 3). C, SAHA and lexatumumab are not toxic to HFF or HUVEC cells. Cells were treated with 100 ng/mL of lexatumumab, 2.5 μmol/L of SAHA, 500 nmol/L of TSA, or the indicated drug combinations for 24 h. Apoptosis was measured as described in (A). Columns, mean; bars, SD (n = 3).

p21, but not p53, is required for lexatumumab and lexatumumab + HDAC inhibitor–induced apoptosis. A hallmark of HDAC inhibitor treatment is a dramatic up-regulation of the CDK inhibitor p21(23), which is also a transcription target induced by the tumor suppressor p53 (24). We therefore tested the potential roles of p53 and p21 by assessing the efficacy of lexatumumab or the lexatumumab + HDAC inhibitor combinations in p21−/− or p53−/− HCT116 colon cancer cell lines. As expected, p21 expression was strongly induced in response to SAHA in HCT116 p21+/+ cells, yet this response was p53-independent (Fig. 3A). The more modest levels of HDAC inhibitor–induced apoptosis was independent of p21 but was partially dependent on p53 (Fig. 3B, C, and D). Notably, p21-deficient HCT116 cells were resistant to lexatumumab-induced apoptosis and were not sensitized by the addition of HDAC inhibitors (Fig. 3B). In contrast, the proapoptotic effects of lexatumumab and lexatumumab + SAHA were equivalent in HCT116 p53+/− and...
HCT116 p53−/− cells (Fig. 3C). Similar findings were observed in clonogenic survival assays (Fig. 3D). Therefore, p21 induction is an important event underlying the ability of HDAC inhibitors to sensitize cancer cells to lexatumumab-induced death.

The induction of p21 by HDAC inhibitors in colon cancer cells is required for the degradation of survivin. To define the role that p21 plays in the response to lexatumumab-induced apoptosis, we next investigated its potential role in DISC formation. It has been previously reported that HDAC inhibitors increase TRAIL-R2 expression (15). Consistent with this finding, we found that SAHA treatment indeed led to a marked increase in TRAIL-R2 expression and this occurred in colon cancer cells lacking p21 (Fig. 4A). Furthermore, no differences in the recruitment of TRAIL-R2, FADD, or caspase-8 to the DISC were detected between p21+/+ and p21−/− HCT116 colon cancer cells (Fig. 4B). We therefore hypothesized that the observed differences in cell death may be due to events occurring downstream of DISC formation, such as changes in the expression of IAPs. Following exposure to SAHA, we detected a dramatic decrease in the expression of survivin, and this response was dependent on p21 (Fig. 5A). In contrast, the expression of the IAPs, cIAP1 and cIAP2, remained unchanged. A very modest reduction in XIAP was also observed independent of p21 (Fig. 5A). To determine the role that survivin may play in lexatumumab and SAHA-induced apoptosis, we knocked down the expression of survivin using siRNA. Immunoblotting confirmed the efficient suppression of survivin in siRNA-targeted cells (Fig. 5B). Reduction of survivin expression modestly enhanced lexatumumab-, SAHA-, and lexatumumab + SAHA–induced apoptosis in the presence of p21, but dramatically sensitized p21-deficient cells to apoptosis, similar to the levels of apoptosis observed in wild-type HCT116 cells (Fig. 5C). Taken together, our results show that survivin expression could promote resistance to lexatumumab and that induction of p21 might lead to decreased survivin levels.

Figure 3. p21 but not p53 is required for HDAC inhibitor–mediated sensitization to lexatumumab-induced apoptosis. A, SAHA dramatically increases the expression of p21. Isogenic p21+/+, p21−/−, p53+/+, and p53−/− HCT116 colon cancer cells were treated with 100 ng/mL of lexatumumab, 2.5 μm/L of SAHA, or both for 16 h and immunoblotting was done as outlined in Materials and Methods. B, p21-deficient HCT116 colon cancer cells were resistant to lexatumumab-induced apoptosis. Cells were treated with 100 ng/mL of lexatumumab, 2.5 μm/L of SAHA, 500 nmol/L of TSA, or the indicated drug combination for 24 h. Apoptosis was measured by active caspase-3 staining followed by flow cytometry or by PI/FACS analysis as described in Materials and Methods. Columns, mean; bars, SD (n = 3). C, lexatumumab-induced apoptosis and SAHA-mediated sensitization to lexatumumab-induced apoptosis was independent of p53. Cells were treated and apoptosis was measured as described in (B). D, p21-deficient HCT116 colon cancer cells were resistant to the effects of lexatumumab and SAHA + lexatumumab on clonogenic survival. Cells were treated with 50 nm/mL of lexatumumab, 500 nmol/L of SAHA, or both for 24 h. Cells were then incubated in fresh medium for 10 d and colonies were stained and scored as described in Materials and Methods. Columns, mean; bars, SD (n = 3).
Apoptosis in GC 3/c1, HCT8, and HT29 colon cancer cell lines

