Transcriptional induction of GRP78/BiP by histone deacetylase inhibitors and resistance to histone deacetylase inhibitor–induced apoptosis

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Abstract
Histone deacetylase (HDAC) inhibitors are emerging as effective therapies in the treatment of cancer, and the role of HDACs in the regulation of promoters is rapidly expanding. GRP78/BiP is a stress inducible endoplasmic reticulum (ER) chaperone with antiapoptotic properties. We present here the mechanism for repression of the Grp78 promoter by HDAC1. Our studies reveal that HDAC inhibitors specifically induce GRP78, and the induction level is amplified by ER stress. Through mutational analysis, we have identified the minimal Grp78 promoter and specific elements responsible for HDAC-mediated repression. We show the involvement of HDAC1 in the negative regulation of the Grp78 promoter not only by its induction in the presence of the HDAC inhibitors trichostatin A and MS-275 but also by exogenous overexpression and small interfering RNA knockdown of specific HDACs. We present the results of chromatin immunoprecipitation analysis that reveals the binding of HDAC1 to the Grp78 promoter before, but not after, ER stress. Furthermore, overexpression of GRP78 confers resistance to HDAC inhibitor–induced apoptosis in cancer cells, and conversely, suppression of GRP78 sensitizes them to HDAC inhibitors. These results define HDAC inhibitors as new agents that up-regulate GRP78 without concomitantly inducing the ER or heat shock stress response, and suppression of GRP78 in tumors may provide a novel, adjunctive option to enhance anticancer therapies that use these compounds. [Mol Cancer Ther 2009;8(5):1086–94]

Introduction
Histone deacetylases (HDACs) represent a promising new target for drug development in cancer therapy. There are four classes of HDACs, which group the 11 known HDACs according to their homology with yeast deacetylases (1). Compounds that inhibit HDACs are currently being tested in clinical trials as primary or adjunctive anticancer agents. Most of the classic HDAC inhibitors result in global acetylation of histone and nonhistone proteins and changes in the gene expression of approximately 2% to 5% of target genes (1). The list of nonhistone proteins known to be affected by differential acetylation is constantly expanding, and it includes E2F1, heat shock protein (HSP)-90, p53, Rb, and YY1, among others (2). The diversity of differentially acetylated proteins may in part explain why treatment of cancer cells with HDAC inhibitors leads to increased differentiation, growth arrest, and apoptosis. Whereas HDAC inhibitors confer therapeutic benefits, they may inadvertently trigger additional events that render the cancer cells resistant to the treatment, such as induction of the prosurvival protein GRP78 (3).

GRP78, also referred to as BiP or HSPA5, is a stress-inducible chaperone located primarily in the endoplasmic reticulum (ER; refs. 4, 5). When cells experience ER stress, such as ER Ca²⁺ efflux or accumulation of unfolded proteins in the ER, a signal transduction cascade is initiated that triggers the unfolded protein response (UPR; ref. 6). The UPR mitigates ER stress by transiently arresting general translation, degrading misfolded proteins, and up-regulating ER chaperones and folding enzymes. If these protective measures are insufficient to reduce ER stress, the UPR will activate apoptotic cell death. A major target of the UPR is the induction of GRP78, which assists in protein folding and assembly, targets misfolded proteins for degradation, binds ER Ca²⁺, and controls the activation of transmembrane ER stress sensors. As a major prosurvival arm of the UPR, GRP78 is emerging as a pivotal regulator of cancer growth and resistance (7, 8). GRP78 transcript and protein levels are elevated in a wide variety of cancers due to intrinsic factors such as altered glucose metabolism in cancer cells and extrinsic factors such as the abnormal pathologic conditions of the tumor microenvironment, which is known to trigger the UPR (9, 10). Through inactivation of the Grp78 allele in endogenous cancer mouse models, GRP78 is shown to be critical for tumor progression (11, 12). Further, overexpression or small interfering RNA (siRNA)–mediated knockdown studies establish that GPR78 confers resistance to a variety of anticancer therapy in tumor as well as tumor-associated endothelial cells (7, 13–17). The transcriptional activation of Grp78 is mediated primarily by highly conserved elements in its promoter.
referred to as the ER stress response elements (ERSE), which serve as binding sites for a multitude of transcription factors, along with chromosomal modifications at the promoter region (18–20). The ERSE is evolutionarily conserved among eukaryotes and consists of 19 nucleotides characterized by a unique tripartite design. The three parts consist of (a) a CCAAT motif that binds NF-Y; (b) a 9-nucleotide GC-rich domain that binds TF-II-I; and (c) a 5-nucleotide sequence that binds YY1 and activating transcription factor 6 (ATF6). The typical mammalian Grp78 promoter contains three such ERSEs, and collectively, they contribute to ER stress–induced transcriptional activation (18, 19).

