Blocking the Raf/MEK/ERK Pathway Sensitizes Acute Myelogenous Leukemia Cells to Lovastatin-Induced Apoptosis

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ABSTRACT

The statin family of drugs are well-established inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase and are used clinically in the control of hypercholesterolemia. Recent evidence, from ourselves and others, shows that statins can also trigger tumor-specific apoptosis by blocking protein geranylgeranylation. We and others have proposed that statins disrupt localization and function of geranylgeranylated proteins responsible for activating signal transduction pathways essential for the growth and/or survival of transformed cells. To explore this further, we have investigated whether the mitogen-activated protein kinase (MAPK) signaling cascades play a role in regulating statin-induced apoptosis. Cells derived from acute myelogenous leukemia (AML) are used as our model system. We show that p38 and c-Jun NH2-terminal kinase/stress-activated kinase MAPK pathways are not altered during lovastatin-induced apoptosis. By contrast, expression of primary and established AML cells to statins results in significant disruption of basal extracellular signal-regulated kinase (ERK) 1/2 phosphorylation. Addition of geranylgeranyl PPi, reverses statin-induced loss of ERK1/2 phosphorylation and apoptosis. By establishing and evaluating the inducible Raf-1:ER system in AML cells, we show that constitutive activation of the Raf/MAPK kinase (MEK)/ERK pathway significantly represses but does not completely block lovastatin-induced apoptosis. Our results strongly suggest statins trigger apoptosis by regulating several signaling pathways, including the Raf/MEK/ERK pathway. Indeed, down-regulation of the Raf/MEK/ERK pathway potentiates statin-induced apoptosis because exposure to the MEK1 inhibitor PD98059 sensitizes AML cells to low, physiologically achievable concentrations of lovastatin. Our study suggests that lovastatin, alone or in combination with a MEK1 inhibitor, may represent a new and immediately available therapeutic approach to combat tumors with activated ERK1/2, such as AML.

INTRODUCTION

The statin family of drugs is immediately available for use as novel, effective anticancer therapeutics. These low molecular weight inhibitors target the rate-limiting enzyme of the mevalonate pathway, 3-hydroxy-3-methylglutaryl-CoA reductase, and have a strong track record as safe and effective agents in the control of hypercholesterolemia (1). Many groups, including our own, have shown that statins can trigger primary and established tumor cells to undergo apoptosis (2–7). Statins have also been shown to suppress tumor growth in animal models of tumorigenesis (8–12). Importantly, nontransformed hematopoietic cells remain fully viable after statin exposure (5, 13). The response to statin treatment of acute malignant disease has been varied. Results of phase I and II clinical trials have shown significant responses, yet dose-limiting toxicities (muscle weakness, myalgia, rhabdomyolysis, anorexia, elevated creatine phosphokinase, nausea, diarrhea, and fatigue) suggest high-dose (>25 mg/kg/d) regimes are not well tolerated (14–17). Because statin-triggered apoptosis is restricted to tumor cells and occurs in a dose- and time-dependent manner (18), it is reasonable to suggest that sustained low-dose treatment regimes will be an effective strategy to target tumor cell death in vivo. Indeed, clinical trials conducted with a sustained low-dose treatment regime support this notion (19, 20). It remains unclear which molecular characteristics will indicate a positive response to statin treatment. To effectively apply this novel anticancer therapeutic to patient care, it is imperative that the mechanism of action be delineated. With this fundamental knowledge, tumors with features that confer sensitivity can be preferentially targeted with statin therapy, alone or in combination with other agents, to eliminate tumor cells without causing collateral damage to neighboring normal cells.

