Histone Deacetylase Inhibitors Induce Cell Death Selectively in Cells That Harbor Activated kRasV12: The Role of Signal Transducers and Activators of Transcription 1 and p21

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Abstract
Histone deacetylase (HDAC) inhibitors (HDACi) show potent and selective antitumor activity despite the fact that they induce histone hyperacetylation in both normal and tumor cells. In this study, we showed that the inducible expression of kRasV12 in nontransformed intestinal epithelial cells significantly lowered the mitochondrial membrane potential (MMP) and sensitized cells to HDACi-induced apoptosis. Consistent with our finding that colon cancer cell lines with mutant Ras have reduced expression of signal transducers and activators of transcription 1 (STAT1), we showed that inducible expression of mutant Ras markedly decreased both basal and inducible expression of STAT1, a transcription factor with tumor suppressor activity. To investigate whether reduced expression of STAT1 in cells that harbor mutant Ras contributes to their increased sensitivity to HDACi, we silenced the expression of STAT1 in HKe-3 cells with small interfering RNA. Despite the fact that silencing of STAT1 was not sufficient to alter the MMP, STAT1 deficiency, like Ras mutations, sensitized cells to apoptosis induced by HDACi. We showed that the induction of p21 by HDACi was significantly impaired in HKe-3 cells with silenced STAT1 expression and showed that the ability of butyrate to activate p21 transcription was diminished in STAT1-deficient HKe-3 cells. Finally, we used cells with targeted deletion of p21 to confirm that p21 protects cells from butyrate-induced apoptosis, strongly suggesting that in these cells STAT1 deficiency promotes butyrate-induced apoptosis through impaired induction of p21. Our data therefore establish that Ras mutations, and consequent reduction in the expression of STAT1, underlie the increased susceptibility of transformed cells to undergo apoptosis in response to treatment with inhibitors of HDAC activity. [Cancer Res 2007;67(18):8477–85]

Introduction
Treatment of tumor cells with histone deacetylase (HDAC) inhibitors (HDACi), drugs that remodel chromatin and thereby modulate the expression of several genes, has been shown to result in growth arrest, differentiation, and/or apoptosis of many cancer cell lines (1, 2). In contrast, normal cells seem to be relatively resistant to HDACi both in vitro and in vivo. The basis for the selective toxicity of HDACi for transformed cells remains unclear. We previously found that mutations in k-Ras, a common genetic change in human tumors, sensitize colon cancer cells to butyrate, an inhibitor of HDAC activity (3). Oncogenic k-Ras has been shown recently to be phosphorylated by protein kinase C, which promotes its translocation to mitochondria, a central organelle in apoptosis, and its association with BCL-x, a potent modulator of programmed cell death (4). In this study, we addressed the significance of constitutive Ras signaling, and Ras downstream target genes, in the responsiveness of colon cancer cells to HDACi.

We reported earlier that a subset of colon cancer cell lines that harbor mutant k-Ras has reduced expression of signal transducers and activators of transcription 1 (STAT1) and of STAT1 target genes and showed that targeted deletion of the mutant Ras allele in HCT116 cells was sufficient to restore the expression of STAT1 (5). We showed that expression of mutant Ras inhibited the basal activity of the STAT1-driven reporter gene and markedly inhibited its responsiveness to IFN-γ (5), showing that activated Ras interferes with STAT1-dependent transcription. This is likely to underlie the decreased expression of IFN-dependent genes in cells harboring an activated k-Ras mutation.

Consistent with our results, genome-wide analysis of mast cells transformed with the H-Ras oncogene revealed strong down-regulation of several IFN-inducible genes (6), and constitutive signaling by phosphatidylinositol 3-kinase and mitogen-activated protein kinase in cells harboring B-Raf mutations has been shown to down-regulate Janus-activated kinase/STAT signaling (7). These findings are significant because, in contrast to STAT3 and STAT5, which are frequently found constitutively activated in leukemias and in solid tumors (8, 9), levels of STAT1 are often found reduced in primary tumors and in established cancer cell lines (5, 10, 11). Although our data showed that Ras mutations are sufficient to inhibit STAT1 expression, STAT1 and its target genes have also been shown to be epigenetically silenced by methylation after cellular immortalization (10), pointing to multiple mechanisms of STAT1 down-regulation in transformed cells.