Interestingly, the induction of GRP78 by an HDAC inhibitor was first discovered in normal rat brain tissue after prolonged treatment with valproic acid, a mood-stabilizing and anticonvulsant drug later found to cause HDAC inhibition (3). However, the mechanism of induction of GRP78 by HDAC inhibitors is not known, and its relevance in anticancer therapy has not been characterized. In this report, we used a panel of cancer cell lines as well as xenograft tumor model to examine modulation of GRP78 expression by HDAC inhibitors. We report here our findings that characterize the specific mechanisms in HDACi-mediated transcriptional induction of GRP78 and the modulation of HDACi-induced apoptosis by GRP78, providing the proof of principle that adjunctive therapies targeting GRP78 could potentially sensitize cancer cells to HDAC inhibitor therapy.

Materials and Methods

Cell Lines and Drug Treatment Conditions

HCT116 and HT29 cell lines were provided by Dr. Robert Ladner (Department of Pathology, University of Southern California Keck School of Medicine, Los Angeles, CA); U87 and LN229 cell lines were obtained from American Tissue Culture Collection. The cells were propagated in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 0.1 mg/mL streptomycin at 37°C and 5% CO2. Thapsigargin (Tg) and trichostatin A (TSA) were obtained from Sigma-Aldrich. Tg was dissolved in DMSO at 1 mmol/L and a final concentration of 300 nmol/L. MS-275, purchased from Calbiochem, was dissolved in DMSO at 500 nmol/L and a final concentration of 500 nmol/L. TSA was dissolved in 5% DMSO final concentration. MS-275, purchased from Calbiochem, was dissolved in DMSO at 1 mmol/L and a final concentration of 500 nmol/L. TSA, MS-275, or 100% DMSO was added to the culture medium 24 h after transfection, and cells were incubated at 37°C for an additional amount of time before harvesting with 50 μL of lysis buffer (Promega). Protein concentrations were determined using BPA reagent (Bio-Rad), and the relative luciferase activity was measured with firefly assay reagent (Promega) and a luminometer. Each transfection was repeated two to five times.

Transient Transfection and Luciferase Assay

The flag-tagged HDAC expression vectors and pGL191 were kindly provided by Dr. Ed Seto (University of Southern Florida, Tampa, FL). All transfections were carried out using Lipofectamine 2000 (Invitrogen) per manufacturer’s guidelines. TSA, MS-275, or 100% DMSO was added to the culture medium 24 h after transfection, and cells were incubated at 37°C for an additional amount of time before harvesting with 50 μL of lysis buffer (Promega). Protein concentrations were determined using BPA reagent (Bio-Rad), and the relative luciferase activity was measured with firefly assay reagent (Promega) and a luminometer. Each transfection was repeated two to five times.

siRNA

The siRNA against Grp78 is 5′-ggaacgcgcaauugauacag-3′ and was previously described (22). The siRNAs against human HDAC1, HDAC2, and HDAC3 were purchased from Applied Biosystems. The control siRNA was purchased from Molecular Probes/Invitrogen and contains an Alexa Fluor fluorescent tag to determine transfection efficiency. HeLa cells were grown to 80% confluence in 12-well dishes and transfected with 10 nmol/L of either control siRNA or gene-specific siRNA, with or without 0.2 μg of −112Luc or pGL181sx reporter plasmid, using Lipofectamine 2000 transfection reagent. The cells were harvested 48 to 72 h after transfection. The experiments were repeated two to three times.
Resistance to HDAC Inhibitors by GRP78 Induction

Reverse Transcription-PCR Analysis

tTotal RNA was extracted using TRIzol (Invitrogen) following the manufacturer’s instructions. First-strand cDNA was synthesized with the Superscript First-Strand Synthesis System for reverse transcription-PCR (Invitrogen). To detect human mRNA, PCR was done using the following primers: Xbp-1 spliced, 5′-GGTCTGCTGATCGGCGGAGC-3′; Grp78, 5′-AACATACATTCAAGTGTTAGTTGAGTG-3′; CHOP, 5′-GCAAGAGTCTGCTTCAAGTG-3′; HDAC1, 5′-GTTCTCCTGGTGTTGTATGC-3′; HDAC2, 5′-GTAATTCCACAGTTTGACAGCA-3′; HDAC3, 5′-CCGGAATGGTGGCCGCTG-3′.