In recent years, we and others have investigated the molecular mechanism of statin-induced apoptosis. Evidence shows this suicide response can be completely reversed by coinucbulation with the immediate product of 3-hydroxy-3-methylglutaryl-CoA reductase, mevalonate, or a downstream product of this biochemical pathway, geranylgeranyl PPi (GGPP; refs. 21–28). One or more geranylgeranylated proteins are thought to be critical to the growth and/or survival of transformed cells, and disruption of these pathways by lovastatin triggers apoptosis in tumor cells (29). It is estimated that ~1% of cellular proteins undergo a posttranslational modification that adds a geranylgeranyl lipid moiety to the CAAX motif at the carboxyl-end of specific protein substrates (30). This modification targets proteins to membranes, and this membrane association is essential for protein function. Geranylgeranylated proteins include signaling molecules such as members of the ras and rho family of proteins; however, the majority of geranylgeranylated substrates remain unknown. Activation of such signaling pathways can occur at a variety of levels, ranging from receptor activation to deregulation of second messengers. Identification of the deregulated pathways in tumors sensitive to lovastatin-induced apoptosis will allow safe and effective application of the statin family of drugs in the clinic.

To determine the pathways that hold a role in statin-induced apoptosis, we have evaluated which of the downstream mitogen-activated protein kinases (MAPKs) contribute to sensitivity. MAPKs are proline-directed protein kinases that mediate the effects of numerous extracellular stimuli on a wide array of biological processes, such as cellular proliferation, differentiation, and death. Three groups of mammalian MAPKs have been studied in detail: extracellular signal-regulated kinases (ERKs), c-Jun NH2-terminal kinases (JNKs) or stress-activated kinases (SAPKs), and p38 MAPKs (reviewed in refs. 31 and 32). The mammalian ERKs (also referred to as p42/44 MAPK) are usually activated by growth factors and mitogenic stimuli, whereas JNK/SAPK and p38 MAPK are usually activated by ultraviolet irradiation, osmotic stress, proinflammatory cytokines, and anticancer drugs. MAPKs are activated by upstream dual-specificity kinases through phosphorylation on both threonine and tyrosine residues. Once activated, MAPKs phosphorylate several transcription factors at
serine and threonine residues, thereby regulating gene expression (reviewed in refs. 31 and 32).

To investigate the molecular mechanism of statin sensitivity, we use cells derived from acute myelogenous leukemia (AML) as our model system. We have shown previously that both primary and established AML cells undergo apoptosis in response to statin exposure, whereas the self-renewal potential of nontransformed primary myelogenous progenitor cells is not affected by exposure to lovastatin (5). Our previous work has also shown that most statins show similar efficacy as apoptotic agonists (33). In this study, we have used lovastatin (a kind gift from Apotex Inc., Toronto, Ontario, Canada) as a representative agent of this class of drugs. We show that lovastatin down-regulates constitutive ERK1/2 phosphorylation in AML cell lines as well as in AML primary blasts. By contrast, lovastatin exposure does not affect the p38 and JNK/SAPK MAPK pathways. By introducing an inducible Raf-1:ER system in AML-3 cells, we demonstrate that down-regulation of ERK1/2 phosphorylation contributes to lovastatin-induced apoptosis. In addition, we show that MEK1 inhibitor PD98059 sensitizes AML cells to low, physiologically achievable concentrations of lovastatin to drive tumor cell apoptosis, suggesting a possible regimen for future therapy.

MATERIALS AND METHODS

Chemical Reagents. Lovastatin was kindly provided by Apotex Inc. The inactive lactone form of lovastatin was converted to the active dihydroxy-open acid form as described previously (7). GGPP, anisomycin, and 4-hydroxymatomixifen (4-HT) were obtained from Sigma (Oakville, Ontario, Canada). MEK1 inhibitor PD98059 was obtained from Calbiochem (La Jolla, CA).