Flavopiridol also dramatically enhanced lexatumumab-induced apoptosis when combined with lexatumumab, and this was equally evident in p21-deficient HCT116 colon cancer cells (Fig. 6A). Reduced cdc2 activity and this required the presence of p21 (Fig. 6A). Consistent with continued cdc2 activity, p21-deficient HCT116 cells remained in G2-M phase when treated with SAHA and lexatumumab, whereas p21+/− HCT116 cells underwent apoptosis (Supplementary Fig. S1). Second, whereas SAHA did not reduce survivin levels in p21-deficient HCT116 cells with or without lexatumumab, the treatment of these cells with flavopiridol or with flavopiridol + lexatumumab led to a total loss in survivin expression (Fig. 6B). Finally, the effects of flavopiridol on survivin expression were associated with a marked apoptotic response when combined with lexatumumab, and this was equally evident in p21-deficient HCT116 colon cancer cells (Fig. 6C). Flavopiridol also dramatically enhanced lexatumumab-induced apoptosis in GC3/c1, HCT8, and HT29 colon cancer cell lines (Supplementary Fig. S2). Collectively, these findings show that survivin is a critical regulator of sensitivity to lexatumumab-induced colon cancer cell death and that reducing its levels (via siRNA or inhibition of cdc2 activity) may be a promising strategy to enhance lexatumumab-induced apoptosis.

Discussion

TRAIL exhibits strong antitumor activity in numerous in vitro and in vivo cancer models (1, 6). Human Genome Sciences has recently developed fully humanized agonistic monoclonal antibodies that specifically recognize and activate TRAIL-R1 (mapatumumab) and TRAIL-R2 (lexatumumab) and are both being evaluated in clinical trials (3, 5, 26). These antibodies may prove to be clinically superior to TRAIL because they selectively bind to their cognate receptors with an affinity 1,000 times greater than that of other TRAIL receptors (3). Therefore, treatment with mapatumumab or lexatumumab may reduce potential toxicity that may result from targeting both TRAIL-R1 and TRAIL-R2 simultaneously. MacFarlane et al. have shown that primary cells from patients with chronic lymphocytic leukemia and mantle cell lymphoma signal primarily through TRAIL-R1 rather than TRAIL-R2, suggesting that mapatumumab may be much more effective than lexatumumab or TRAIL in these malignancies (27, 28). Along these lines, our study revealed that colon cancer cells express high surface levels of TRAIL-R2 compared with the expression of other TRAIL receptors. Accordingly, lexatumumab induced apoptosis much more effectively than mapatumumab in this panel of colon cancer cell lines. Taken together, these findings suggest that individually tailored therapy, which specifically targets either TRAIL-R1 or TRAIL-R2, should be considered following determination of their surface expression in different cancers.

Lexatumumab induced similar levels of apoptosis in the TRAIL-sensitive colon cancer cell lines (HCT116 and GC3/c1), but interestingly, also induced moderate levels of apoptosis in two TRAIL-resistant tumor lines (Fig. 1B and C).6 We are currently investigating the potential mechanistic differences between lexatumumab and TRAIL-induced apoptosis. Importantly, despite its greater potency than TRAIL in some colon carcinoma cell lines and its inability to bind to decoy receptors, lexatumumab displayed minimal toxicity to the nontransformed HFF and HUVEC cell lines (Fig. 2C). Given this, the selectivity of lexatumumab in colon carcinoma cells seemed to correlate with the presence of TRAIL-R2 surface expression rather than the presence or absence of decoy receptors.

Despite the potential of TRAIL-based therapies, many tumors are resistant to TRAIL (i.e., HCT8 and HT29 colon carcinoma cells), suggesting that the potential of TRAIL may be best exploited by using it in combination with conventional chemotherapy. Consistent with this idea, numerous reports have shown synergistic anticancer activity when TRAIL was given in combination with various chemotherapeutic agents (1, 7, 29). This cast includes HDAC inhibitors, which are especially promising given that, similar to TRAIL, these agents also display selectivity for inducing apoptosis in tumor cells (23). Here, we have shown that two HDAC inhibitors, SAHA and TSA, greatly enhance the anticancer activity of lexatumumab in all of the colon carcinoma cell lines tested. Although we did not perform a formal synergy analysis of the combination of lexatumumab and HDAC inhibitors, the greater levels of apoptosis with combination treatment were extremely significantly when compared with single-agent treatment. HDAC inhibitors have been reported to induce p21 expression independent of p53 through the transcription factor Sp1 (30), and here, we have shown that SAHA also robustly induces p21 expression in colon cancer cells in a p53-independent fashion. Furthermore, p21, but not p53, is required for the increased levels of apoptosis observed between SAHA and lexatumumab in compromising the growth and survival of colon cancer cells. Somewhat similar findings have been reported by others who have shown that sensitivity to TRAIL and resveratrol is reduced in p21-deficient cells (31), and in accordance with these results, cancer cells display

6 Unpublished observations.
increased sensitivity to TRAIL when arrested in the G1 phase of the cell cycle (32, 33). Thus, whereas induction of p21 is conventionally thought to promote cell survival (34), our studies and those of others suggest that p21 also has proapoptotic functions (35–37).