Chromatin Immunoprecipitation
Stably transfected HeLa cells grown to 80% confluence in 15-cm plates were subjected to chromatin immunoprecipitation assay with antibodies against HDAC1 (Upstate) as previously described (19). Purified DNA from the input and immunoprecipitation samples was subjected to quantitative PCR with SYBR green and the results were analyzed per manufacturer’s recommendations (Perkin-Elmer). The primers used were as follows: for Grp78, primer c, 5′-CATTGTTGCCTAAAGAATGACCAG (forward), and primer d, 5′-AGTATCGAGCGCGCCGTCGC (reverse); RVprimer3 (primer a), 5′-CTAGCATAAGTGCCTGTCCC-3′; and GLprimer2 (primer b), 5′-GGTAGACAGCCAAAACATAAAG-3′.

Xenograft Mouse Models
Nude athymic mice were injected s.c. with 2 × 10⁶ MDA-MB-435 human breast cancer cells in 0.1 mL serum free medium. The injection of TSA at 500 μg/kg body weight or the solvent DMSO for 4 d was started once palpable tumors were formed.

Apoptotic Assay
For mitochondrial membrane potential staining by the JC-1 assay, the Mitochondrial Permeability Transition Detection Kit (Immunochemistry) was used per manufacturer’s protocol. Following wash with PBS, the cells were examined under a fluorescence microscope. Each assay was done in triplicate.

Results

HDAC Inhibitors Specifically Induce GRP78 but not Other Stress Response Pathways

GRP78 protein levels were examined by Western blots in a panel of cancer cell lines, which were either untreated or treated with 500 nmol/L TSA, a pan-HDAC inhibitor, for 24 hours. GRP78 induction was observed in varying levels in all five cancer cell lines tested, which include the human colon cancer cell lines HCT116 (wt p53) and HT29 (p53 mutant), the human breast adenocarcinoma cell line MCF-7, and the human glioma cell lines LN229 and U87 (Fig. 1A). To test whether TSA induces GRP78 at the transcript level, HCT116 cells were treated with TSA and, as a positive control, treated with the 300 nmol/L of the ER stress inducer Tg. As expected, Tg treatment results in up-regulation of Grp78 transcript, as well as of the transcript levels of UPR targets CHOP and the spliced form of XBP-1 (Fig. 1B). In contrast, whereas TSA up-regulated Grp78 mRNA level, CHOP and spliced XBP-1 mRNA levels were unaffected by TSA. To investigate the effect of TSA on tumor cells in vivo, nude mice with MDA-MB-435 xenografts were injected with TSA for 4 days. Western blot analysis of the tumor lysates shows induction of GRP78 but not the stress-inducible heat shock protein HSP70 (Fig. 1C). The tumor microenvironment is subject to ER stress (10). To test whether ER stress modulates HDAC inhibitor induction of GRP78, HCT116 cells were pretreated with increasing amounts of the class I HDAC inhibitor MS-275 for 24 hours, followed by Tg treatment. At 0.5 μmol/L MS-275, an additive effect was observed, whereas at 1.5 μmol/L MS-275, a synergistic effect was observed (Fig. 1D).

Identification of the HDAC Inhibitor Response Elements in the Grp78 Promoter
Transcriptional induction of the rat Grp78 promoter primarily requires 170 bp of the promoter sequence upstream of the TATA element (18). Within this region are three ERSEs, with ERSE1 being the most proximal to the TATA element.
To map the location of the HDAC inhibitor response element, luciferase reporter plasmids containing 5’ deletion or specific ERSE mutations of the rat Grp78 promoter as indicated were transiently transfected into HeLa cells, and luciferase activities were monitored 18 h after TSA or MS-275 treatment. For inactivation of ERSE, the CCAAT sequence was mutated. The luciferase activity for each construct in nontreated cells was set as 1. Columns, fold induction by TSA or MS-275; bars, SD. A, the minimal HDAC inhibitor–inducible Grp78 promoter −112Luc was used as a template for successive mutation of putative transcription factor binding sites within the ERSE. The putative transcription factor binding sites are indicated. Following transfection and treatment with TSA or MS-275, the luciferase activities were determined. Columns, fold induction; bars, SD.