Retrovirus Construction, Production, and Infection. To generate retroviral vectors carrying Raf-1YY:ER and Raf-1DD:ER, the regions encoding these fusion proteins were subcloned from plasmids carrying GFPΔRaf-1YY:ER and GFPΔRaf-1DD:ER (a kind gift from Dr. Martin McMahon, University of California San Francisco, San Francisco, CA) into the pBMNi- lytesyr2-CD8 vector (a kind gift from Dr. Garry Nolan, ref. 34). Using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA), Kozak consensus sequences containing start codons were then introduced into the vectors to generate novel pBMNiRaf-1YY:ERiresGFP and pBMNiRaf-1DD: ERiresGFP retroviral vectors. The new constructs were confirmed by DNA sequencing. To produce infectious replication-deficient ecotropic retroviral particles, the new pBMNiRaf-1YY:ERiresGFP and pBMNiRaf-1DD:ERiresGFP retroviral constructs were transfected by the calcium phosphate method into the Phoenix ecotropic packaging cell line, and viral supernatant was harvested 48 hours later. This virus was then used immediately to infect AML-3 EcoR cells for 45 minutes in the presence of 8 μg/mL Polybrene by spin infection.4 Infected cells were isolated by fluorescence-activated cell sorting for the green fluorescent protein (GFP) marker 3 days postinfection. GFP-positive cells were isolated with a Becton Dickinson FACStarPLUS cell sorter (Becton Dickinson, San Jose, CA). BDIS CellQuest software was used for acquisition and analysis of data.

Quantitative Measurement of Apoptosis Using the Terminal Deoxynucleotidyl Transferase-Mediated Nick End Labeling Assay. Analysis of apoptosis by the terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay was performed as described previously (33). Briefly, the cells were exposed for the indicated times, harvested, and fixed by incubation in 4% formaldehyde for 15 minutes on ice. Subsequently, the cells were exposed for the indicated times, harvested, and fixed by incubation in 4% formaldehyde for 15 minutes on ice. Subsequently, the cells were washed, resuspended in 70% ethanol (EOH), and stored at −20°C for up to 1 week. For analysis, 104 cells were incubated with 0.02 mmol/L biotin-dUTP and 12.5 units of terminal deoxynucleotidyltransferase enzyme in a reaction buffer [200 mmol/L potassium cacodylate, 25 mmol/L Tris-HCl, and 2.5 μg/mL BSA (pH 6.6)], 2.5 mmol/L CoCl2, and 0.01 mmol/L dTTP (Roche Applied Science, Laval, Quebec, Canada) for 45 minutes at 37°C. The cells were then washed twice with PBS, labeled with avidin-FITC for 60 minutes at room temperature, washed again, and analyzed using a FACScalibur cytometer (Becton Dickinson).

RESULTS

Lovastatin Disrupts Constitutive ERK1/2 Phosphorylation before Cells Undergo Apoptosis. To evaluate the role MAPK signaling may play in the mechanism of lovastatin-induced apoptosis, the expression and activation of ERK1/2, p38, and JNK/SAPK MAPKs were measured after exposure of AML-3 cells to 20 μmol/L lovastatin for a period of 30 minutes to 24 hours (Fig. 1A). Quantitative measurement of apoptosis was also performed by TUNEL assay and 29.8 ± 1.0% of the population was shown to undergo apoptosis after 24 hours (Fig. 1B). In addition, cells were treated with 1.0 μg/mL
Lovastatin Down-Regulation of ERK1/2 Phosphorylation Occurs with Similar Kinetics to the Decrease in Protein Geranylgeranylation Triggered by Lovastatin-Induced Apoptosis and Is Reversible by the Addition of GGPP. To evaluate whether the kinetics of lovastatin-induced ERK1/2 dephosphorylation are associated with the decrease of protein geranylgeranylation evident as cells undergo apoptosis, a detailed time course analysis was conducted of AML-3 cells exposed to 20 μmol/L lovastatin. Protein geranylgeranylation was evaluated by the presence of processed and unprocessed forms of Rap1A, a protein substrate that is exclusively lipidated by geranylgeranylation (35). PARP cleavage was monitored as an indicator of caspase activation during apoptosis. As shown in Fig. 2A, unprocessed Rap1A was evident as early as 6 hours after lovastatin exposure, suggesting the depletion of internal GGPP pools occurred at or just before this time and precluded the posttranslational geranylgeranylation of this substrate. Resolution of processed and unprocessed forms of Rap1A was highly reproducible and consistently showed a notable shift 6 hours after exposure to lovastatin. PARP cleavage was clearly evident 24 hours after lovastatin exposure (Fig. 2A), which is consistent with TUNEL analysis showing that cells undergo apoptosis at this time (Fig. 1B). Moreover, as shown in Fig. 2B, exposure to 20 μmol/LLovastatin (48 hours) completely disrupted ERK1/2 phosphorylation, and it induced complete PARP cleavage compared with control AML-3 cells, whereas coincubation of 10 μmol/L GGPP with lovastatin reversed both loss of ERK1/2 phosphorylation and PARP cleavage (Fig. 2B). As expected, exposure to GGPP alone had no effect on either ERK1/2 phosphorylation or cell viability compared with control (26). These results indicate that blocking protein geranylgeranylation in response to lovastatin is highly associated with the decrease in ERK1/2 phosphorylation, and these events occur before caspase activation and apoptosis.