STAT1 is a transcription factor that regulates the expression of several genes involved in proliferation, apoptosis, and differentiation (12), including the cyclin-dependent kinase (cdk) inhibitor p21, which harbors conserved STAT1-responsive elements in its promoter region (13). In many cell lines, the ability of STAT1 to induce the expression of p21 seems to be fundamental for STAT1-mediated growth arrest. For example, IFN-γ failed to inhibit growth of STAT1-deficient U3A cells but regained antiproliferative properties on STAT1 reintroduction (13). In addition, hypermethylation of the STAT-responsive element located within the CpG island in the p21 promoter was associated both with decreased constitutive expression of p21 as well as with IFN-γ–induced activation of p21 in rhabdomyosarcoma cell lines (14).
STAT1-deficient mice are prone to develop epithelial tumors, confirming the tumor suppressor properties of STAT1 (15), and we showed that a deficiency in p21 promotes formation of intestinal tumors, initiated by mutation in the APC tumor suppressor gene (16).

We have shown earlier that colon cancer cell lines that harbor Ras mutations have reduced levels of STAT1 (5) and that constitutive Ras signaling modulates the responsiveness of cells to the chemopreventive agent butyrate (3). In this study, we used nontransformed intestinal epithelial cells (IEC) with inducible expression of oncogenic kRasV12, as well as a colon cancer cell line with silenced STAT1 expression, to dissect the role of mutant Ras and STAT1 in the responsiveness of cells to inhibitors of HDAC activity. We showed that silencing of STAT1 expression, like Ras mutations, promotes apoptosis in response to inhibitors of HDAC activity, suggesting that Ras modulates apoptosis, at least in part, through down-regulation of STAT1 expression.

Inhibitors of HDAC activity are promising chemotherapeutic compounds and several are in clinical trials for a number of malignancies (2). An important characteristic of HDACIs is that they induce apoptosis preferentially in transformed cells. Our data show that mutations in k-Ras and the subsequent down-regulation of STAT1 and perturbed activation of p21 in STAT1-deficient cells may constitute the molecular basis for the selectivity of HDACIs.

Materials and Methods

Cell culture and Western blot analysis. The HCT116 colorectal carcinoma cell line and its clonal derivative HKe-3 that lacks the mutant k-Ras allele (17) were cultured under standard conditions in MEM supplemented with 10% FCS and antibiotics. IEC-iKRas cells, a generous gift from Raymond DuBois, (Vanderbilt University, Nashville, TN) were grown in DMEM supplemented with 10% FCS; stimulation with isopropyl-L-thio-B-D-galactopyranoside (IPTG; 5 mmol/L) was done for 24 h. Western blot analysis was done using standard procedures. Briefly, 50 μg of total cell lysates were fractionated in 10% SDS-polyacrylamide gels and transferred to nitrocellulose membrane. The membranes were incubated with antibodies for 1 h at room temperature or overnight at 4°C and enhanced chemiluminescence (Amersham) was used for visualization of immune complexes.

Immunofluorescence. Cells were grown on chamber slides, serum starved for 16 h, and either left untreated or treated as indicated. Cells were fixed in ice-cold methanol-acetic acid solution (95:5, v/v) for 20 min at −20°C. Incubation with antibody that recognizes activated caspase-3 (Cell Signaling) was done for 1 h at 37°C. Slides were washed with PBS and incubated with a secondary antirabbit antibody conjugated to FITC for 45 min at 37°C. Samples were examined with a fluorescent microscope and images were acquired with a SPOT CCD camera and analyzed by SPOT software.