(Fig. 2A). To map the location of the HDAC inhibitor response element, luciferase reporter plasmids −169Luc, −144Luc, −112Luc, and −79Luc, containing three, two, one, or no ERSE, respectively, were constructed. Additionally, −52Luc, containing only the sequence immediately downstream of the TATA element to the transcription start site, was constructed. These were transfected into HeLa cells and, 24 hours later, were either untreated or treated with TSA or MS-275. As shown in Fig. 2A, the −112Luc promoter was the minimal promoter fragment sufficiently inducible at 6- to 7.5-fold over control levels, and this was similar to the level of induction observed in the −144Luc and −169Luc plasmids. The −79Luc and −52Luc showed no response to HDAC inhibitor treatment. Additionally, the results obtained with TSA directly parallel those obtained with MS-275.

Previously, it was determined that a 2-bp mutation of the CCAAT sequence site within the ERSE inactivates its transcriptional activity (18). To assess the contribution of the individual ERSEs, plasmids containing mutated CCAAT sequence of specific ERSEs were constructed (Fig. 2A). We observed that inactivation of ERSE2 in −169(ERSE2m) did not have any effect, whereas inactivation of ERSE1 in −169(ERSE1m) substantially reduced induction of the Grp78 promoter by both TSA and MS-275. Similarly, inactivation
of ERSE1 with intact ERSE2 in −144(ERSE1m) resulted in minimal induction by TSA or MS-275 (Fig. 2A). Collectively, these results establish that ERSE1 is essential for Grp78 induction by HDAC inhibitors, and that class 1 HDAC is likely to be responsible for suppression of the Grp78 promoter.

To determine the specific elements within the tripartite-structured ERSE1 responsible for the induction of the Grp78 promoter by HDAC inhibitors, the −112Luc plasmid was used as a template for creating plasmids containing mutations that prevented the binding of transcription factors with specific affinity for elements within ERSE1. The constructs mut1, mut2, and mut3 contained mutations in the NF-Y, TF-II-1/Sp, and YY1/ATF6 binding sites, respectively (Fig. 2B). An additional mutant, mut4, contained mutations in both the YY1/ATF6 and TFII-1/Sp binding sites. Transfections were carried out under similar conditions to Fig. 2A. We observed that the CCAAT sequence representing NF-Y binding site is most important for HDAC inhibitor–induced transcription of the −112Luc promoter fragment because its mutation (mut1) abolished the promoter induction by both TSA and MS-275 (Fig. 2B). Mutation of the TF-II-1/Sp site (mut2) was without effect and a 30% reduction of induction was observed for mutation of the YY1/ATF6 site (mut3). Interestingly, when these sites were mutated in combination (mut4), a 70% reduction was observed.

**Grp78 Promoter Repression Is Mediated by HDAC1**

Our observation in transfection assays that MS-275, an HDAC inhibitor that only blocks HDAC1, HDAC2, and HDAC3 at the concentrations used in our experiments (23), is able to induce the Grp78 promoter similar to the level of TSA provides the first hint that this class of HDACs may be responsible for Grp78 promoter repression. To identify the HDAC involved, HeLa cells were transfected with flag-tagged HDAC1, HDAC2, and HDAC3 expressing plasmids along with −169Luc, −112Luc, and −79Luc, and the luciferase activities were determined. As a control, the HDAC3-repressible luciferase reporter plasmid pGL191Luc (24) was analyzed in parallel. Before transfection, the expression level of each of the HDAC was determined to be similar by Western blot analysis (Fig. 3A, inset). For each HDAC, varying doses were tested for effect on the promoter activity (Fig. 3A). For the Grp78 promoter, overexpression of HDAC1 at the...