To evaluate whether the down-regulation of ERK1/2 phosphorylation in response to lovastatin is restricted to AML-3 cells or is evident in other AML cells, similar analyses were conducted in lovastatin-sensitive NB-4 cells (5). Indeed, exposure of NB-4 cells to 20 μmol/L lovastatin blocked basal ERK1/2 phosphorylation and induced PARP cleavage (Fig. 2C). Coincubation of 10 μmol/L GGPP with lovastatin for 48 hours reversed lovastatin-triggered loss of ERK1/2 phosphorylation and PARP cleavage in NB-4 cells (Fig. 2D). These results indicate that the down-regulation of ERK1/2 phosphorylation is highly associated with lovastatin depletion of geranylgeranylation as well as the induction of apoptosis in AML cell lines.

Generation of AML-3 Cell Lines Stably Expressing Inducible Raf-1:ER. To evaluate whether the decrease in ERK1/2 phosphorylation in response to lovastatin was functionally important to lovastatin-induced apoptosis, we took a molecular approach and evaluated the effect of conditional activation of ERK1/2 in AML-3 cells in the presence and absence of lovastatin. To establish conditional activation of the Raf/MAPK kinase (MEK)/ERK kinase cascade, we ectopically expressed the inducible Raf-1:ER fusion protein in AML-3 cells using the pBMNiresGFP retroviral vector. The two forms of Raf-1 that were analyzed include a variant (Raf-1DD) in which the pair of adjacent regulatory tyrosine residues were mutated to aspartic acid (DD) to constitutively activate Raf-1 (Fig. 3A) as well as wild-type (Raf-1YY; ref. 36). After retroviral infection, pooled cells ≥95% GFP positive were collected. Without inducing the expression of the constructs, the growth rate and apoptotic response to lovastatin in AML-3 Raf-1DD:ER and AML-3 Raf-1YY:ER stable cell lines were similar to those of the parental AML-3 and AML-3 EcoR cell lines (data not shown).

To evaluate the activity of the inducible stable cell lines of AML-3 cells expressing Raf-1DD:ER or Raf-1YY:ER, we treated the cells with increasing concentrations of 4-HT for 24 hours. After 4-HT...
exposure, activation of the Raf/MEK/ERK pathway was monitored by measuring ERK1/2 phosphorylation by immunoblot analysis. As shown in Fig. 3B, ERK1/2 phosphorylation occurred in a dose-dependent manner in response to 4-HT in AML-3 Raf-1DD:ER cells, with activation first evident at doses as low as 2 nmol/L and maximal activation achieved at 50 nmol/L 4-HT. Time course analysis showed that the addition of 100 nmol/L 4-HT induced a rapid ERK1/2 phosphorylation as early as 30 minutes, which was maximal by 24 hours (Fig. 3C). AML-3 Raf-1YY:ER cells showed a similar response to 4-HT (data not shown). These results demonstrate that the Raf/MEK/ERK pathway can be activated by 4-HT in our experimental system. Activation of Raf-1:ER with 4-HT was specific because phosphorylation of several other kinases including p38 and JNK/SAPK was not detected (data not shown).