Transient transfections and reporter gene assays. Cells were transfected with a pool of small interfering RNA (siRNA) specific for STAT1 or IRF1 (Dharmacon) using the calcium phosphate method (Profection Mammalian Transfection System, Promega) as we described before (18). Transient transfection experiments using the 2.4-kb genomic fragment containing the p21 promoter cloned upstream of the LUC reporter gene (19) were done in 12-well plates in the presence or absence of STAT1-specific siRNA.

Apoptosis assay. Cells were resuspended in hypotonic buffer (0.1% Triton X-100, 0.1% sodium citrate) and stained with propidium iodide (50 μg/mL) for 4 h at 4°C as described before (20). Samples were filtered through a nylon mesh (40-μm pore size) and analyzed by flow cytometry. Cell cycle distribution and the extent of apoptosis (cells with a sub-G1 DNA content) were analyzed by the ModFit software. Mitochondrial membrane potential (MMP) was determined by flow cytometry using the fluorescent dye JC1 (Invitrogen). Cells were stained with 1 μmol/L JC1 for 1 h at 37°C, washed with PBS, and analyzed by fluorescence in the FL2 channel.

Results

Activation of oncogenic Ras in nontransformed epithelial cells is sufficient to prime cells to undergo apoptosis in response to inhibitors of HDAC activity. We recently reported that the extent of apoptosis in response to butyrate, an inhibitor of HDAC activity, is increased in colon cancer cell lines that carry mutant Ras (3). To determine whether oncogenic Ras plays a general role in apoptosis induced by HDACI, we used nontransformed IEC-iKRas intestinal cells with inducible expression of oncogenic k-Ras (21). As described for the IEC-iKRas cells (21), expression of oncogenic Ras by addition of IPTG was extensive and tightly regulated, and as expected, cells with activated Ras displayed enhanced proliferation (Supplementary Fig. S1A and B).

To determine whether induction of oncogenic Ras alters the response of cells to HDACI, we treated cells with 3 mmol/L butyrate or 1 μmol/L suberoylanilide hydroxamic acid (SAHA) for 24, 48, and 72 h in the presence or absence of IPTG (5 mmol/L). The results were expressed as growth index, which is the ratio of the number of cells in treated cultures to the number of control cells. As shown in Fig. 1A, both butyrate and SAHA induced death preferentially in cells that were induced with IPTG to express mutant Ras. Consistent with these data, both SAHA and butyrate inhibit the proportion of cells in S phase preferentially in cells expressing oncogenic Ras (Fig. 1B). Induction of Ras also sensitized cells to apoptosis induced by trichostatin A (TSA) and apicidin, two structurally unrelated inhibitors of HDAC activity (data not shown).

These findings are consistent with our published report that HCT116 cells, which harbor an activating mutation in k-Ras, respond to butyrate with increased apoptosis when compared with HKh2 and HKe-3 cells, two clones derived from HCT116 cells in which the mutant Ras allele has been deleted (3). Therefore, our findings show that activation of Ras during transformation of colonic epithelial cells is sufficient to sensitize cells to apoptosis induced by several HDACIs.

Because mutant k-Ras has recently been shown to localize to mitochondria (4), we tested whether the induction of kRasV12 modulates mitochondrial functions, such as the MMP, a key indicator of cell viability. As shown in Fig. 2A, the induction of mutant Ras significantly reduced the MMP in IEC-iKRas cells but not in the parental IEC6 cell line, excluding the possibility that the decrease in MMP was caused by the addition of IPTG. In addition, the decrease in the MMP was progressive, becoming apparent only 24 h after addition of IPTG (Fig. 2B), which coincided with the kinetics of induction of mutant Ras in these cells (data not shown; ref. 21). Consistently, we showed that HCT116 cells, which carry mutant Ras, have a lower resting MMP when compared with HKe-3 cells, the isogenic clone with a targeted deletion of the mutant Ras allele (data not shown).