**Figure 3.** Identification of HDAC1 as a repressor of Grp78 promoter activity. A, flag-tagged HDAC1, HDAC2, and HDAC3 expression plasmids at increasing amounts (in micrograms) as indicated were transfected into HeLa cells along with −79Luc, −112Luc, −169Luc, or the HDAC3-repressible human GDF11 promoter reporter plasmid pGL191Luc. The transfected HDAC levels were determined by Western blot analysis (Fig. 3A, inset). The cells were harvested 72 h after transfection. Columns, relative luciferase activity; bars, SD. B, HeLa cells were transfected with siRNA against HDAC1, HDAC2, or HDAC3 expression plasmids at increasing amounts (in micrograms) as indicated were transfected into HeLa cells along with −79Luc, −112Luc, −169Luc, or the HDAC3-repressible human GDF11 promoter reporter plasmid pGL191Luc. The transfected HDAC levels were determined by Western blot analysis (Fig. 3A, inset). The cells were harvested 72 h after transfection. Columns, relative luciferase activity; bars, SD. C, HeLa cells were transfected with siRNA against HDAC1, HDAC2, or HDAC3, along with reporter plasmid −112Luc, −79Luc, or pGL191Luc. The cells were harvested after 72 h and luciferase activity was determined. Fold induction in comparison with control siRNA; bars, SD.

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**Resistance to HDAC Inhibitors by GRP78 Induction**

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higher doses substantially repressed both the −112Luc and −169Luc reporter activities. No change was seen in the −79Luc and pGL191 activities for HDAC1, and neither promoter responded to HDAC2 overexpression. The Gdf11 promoter (pGL191) was suppressed by overexpression of HDAC3 in a dosage-dependent manner, as was expected (24), but the Grp78 promoter did not. These results show that HDAC1 is a repressor of the Grp78 promoter activity.

To confirm that HDAC1 is necessary for repression of the Grp78 promoter, siRNA oligos directed against human HDAC1, HDAC2, or HDAC3 were transfected into HeLa cells along with the Grp78 promoter reporter plasmid. The pGL191 Gdf11 reporter was also transfected as a control. The siRNAs against HDAC1, HDAC2, and HDAC3 sufficiently inhibited the expression of their respective targets as determined by quantitative reverse transcription-PCR (Fig. 3B). In agreement with the data showing that overexpression of HDAC1 represses −112Luc, depletion of HDAC1, but not HDAC2 or HDAC3, increased the activity of −112Luc. Additionally, the pGL191 Gdf11 promoter responded to the depletion of HDAC3, but not HDAC1 or HDAC2, as expected (Fig. 3C). These findings identify HDAC1 as the primary HDAC responsible for transcriptional repression of the Grp78 promoter, and it acts through the sequence spanning −112 and −79, which contains ERSE1.

**HDAC1 Differentially Occupies the Grp78 Promoter at the CCAAT Element in ERSE1**

One mechanism for the repression of the Grp78 promoter by HDCA1 is that it binds to ERSE1 and inhibits acetylation of transcription factors and/or chromatin associated with the Grp78 promoter. When the Grp78 promoter is activated by Tg or TSA, this suppression is relieved through removal of HDAC1 binding to the promoter element. One explanation why CCAAT mutation of ERSE1 abolishes induction by HDAC inhibitors is that it is required for HDAC1 binding. To test these predictions, chromatin immunoprecipitation assays for HDAC1 were done in HeLa cells stably transfected with −112Luc or mut1Luc, where the CCAAT sequence was mutated. Before immunoprecipitation with antibody against human HDAC1, the transfected cells were either untreated or treated with TSA for 12 hours or Tg for 4 hours. The isolated DNA was amplified with primers matching pGL3 basic that spanned either the region encompassing the Grp78 promoter insert of the −112Luc and mut1Luc (labeled a and b) or the sequence just upstream of ERSE3 and just downstream of the TATA box (labeled c and d), with the latter set of primers detecting HDAC1 binding to the endogenous Grp78 promoter in the transfected cells (Fig. 4A). We observed HDAC1 binding to the −112Luc promoter before, but not after, TSA or Tg treatment, and this binding is abolished by a mutation in the NF-Y binding site in mut1Luc (Fig. 4B). Concurrently, HDAC1 binds to the endogenous Grp78 promoter in both the −112Luc and the m1Luc stably transformed cells, but binding was no longer detected after treatment with TSA or Tg (Fig. 4C).
Knockdown of GRP78 Induction Sensitizes Cancer Cells to TSA-Induced Apoptosis