Activation of Raf/MEK/ERK Pathway Prevents Lovastatin Down-Regulation of ERK1/2 Phosphorylation and the Full Apoptotic Response. Using the Raf-1:ER system in AML-3, we evaluated the role of the Raf/MEK/ERK pathway in lovastatin-induced apoptosis. As expected, activation of Raf-1DD:ER alone resulted in increased activation of ERK1/2 in response to elevated concentrations of 4-HT (Fig. 3D, Lanes 1 and 2). To determine whether the down-regulation of ERK1/2 phosphorylation was functionally important to lovastatin-induced apoptosis, the AML-3 Raf-1DD:ER cells were exposed to 2–100 nmol/L 4-HT for 24 hours and then treated with 20 μmol/L lovastatin for an additional 24 hours. ERK1/2 phosphorylation was used to measure activation of the Raf/MEK/ERK pathway, whereas PARP cleavage was used to evaluate caspase activation and apoptosis. When ERK1/2 activation was low (2–10 nmol/L 4-HT), the extent of PARP cleavage induced by lovastatin was similar to that induced by the control (Fig. 3D, Lanes 3–6). However, in response to higher concentrations of 4-HT (20–100 nmol/L), abundant ERK1/2 activation was achieved, and the extent of PARP cleavage induced by lovastatin was substantially decreased in a dose-dependent manner (Fig. 3D, Lanes 7–9). Interestingly, complete abrogation was not achieved at the maximum levels of activation. Moreover, ectopic expression of vector or Raf-1DD:ER in the absence of 4-HT had no effect on lovastatin-induced apoptosis (Fig. 3E, Lanes 1–4); however, in the presence of 100 nmol/L 4-HT, Raf-1:ER activation was triggered in AML-3 Raf-1DD:ER cells, and lovastatin-induced PARP cleavage was decreased but not completely reversed, compared with control AML-3 GFP cells (Fig. 3E, Lanes 5–8). Enforced activation of Raf did not have an effect on Rap1A processing (data not shown). Similar results were obtained using AML-3 Raf-1YY:ER cells (data not shown).

Lovastatin Disrupts Constitutive ERK1/2 Phosphorylation and Induces PARP Cleavage in AML Primary Blast Cells. To determine whether lovastatin triggers a down-regulation of ERK1/2 phosphorylation in primary patient blasts, we examined the effect of lovastatin exposure on six randomly archived primary AML patient cells. As shown in Fig. 2, lovastatin down-regulation of ERK1/2 phosphorylation is highly associated with apoptosis triggered by lovastatin depletion of protein geranylgeranylation and is reversible by the addition of GGPP.
samples. The effects of freezing on the viability of these patient blasts were minimal. The six samples were exposed to EtOH control or lovastatin for 48 hours and then analyzed by immunoblot for ERK1/2 expression and activation, PARP cleavage as an indicator of apoptosis, and actin expression as a loading control. AML-3 cells were included as a positive control. Analysis of patient samples showed that medium to high basal ERK1/2 phosphorylation was evident in four primary AML samples [Fig. 4A and B; patients 1 (Lane 3), 2 (Lane 7), 3 (Lane 11), and 5 (Lane 17)], whereas two patient samples were shown to express low or undetectable basal ERK1/2 activity [Fig. 4A and B; patients 4 (Lane 13) and 6 (Lane 19)]. Exposure to lovastatin down-regulated basal ERK1/2 phosphorylation and induced PARP cleavage in the four AML samples with high or medium basal ERK1/2 activity (Fig. 4, compare Lanes 3 and 4, 7 and 8, 11 and 12, and 17 and 18). By contrast, exposure toLovastatin did not further down-regulate the already low levels of ERK1/2 phosphorylation evident in two primary patient samples, yet PARP cleavage was evident (Fig. 4, compare Lanes 13 and 14 and Lanes 19 and 20). Table 1 summarizes the six AML patient samples analyzed for patient age and gender, World Health Organization classification, total white blood cell and blast counts in peripheral blood, presence of ERK1/2 phosphorylation levels down-regulated by lovastatin, and PARP cleavage in response to lovastatin exposure (fold increase). Thus, constitutive ERK1/2 phosphorylation is evident in primary AML
blasts, and lovastatin triggers a down-regulation of ERK1/2 activation, which is associated with the magnitude of the apoptotic response.