We next examined whether the presence of oncogenic Ras perturbs changes in the MMP in response to HDACI in IEC-iKRas cells. As shown in Fig. 2C, treatment of cells with butyrate or SAHA increased the MMP in the absence of IPTG but decreased it in cells induced by IPTG. Because dissipation of the MMP is an initial step in triggering an apoptotic cascade, this observation directly links the expression of mutant Ras to the ability of HDACIs to induce apoptosis. Moreover, these data are consistent with our findings that differences in the intrinsic MMP are linked to the biological responsiveness of cells to butyrate (22).

Silencing of STAT1 in a colorectal cancer cell line promotes butyrate-induced apoptosis. We have previously reported that the expression of STAT1 is reduced in colon cancer cell lines that
harbor oncogenic Ras mutations (5). Using IEC-iKRas cells with inducible activated Ras (Supplementary Fig. S1), we confirmed that the inducible expression of mutant Ras by IPTG is sufficient to down-regulate both basal and IFN-γ-inducible STAT1 expression (Fig. 3A). We validated functional Ras signaling on IPTG induction by showing phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 in IPTG-treated cells (Fig. 3B). These results therefore confirmed that Ras-induced transformation of epithelial cells leads to down-regulation of STAT1 expression, establishing STAT1 as a potentially important effector of Ras signaling.

To determine whether the reduced levels of STAT1 in Ras-transformed cells contribute to their enhanced sensitivity to butyrate, we silenced STAT1 expression. However, silencing of STAT1 in IECs was not very efficient and was, in addition, transient, which did not allow us to use this system to show the role of STAT1 in HDACi-induced apoptosis. Therefore, we silenced STAT1 expression in HKe-3 cells, a cell line derived from HCT116 cells by targeted deletion of the mutant Ras allele (17). STAT1 expression was silenced by RNA interference using a pool of siRNAs directed against STAT1 as we described before (23). We achieved 80% to 90% inhibition of both the basal and IFN-γ-inducible expression of STAT1 at 25 nmol/L STAT1 siRNA (Fig. 4A). Silencing persisted for at least 136 h (data not shown), which allowed us to investigate the biological significance of STAT1 deficiency in HKe-3 cells. We showed that silencing of STAT1 in HKe-3 cells was not sufficient to alter the MMP (Fig. 4B). Consistent with our data shown in Fig. 2, HCTT16 cells, which harbor mutant Ras, have lower MMP compared with the HKe-3 cells with targeted deletion of the mutant Ras allele (Fig. 4B),

Figure 1. Oncogenic activation of RasV12 sensitizes cells to apoptosis induced by HDACi. A, cells were treated with 3 mmol/L butyrate (Bu) or 1 μmol/L SAHA and the number of cells was determined 24, 48, and 72 h after treatment. Growth index (GI) represents the ratio between the number of cells in treated and untreated (CTRL) cultures. Pictures were taken 48 h after treatment. B, the proportion of cells in S phase in cells treated with butyrate or SAHA for 24 h was determined by flow cytometry. Bars, calculated from three independent experiments.
showing that complex changes on Ras signaling are required to alter the MMP.

We next compared the extent of butyrate-induced apoptosis in cells transfected with nontargeted siRNA or siRNA specific for STAT1. We previously reported that HKe-3 cells are, due to a targeted deletion of the mutant Ras allele, relatively resistant to butyrate and that they express relatively high levels of STAT1 (3). As shown in Fig. 4C and D, silencing of STAT1 was sufficient to sensitize HKe-3 cells to butyrate-induced apoptosis. These data suggest that reduced expression of STAT1 in Ras-transformed cells is at least in part responsible for enhanced apoptosis of these cells in response to butyrate.

Butyrate, through its ability to induce growth arrest, differentiation, and apoptosis in transformed cells, acts as a physiologic chemopreventive agent. Next, we determined whether STAT1 also regulates the responsiveness of cells to an important pharmacologic chemopreventive agent, sulindac. Control cells, or cells transfected with STAT1 siRNA, were treated with sulindac sulfide...
for 24 h and the extent of caspase-3 activation was determined by immunofluorescence using antibody that specifically recognizes cleaved, activated caspase-3. As shown in Supplementary Fig. S2A, the extent of activation of caspase-3 was significantly higher in cells with silenced STAT1 expression. Consistently, the amount of cleaved poly(ADP-ribose) polymerase (PARP), a caspase substrate, was enhanced in STAT1-deficient cells (Supplementary Fig. S2B).