Because up-regulation of GRP78 is a potential mechanism for resistance to TSA-induced apoptosis, we determined whether suppression of GRP78 expression can sensitize cancer cells to TSA-mediated apoptosis. Annealed siRNA oligos against Grp78 (siGrp78) or a random control (siCtrl) were transfected into the breast carcinoma cell line MDA-MB-435 before treatment with 400 nmol/L TSA for 24 hours. Apoptotic cells were identified using mitochondria membrane potential assay. The MDA-MB-435 cells were resistant to this TSA treatment regimen as evidenced by the low percent (<10%) of apoptotic cells in cells treated with siCtrl (Fig. 6A). However, when GRP78 expression was knocked down by siGrp78, about 75% of the cells entered apoptosis (Fig. 5A). A similar experiment was carried out in colon carcinoma HCT116 cells, and PARP cleavage was used for detection of apoptosis. Before TSA treatment, no PARP cleavage was detected. In the cells where Grp78 was targeted by siGrp78, there was significant cleavage of PARP after TSA treatment compared with the cells transfected with siCtrl (Fig. 5B). Therefore, in two different cancer cell lines, using two different assays for detection of apoptosis, knockdown of GRP78 sensitizes the cancer cells to TSA-induced apoptosis.

Discussion

HDAC inhibitors represent a new class of anticancer compounds with great therapeutic potential, with the HDAC inhibitor suberoylanilide hydroxamic acid being the first HDAC inhibitor approved by the Food and Drug Administration for the treatment of cutaneous T-cell lymphoma (26). Here we show that in a wide variety of cancer cells ranging from colon, breast, to brain tumor, HDAC inhibitors TSA and MS-275 induce the UPR target GRP78, a major antiapoptotic protein representing the prosurvival arm of the UPR. Interestingly, the action of the HDAC inhibitors is distinct from that of ER stress inducers, which in addition to inducing GRP78 also induces the UPR signaling pathways, as exemplified by CHOP induction and generation of the spliced form of XBP-1 (6). The observation that HSP70, a major indicator of cytosolic stress, is not induced by HDAC inhibitors further suggests that the up-regulation of GRP78 by HDAC inhibitors is not a result of major cellular stress. In addition to cells in culture, GRP78 is induced by HDAC

![Figure 5](image-url)

Overexpression of GRP78 protects 293T cells from TSA-induced apoptosis. (A) Cell lysates prepared from 293T cells transfected with either 1.0 μg of a plasmid expressing His-tagged GRP78 or same amount of empty vector pcDNA3 were subjected to Western blot with anti-KDEL, anti-His, and anti-β-actin antibodies. The anti-KDEL antibody recognized the COOH-terminal KDEL motif of GRP78, GRP94, and protein disulfide isomerase (PDI). (B) Empty vector pcDNA3 or increasing amounts of the plasmid expressing His-GRP78 (0.25, 0.5, and 1.0 μg) as indicated were transfected to 293T cells. Empty vector was added to adjust the total amount of plasmids to be the same. Twenty-four hours after transient transfection, the cells were treated with 500 nmol/L TSA for 48 h and then subjected to mitochondrial membrane potential staining using the JC-1 assay, which detects cells at early stage of apoptosis. Red fluorescence, viable cells; green fluorescence, apoptotic cells. (C) The percent of apoptotic cells under each condition in B was quantitated and plotted against the transfected amount of GRP78. Open and solid columns, no treatment and TSA treatment, respectively; bars, SD.
The CCAAT sequence, which in the case of initially to the regulation and that await future investigation. Other sequences within the ERSE may also contribute par-

drial membrane potential staining using the JC-1 assay. B, cent of apoptotic cells under each condition as determined by mitochon-

and which HDAC represses the Grp78 promoter and the functional consequence of GRP78 induction to HDAC inhibitor therapy in cancer cells.