**MEK1 Inhibitor PD98059 Sensitizes Tumor Cells to Lovastatin-Induced Apoptosis.** To evaluate whether direct inhibition of the Raf/MEK/ERK pathway can increase the efficacy of lovastatin to trigger apoptosis, we evaluated AML cells after exposure to the MEK inhibitor PD98059, alone or in combination with lovastatin. The concentration of lovastatin used for these studies is achievable in human plasma (14). Exposure of AML-3 cells to 20 μmol/L PD98059 or 4 μmol/L lovastatin for 48 hours results in approximately 5% and 25% apoptosis, respectively. However, pretreating cells for 1 hour with the MEK inhibitor followed by a mixture of both PD98059 and lovastatin results in a greater percentage of cells undergoing apoptosis than that seen with either agent alone for a similar time period.

PD98059 (10, 20, and 50 μmol/L) also potentiated 2 or 10 μmol/L lovastatin-induced apoptosis in AML-3 cells (data not shown). Another MEK1-specific inhibitor, U0126, also potentiates lovastatin-induced apoptosis in AML-3 cells (data not shown). As expected, the down-regulation of ERK1/2 phosphorylation was evident in response to these agents when used individually or in combination (Fig. 5B).

**DISCUSSION**

We show here that lovastatin can effectively and specifically down-regulate the ERK MAPK pathway and that this activity directly contributes to the apoptotic response of AML cells after exposure to lovastatin.
lovastatin. Other MAPK pathways (p38 and JNK/SAPK) are not affected by lovastatin during the apoptotic response. Furthermore, lovastatin down-regulates basal ERK1/2 phosphorylation with kinetics consistent with the loss of this signal transduction pathway contributing to lovastatin-induced apoptosis in tumor cells. Indeed, we show that ectopic expression of Raf-1:ER in AML cells suppresses lovastatin-induced apoptosis. Importantly, the down-regulation of ERK1/2 phosphorylation by lovastatin is evident in primary patient samples. Moreover, evidence suggests that blast cells with high basal levels of phosphorylated ERK1/2 are particularly sensitive to lovastatin-triggered apoptosis. Taken together, our results suggest that lovastatin down-regulation of ERK1/2 phosphorylation contributes to the apoptotic response of transformed cells that are dependent on activation of the Raf/MEK/ERK pathway for growth and/or survival.

Indeed, constitutive ERK1/2 activation has been shown to play an important role in the progression of tumorigenesis in many different cancer types such as head and neck, colon, pancreatic, lung, and ovarian cancer and AML (37–41), and inhibitors of the Raf/MEK/ERK pathway have been developed (42). Interestingly, many tumor types that undergo apoptosis in response to statins are associated with constitutive activation of the Raf/MEK/ERK pathway. These include but are not limited to head and neck carcinomas, pancreatic cancers, colon cancers, and AML. In addition, the AML patient samples with high basal ERK1/2 activation in our study appear to be more sensitive to lovastatin-induced apoptosis than those with lower basal ERK1/2 activation. This suggests that constitutive ERK1/2 activation appears to be a marker of lovastatin-sensitive tumor types. The down-regulation of constitutive ERK1/2 activity may be a common mechanism of action of statin-induced apoptosis among these tumor types (43–45).