These data established that STAT1 can protect cells from apoptosis not only in response to HDACi but also to a pharmacologic inducer of programmed cell death. However, our data also revealed that silencing of STAT1 is not sufficient to lower the MMP (Fig. 4B). Therefore, how does STAT1 protect HKe-3 cells from apoptosis in response to HDACi?

Silencing of STAT1 in HKe-3 cells interferes with induction of p21 in response to butyrate. p21 is known to play an important role in butyrate-induced growth arrest as well as in butyrate-induced apoptosis (24, 25). Because STAT1 has been shown to regulate transcription of the p21 gene (13), we determined whether STAT1 deficiency perturbs the induction of p21 in response to butyrate and sulindac sulfide in IECs. HKe-3 cells, in which the mutated Ras allele has been deleted, have therefore elevated levels of STAT1 compared with the HCT116 cells, were transfected with nontargeting siRNA or siRNA specific for STAT1, and were either left untreated or treated with 3 mmol/L butyrate or 150 μmol/L sulindac sulfide. The levels of cleaved PARP, a marker of apoptosis, and the levels of p21 were determined by immunoblotting 24 and 48 h after treatment. Consistent with results shown in Fig. 4 and Supplementary Fig. S2, STAT1-deficient cells underwent enhanced apoptosis in response to both butyrate and sulindac sulfide, as shown by enhanced cleavage of PARP in cells with silenced expression of STAT1 (Fig. 5A). In addition, we showed that p21 induction in response to butyrate and sulindac sulfide was significantly impaired in STAT1-deficient cells, showing that STAT1 plays a crucial role in p21 induction in response to both butyrate and sulindac sulfide.

To determine whether STAT1 is required for transcriptional activation of p21 in response to butyrate, we transfected cells with a p21 promoter reporter construct in the presence of nontargeting siRNA or siRNA specific for STAT1. Butyrate induced the activity of the p21 promoter in a dose-dependent manner, and we showed that silencing of STAT1 severely impaired the transcriptional activation of the p21 promoter in response to butyrate (Fig. 5B). This result established that STAT1 plays an important role in transcriptional activation of p21 in response to butyrate.

One of the important downstream effectors of STAT1 is IRF1, a transcription factor that has been shown to regulate the activity of the p21 promoter (26) and that is expressed, as we showed, in a STAT1-dependent manner (23). To determine whether IRF1 mediates the ability of STAT1 to regulate p21, we silenced IRF1 in HKe-3 cells and examined the responsiveness of IRF1-deficient cells to butyrate. Our data revealed that, in contrast to STAT1, silencing of IRF1 did not interfere with p21 induction in response to butyrate (Supplementary Fig. S3A and B) and did not modulate butyrate-mediated apoptosis (data not shown). These data exclude the possibility that STAT1 regulates p21 activation through induction of IRF1 and support our hypothesis that STAT1 may be a direct regulator of p21 transcription. Indeed, our preliminary data suggest that overexpression of STAT1 activates p21 promoter activity (data not shown).

p21 protects cells from apoptosis induced by butyrate and other inhibitors of HDAC activity. The best understood biological activity of butyrate is inhibition of HDAC activity (27). Therefore, we next determined whether two structurally unrelated inhibitors of HDAC activity, TSA and SAHA, also require STAT1 for the induction of p21 and whether STAT1 deficiency modulates their biological activity. Cells were transfected with nontargeting siRNA or siRNA specific for STAT1 as described earlier and treated with butyrate (3 mmol/L), TSA (0.5 or 1 μmol/L), or SAHA (1 or 2 μmol/L) for 24 h. As shown in Fig. 6A, all three inhibitors of HDAC activity induced significantly higher levels of apoptosis in STAT1-deficient cells and they all failed to induce p21 in STAT1-deficient cells. In contrast, the levels of another inhibitor of cdk activity, p27, were not affected by STAT1 deficiency.