A recent study reported that HDAC inhibitors up-regulated TRAIL-R2 and thus, promoted sensitivity to TRAIL-induced apoptosis (13), whereas we have shown here that the ability of SAHA to increase TRAIL-R2 levels was independent of p21. Furthermore, recruitment of TRAIL-R2, FADD, and caspase-8 to the DISC following lexatumumab treatment was not impaired in the absence of p21. These results suggest that p21 may promote lexatumumab-induced cell death at a point downstream of DISC formation, and other studies have shown that HDAC inhibitors down-regulate the expression of the IAPs, XIAP and survivin (38–41). In agreement with these reports, we also observed a decrease in these two proteins following treatment with SAHA. However, we have shown here that the ability of SAHA to decrease survivin expression was dependent on p21, whereas the more modest down-regulation of XIAP was not (Fig. 5A). Finally, knockdown of survivin sensitizes otherwise resistant p21-deficient cells to apoptosis induced by lexatumumab, SAHA, and the drug combination. Therefore, survivin seems to block lexatumumab-induced apoptosis and p21 antagonizes this process by reducing survivin expression (Fig. 6D).

Survivin is phosphorylated on Thr34 by cdc2, which results in increased stability of this antiapoptotic protein (25). We reasoned that p21 might modulate survivin expression by inhibiting cdc2 activity. Indeed, cdc2 activity was strongly reduced following treatment with SAHA, and this was largely dependent on p21. In addition, p21-deficient HCT116 cells remained in G2-M phase rather than undergoing apoptosis following treatment with lexatumumab and SAHA (Supplementary Fig. S1). Earlier investigations have suggested links between cdc2, survivin, and TRAIL sensitivity (39, 42–45), and we found that these were clearly operational in colon carcinoma in which the CDK inhibitor flavopiridol provokes reductions in survivin expression and enhances lexatumumab-induced apoptosis independent of p21. Investigation of the efficacy of lexatumumab in combination with SAHA or flavopiridol in colon cancer xenograft models in vivo are planned as a follow-up to the current study. Collectively then, our data suggest that down-regulation of survivin is a major mechanism by which HDAC inhibitors enhance the anticancer activity of lexatumumab and that this occurs via the induction of p21 and subsequent inhibition of cdc2 activity (Fig. 6D). Furthermore, because colon

Figure 5. Knockdown of survivin augments lexatumumab-induced apoptosis. A, p21 is required for SAHA to repress survivin levels. Cells were treated with 2.5 μmol/L of SAHA for the indicated times. Expression levels of various IAPs were determined by immunoblotting. B and C, knockdown of survivin enhances lexatumumab-induced apoptosis even in the absence of p21. Cells were treated with siRNA against survivin or luciferase (nontarget control) as described in Materials and Methods. Knockdown of survivin levels was determined by immunoblotting. Following knockdown, cells were treated with 100 ng/mL of lexatumumab, 2.5 μmol/L of SAHA, or both for 24 h. Apoptosis was measured by active caspase-3 staining followed by flow cytometry or PI-FACS analysis. C, columns, mean; bars, SD (n = 3).
carcinoma cells preferentially signal through TRAIL-R2, our studies suggest that clinical investigations of lexatumumab in this arena are warranted. We have also shown that survivin seems to be a key mediator of resistance to lexatumumab and that targeting its expression directly (via siRNA) or indirectly (by HDAC inhibitors or CDK inhibitors) may greatly potentiate the efficacy of lexatumumab in clinical trials.

Acknowledgments


Grant support: NIH RO1 awards (CA32613 and CA76379), Cancer Center (CORE) Support grant (CA21765), and by the American Lebanese Syrian Associated Charities.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References


Histone Deacetylase Inhibitors Enhance Lexatumumab-Induced Apoptosis via a p21 Cip1-Dependent Decrease in Survivin Levels


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/67/14/6987

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2007/07/26/67.14.6987.DC1

Cited articles
This article cites 45 articles, 20 of which you can access for free at:
http://cancerres.aacrjournals.org/content/67/14/6987.full.html#ref-list-1

Citing articles
This article has been cited by 11 HighWire-hosted articles. Access the articles at:
/content/67/14/6987.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.