Thus, statins are immediately available as anticancer agents and may be an effective therapeutic to combat the wide range of human tumors harboring an activated ERK pathway.

Because lovastatin-induced apoptosis and the down-regulation of ERK1/2 activation can be reversed by ectopic addition of GGPP, it is thought that lovastatin targets a geranylgeranylated protein(s) that plays an essential role in the constitutive activation of the Raf/MEK/ERK pathway. The Raf/MEK/ERK pathway is commonly activated by geranylgeranylated proteins in the Ras family (46), which occur in approximately 30% of cancers (47). However, the association has not been found to be consistent in all tumor types (48, 49), nor has an association with ras mutation and expression status in primary AML cells been found to be consistent with sensitivity to lovastatin-induced apoptosis (50). This would suggest that one or more of the remaining 1% of proteins that are geranylgeranylated within the cell (30) may be activating the Raf/MEK/ERK pathway. Alternatively, activation of the Raf/MEK/ERK pathway may be achieved by deregulation at many other levels, including upstream growth factors and/or survival signals, their respective receptors, or intracellular signaling molecules.

Our work also shows that lovastatin does not trigger apoptosis by down-regulating the Raf/MEK/ERK signaling cascade alone. This is shown by three pieces of evidence. First, ectopic activation of the inducible Raf-1:ER suppressed but did not completely block lovastatin-induced apoptosis. Second, all six primary patient samples were responsive to lovastatin-induced apoptosis, including one that showed little to no constitutive ERK1/2 phosphorylation, and another that did not display ERK1/2 down-regulation in response to lovastatin exposure. Third, direct inhibition of the Raf/MEK/ERK pathway with a MEK inhibitor did not mimic lovastatin at the level of biological response. The MEK inhibitor was a poor inducer of apoptosis compared with lovastatin. Finally, the addition of lovastatin to PD98059 does not enhance inactivation of ERK, yet increased apoptosis is observed. The additional pathways affected by lovastatin to trigger apoptosis remain unclear; however, they likely involve proteins that are geranylgeranylated. Understanding the complete mechanism of lovastatin-induced apoptosis is presently under further investigation.

Despite the complexity of the mechanism of lovastatin-induced apoptosis, blocking ERK1/2 phosphorylation appears to be a key element of overall potency. Indeed, direct disruption of the pathway with a MEK inhibitor synergizes with lovastatin to drive apoptosis. These results suggest that abrogation of the ERK pathway is a key determinant of lovastatin efficacy and that down-regulation of this pathway enables lovastatin to fully drive apoptosis by additional mechanisms of action. This insight is important to the clinical application of statins. Phase I clinical trials with statins alone show that high-dose regimens are not well tolerated because of dose-limiting toxicities (14, 17). Combining statins with inhibitors of the Raf/MEK/ERK pathway at clinically achievable doses significantly increases the efficacy of kill and strongly suggests that statins can play an important role in patient care. Both inhibitors of the Raf/MEK/ERK pathway and statins are available for immediate application to the clinic. This combination may function as a potent, tumor-specific trigger of apoptosis because neither MEK inhibitors nor statins affect normal cells.
yet the two combine to trigger tumor cells to undergo apoptosis. The results of this study suggest that additional analysis of this drug mixture is warranted to determine the efficacy of this combination for clinical use. Tumors to target include those, such as AML, that harbor constitutive ERK1/2 phosphorylation, show sensitivity to MEK inhibitors, and are responsive to statin-induced apoptosis.

ACKNOWLEDGMENTS

The authors are grateful to Drs. M. McMahon and Garry Nolan for providing vectors, to Z. Hu (Ontario Cancer Institute, Toronto, Ontario, Canada) for technical assistance, and to the Penn Lab members for critically reviewing the manuscript. The authors apologize to those whose work was not cited due to space constraints.

REFERENCES

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Cancer Res 2004;64:6461-6468.

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