These data suggested that STAT1 protects cells from apoptosis through its ability to contribute to p21 induction in response to treatment of cells with HDACi. We confirmed the protective role of p21 by showing that HCT116 cells with deletion of the p21 gene respond with increased apoptosis to butyrate (Fig. 6B). In contrast, deficiency in p53 did not affect butyrate-induced apoptosis (data not shown), excluding the role of p53 in p21 induction.

Altogether, our data suggest that STAT1 protects IECs from apoptosis through its ability to support p21 induction in response to a variety of stimuli and that signaling by oncogenic Ras promotes apoptosis in response to HDACi at least in part through its down-regulation of STAT1 expression and consequent loss of p21 induction.

Discussion

There is growing evidence that epigenetic changes that occur during transformation are as crucial for the progression of malignant disease as genetic alterations. Inhibitors of HDAC activity (HDACi), such as butyrate, TSA, and SAHA, are drugs that
induce histone acetylation, modulate the expression of several genes, and thereby normalize the epigenetic state in tumor cells. HDACis exert their potent and selective antineoplastic activity through their ability to inhibit growth and to induce apoptosis of cancer cells but also through inhibition of angiogenesis, induction of differentiation, and activation of the host immune response (2). In addition, HDACis have been shown to exert anti-inflammatory properties through suppression of important inflammatory cytokines (28) or inhibition of cytokine signaling (29–31) and their ability to inhibit inflammation is likely to contribute to their antitumorigenic activity.

Although acetylation of histones is generally thought to reactivate gene expression, genome-wide expression analysis has revealed that a comparable number of genes were repressed and induced in cells that were exposed to structurally unrelated inhibitors of HDAC activity (1, 32). Another intriguing feature of HDACi is that, despite the fact that they induce similar hyperacetylation of histones in both normal and tumor cells, HDACis show remarkable specificity for transformed tumor cells (1, 2). The molecular basis for this selectivity has not been revealed. For example, despite the fact that HDACis have been shown to activate a death receptor pathway in leukemic cells, but not in normal hematopoietic progenitors, the expression of oncogenic fusion proteins AML1/ETO and PML/RAR was not sufficient to confer HDACi sensitivity (33, 34).

Here, we present data that show that inducible expression of oncogenic kRasV12 in nontransformed IECs significantly lowers the MMP and that acquisition of the mutant Ras is sufficient to sensitize cells to HDACi-induced apoptosis.

We identified STAT1 as a downstream target of signaling by mutant Ras, whose silencing, like activation of oncogenic Ras, sensitizes cells to HDACi-induced apoptosis. Our work established that, in STAT1-deficient HKe-3 cells, HDACis failed to induce p21 expression, showing that in these cells STAT1 is required for p21 induction in response to HDACi. STAT1-null mouse embryonal fibroblasts and STAT1-deficient fibrosarcoma cells exhibit significantly lower expression of p21, showing that STAT1 is required for basal expression of p21 (35). Likewise, the basal expression of p21 in the intestine of STAT1-deficient mice was lower, and STAT1-null mice also displayed a lower level of inducible p21 in response to intestinal injury (36). Another group has recently reported that oncogenic H-Ras also promotes HDACi-induced apoptosis and that induction of p21 in response to HDACi is impaired in cells that harbor mutant H-Ras (37).

Although we showed that the ability of HDACis to activate transcription of p21 is severely impaired in STAT1-deficient cells, the mechanism of STAT1-dependent activation of p21 remains to be established. As we reported in HKe-3 cells, STAT1 is constitutively phosphorylated on serine but not on tyrosine (31). Although tyrosine phosphorylation remains a principal mechanism whereby...
STAT1 dimerizes and translocates to the nucleus, STAT1 has been shown to regulate gene expression in its monomeric, unphosphorylated form (38). We showed that treatment of cells with butyrate results in up-regulation of STAT1 expression (Fig. 5) but does not modulate the activation of STAT1 (data not shown). Similarly, phosphorylation of STAT1 has been shown dispensable for its ability to regulate oxysterol-induced apoptosis via the p21/caspase-3–dependent pathway (35).