Here we identified ERSE1, the most proximal ERSE to the TATA element of the Grp78 promoter, as the most critical for the HDAC inhibitor response and, within the ERSE tripartite structure, the CCAAT motif as the essential control element. Other sequences within the ERSE may also contribute partially to the regulation and that await future investigation. The CCAAT sequence, which in the case of Grp78 promoter

binds the transcription factor NF-Y (18, 19), is a critical element for HDAC inhibitor–mediated induction of the GADD45, multidrug resistance 1, and GTPase RhoB promoters, among others (1). Through the use of the class I HDAC inhibitor MS-275, overexpression, and specific knockdown of individual HDACs, we identified HDAC1 as the key repressor of the Grp78 promoter. This is an important finding because although a multitude of activating transcription factors for the Grp78 promoter have been identified, including NF-Y, ATF6, YY1, and TF-II-I (19), little is known on how the Grp78 promoter is maintained at low basal level in non-stressed cells. The discovery here that HDAC1 binds the Grp78 promoter constitutively under normal culture conditions but departs from the promoter when it is activated provides the first evidence that acetylation inhibition is a novel mechanism for suppression of this multifunctional protein. Interestingly, it has been reported that HDAC1 regulates itself by self-mediated repression, using a NF-Y site along with a distal Sp-1 site to its own promoter (27).

While the precise mechanism whereby HDAC1 regulates the Grp78 promoter awaits further investigation, possible candidates for HDAC1 include the chromatin and transcription factors associated with the Grp78 promoter. Our mutational analysis of the ERSE1 shows that the NF-Y binding site is required for HDAC1 binding to the Grp78 promoter, suggesting that NF-Y mediates binding of HDAC1, which, through protein-protein interaction, exerts its effect on the transcription factors and the chromatin that associates with the Grp78 promoter. Consistent with this notion, NF-Y, Sp proteins, and YY1 have been reported to be targets for HDAC modifications (28–30). As for the chromatin, recently, it has been determined through single molecule footprinting that the 350-bp region upstream of the transcription initiation site spanning the three ERSEs of the Grp78 promoter is constitutively depleted of nucleosomes; however, nucleosome footprints are detected immediately downstream of the transcription initiation site, as well as further upstream of promoter (28). These chromatin structures, as well as the ERSE binding factors, are prime targets of HDAC1 modification although this remains to be determined.

GRP78 promotes tumor growth by enhancing cell proliferation and tumor angiogenesis and simultaneously inhibits stress-induced apoptosis (11). The ability of GRP78 to block apoptosis includes its binding and inactivation of proapop-

otic components such as BIK and caspase-7 that locate to the ER, as well as suppression of induction of CHOP, which mediates the apoptotic arm of the UPR (13, 14). Recently, GRP78 is also identified as a regulator of autophagy due to its role in maintaining ER integrity and homeostasis (31). The potent prosurvival property of GRP78 explains why it is found to confer drug resistance to a wide variety of cancer cells, as well as dormant cancer cells and blood vessels associated with tumor (7, 8, 15–17). Antiangiogen-

esis therapy, although promising as an anticancer therapy, inadvertently induces GRP78 in the treated tumor due to nutrient and oxygen deprivation, leading to the prediction that GRP78 induction by these agents may result in drug resistance (9). Here we show that GRP78 overexpression

\[ {\text{Figure 6. Knockdown of GRP78 sensitizes cancer cells to HDAC inhibitor–mediated apoptosis. A, MDA-MB-435 cells were transfected with siRNA against GRP78 (siGrp78) or a random control (siCtrl) and, 48 h later, were either nontreated (−) or treated (+) with TSA for 24 h. Top, GRP78 levels in the transfected cells with β-actin as loading control. Bottom, percent of apoptotic cells under each condition as determined by mitochondrial membrane potential staining using the JC-1 assay. B, HCT116 cells were subjected to the same conditions as in A and the cell lysates were assayed for PARP cleavage by Western blot. The normal length (116 kDa) and apoptotic, cleaved forms (85 kDa) of PARP are indicated. GAPDH was used as a control.}} \]
leads to resistance of TSA-induced apoptosis. Our results with siRNA against GRP78 show that lowering GRP78 levels significantly increases the apoptotic effects of the HDAC inhibitor TSA. Compounds and agents that can specifically block GRP78 expression and/or its activity have been reported and are being evaluated (8, 32–37). Their use as an adjunct to HDAC inhibitor therapy to reduce resistance warrants vigorous investigation. Conversely, agents that induce GRP78 have been reported to be neuroprotective (38). That may explain the therapeutic benefits of valproic acid and suggest that other HDAC inhibitors that up-regulate GRP78 expression or activity may be potential agents for organ or tissue protection during stress.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**

Molecular Cancer Therapeutics

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