The regulatory region of p21 harbors multiple STAT1-binding sites (13), suggesting that STAT1 may act as a direct regulator of p21 transcription in response to HDACi. Transient transfection studies using a p21 promoter reporter construct have shown that induction of p21 by TSA requires an Sp1 site located in the p21 promoter region (19, 39). Sp1 and STAT1 have been shown to physically interact and to synergistically regulate the expression of the intercellular adhesion molecule 1 in response to IFN-γ (40). Whether Sp1 and STAT1 also cooperate in the induction of p21 in response to treatment with HDACi is not yet resolved.

We showed that both STAT1-deficient and p21-deficient cells respond to HDACi with an increased extent of apoptosis. We hypothesize that, in the absence of STAT1, like in the absence of p21, cells fail to undergo growth arrest in response to HDACIs but are instead driven to apoptosis (Fig. 6C). We showed that the expression of STAT1 is markedly reduced in IECs on activation of oncogenic Ras, therefore identifying a biological situation with perturbed expression of STAT1.

We showed that STAT1 deficiency also interfered with p21 induction in response to camptothecin, a commonly used chemopreventive and chemotherapeutic agent, establishing STAT1 as a transcription factor that plays a critical role in p21 induction in response to a variety of stimuli. Because we reported that p21 determines the responsiveness of colon cancer cells to camptothecin (41), it is likely that STAT1, like p21, will regulate the responsiveness of cells to camptothecin. Experiments to address this question are under way.

STAT1 was cloned as a transcription factor required for signaling by IFN (42). Its role in apoptosis, however, is not without precedent. Although STAT1 is required for the basal expression of caspases (38), we did not observe a significant effect of STAT1 deficiency on the expression of caspase-3, caspase-7, caspase-8, or caspase-9 in the HKe-3 cells (data not shown). Elevated levels of STAT1 have been shown to protect head and neck squamous carcinoma cells from radiation-induced apoptosis (43), and silencing of STAT1 sensitized prostate carcinoma cell lines to docetaxel-induced apoptosis (44). Recently, STAT1 has been shown to be acetylated in cells treated with HDACi, and its expression was induced selectively in melanoma cell lines that were sensitive to HDACi (45). In this report, the authors showed that STAT1 binds to and sequesters the NF-κB p65 subunit from the nucleus, thereby interfering with the antiapoptotic activity of NF-κB and consequently sensitizing cells to HDACi-induced apoptosis (45). It therefore seems that the interaction of STAT1 with other signaling pathways may dictate the role of STAT1 in HDACi-induced apoptosis.

In summary, our data provide the mechanistic explanation for the selective toxicity of HDACi for tumor cells. We have shown that
the acquisition of oncogenic k-Ras, which occurs in >50% of all human tumors, is sufficient to sensitize colon cancer cells to apoptosis in response to HDACi. Furthermore, we identified STAT1 as a downstream target of Ras signaling in HCT116 cells, whose deregulation is sufficient to confer sensitivity to tumor cells, through its requirement to support the induction of p21 in response to HDACis.

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**Figure 6.** Induction of p21 in response to HDACis protects cells from apoptosis. A, HKe-3 cells were transfected with nontargeting or STAT1-specific siRNA and treated with butyrate, TSA, or SAHA as indicated. The amount of apoptosis was calculated and the levels of p21 and p27 were determined by immunoblotting. B, the amount of butyrate-induced apoptosis was determined in HCT116 p21+/+ and HCT116 p21−/− cells as indicated. The experiment was repeated thrice. C, schematic representation of the role of mutant k-Ras in apoptosis in response to HDACi. WT, wild-type.

**References**


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