

# Selective inhibitors of MEK1/ERK<sup>44/42</sup> and p38 mitogen-activated protein kinases potentiate apoptosis induction by sulindac sulfide in human colon carcinoma cells

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## Abstract

The nonsteroidal anti-inflammatory drug (NSAID) sulindac prevents experimental colon cancer and can regress precancerous polyps in humans. Sulindac sulfide inhibits cyclooxygenase (COX)-mediated prostaglandin synthesis and retards the growth of cultured colon cell lines primarily by inducing apoptosis. Given the known role of mitogen-activated protein kinase (MAPK) in signal transduction and the regulation of cell survival and death, we determined the effect of sulindac sulfide on MAPK activation, COX-2 expression, and apoptosis induction in HCA-7 human colon cancer cells. Sulindac sulfide treatment was associated with activation of ERK<sup>p44/42</sup> and p38 MAPK in a dosage- and time-dependent manner, and also activated upstream MEK. Similar results were seen in HCT-15 cells and also with the selective COX-2 inhibitor NS398. ERK<sup>p44/42</sup> and p38 activation were accompanied by an induction of COX-2 protein expression. Selective inhibitors of sulindac sulfide-induced ERK<sup>p44/42</sup> (PD98059) and p38 MAPK (SB203580) activation also suppressed the induction of COX-2 by this NSAID. Furthermore, both MAPK inhibitors significantly augmented sulindac sulfide-induced apoptosis, as did suppression of constitutive COX-2 using antisense oligonucleotides. In conclusion, MEK/ERK and p38 MAPK activation mediate COX-2 induction by sulindac sulfide. Selective inhibitors of these MAPKs potentiate apoptosis induction by this NSAID, suggesting a novel strategy for the prevention or treatment of colorectal cancer. [Mol Cancer Ther 2005;4(1):51–59]

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## Introduction

Epidemiologic studies have consistently shown that frequent and prolonged use of nonsteroidal anti-inflammatory drug (NSAID) can reduce the incidence of colorectal adenomas and carcinomas (reviewed in ref. 1). Furthermore, the NSAID sulindac has been shown to regress colorectal polyps in patients with familial adenomatous polyposis (2). Sulindac is a pro-drug that is metabolized *in vivo* to its sulfide and sulfone derivatives. Only the active sulfide derivative inhibits cyclooxygenase (COX) enzymes that regulate prostaglandin synthesis from arachidonic acid (3). The inducible COX-2 isoform is overexpressed in most human colorectal cancers and has been shown to modulate apoptosis, angiogenesis, and tumor cell invasiveness (reviewed in ref. 1). These effects may underlie its role in tumorigenesis. Sulindac sulfide has been shown to inhibit the growth of cultured colon cancer cells primarily by inducing apoptosis (4, 5). Whereas this drug is a potent inhibitor of COX enzymatic activity, its effect upon COX-2 protein expression remain unknown. COX-2 is regulated by transcriptional and post-transcriptional events (6). Evidence suggests that COX-2 induction can be mediated by mitogen-activated protein kinase (MAPK; refs. 7–11). In this regard, MAPK pathways [ERK, p38, and c-Jun NH<sub>2</sub>-terminal kinase (JNK)] have been shown to be involved in COX-2 induction in a stimulus- and cell type-specific manner. However, limited and conflicting data exist regarding the relationship of MAPKs with COX-2 induction in NSAID-treated tumor cells (12–14). Furthermore, the role of MAPK pathways in relation to NSAID-induced apoptosis remains unknown.

The MAPK family is a well-characterized signaling pathway regulating cell survival and cell death (15). The MAPK family comprises extracellular signal-regulated kinase (ERK<sup>p44/42</sup>), p38 MAPK, and JNK. MAPK subfamilies can be activated by a variety of extracellular stimuli and recent data suggest that MAPKs may mediate apoptotic signaling induced by antineoplastic drugs (16). The ERK pathway is activated by various stimuli, including growth factors (17), lipopolysaccharide (18), and chemotherapeutic agents (19, 20). Microtubule or actin-interfering agents were found to stimulate MAPK signaling and AP-1 activity, which led to increased COX-2 expression in mammary epithelial cells (21). ERK<sup>p44/42</sup> activation is associated with cell proliferation, differentiation, and cell survival. In contrast, p38 MAPK and JNK/stress-activated protein kinase are activated in response to cellular stress (induced by UV irradiation, chemotherapeutic agents, and pro-inflammatory cytokines) and are involved in growth arrest and, in some contexts, apoptosis (22–24).

We (25) and others (26) have shown that sulindac sulfide can increase the level of membrane death receptor 5 and can activate caspase-8 to engage the extrinsic apoptotic pathway. Furthermore, sulindac sulfide can trigger the intrinsic mitochondrial apoptotic pathway with cytosolic release of cytochrome *c* (27). Studies have also shown that sulindac can induce apoptosis *in vivo*. In this regard, inhibition of intestinal tumorigenesis in animal models is accompanied by increases in the extent of enterocyte apoptosis (28, 29). Prolonged treatment with sulindac or celecoxib are also associated with modulation of apoptosis in colorectal epithelial from familial adenomatous polyposis patients (30, 31). However, the signal transduction pathways used by sulindac to trigger apoptosis, including the potential role of MAPKs, remain unknown. Whereas sulindac inhibits COX enzymatic activity, several studies have shown that NSAIDs exert their antiproliferative and proapoptotic effects by a COX-independent mechanism (1, 27, 32).

In this study, we examined the role of ERK<sup>P44/42</sup> and p38 MAPKs in the regulation of sulindac sulfide-induced COX-2 expression in human colon cancer cells. The influence of MAPK signaling upon apoptosis induction by this NSAID was also studied. Specifically, we determined the effects of selective inhibitors of MEK1/ERK<sup>P44/42</sup> (PD98059) and p38 (SB203580) upon alterations in cell viability and apoptosis induced by sulindac sulfide.

## Materials and Methods

### Cell Culture

HCA-7 cells (obtained from S. Kirkland, United Kingdom) and HCT-15 (American Type Culture Collection, Gaithersburg, MD) human colon cancer cell lines were grown in RPMI 1640 (Life Technologies, Gaithersburg, MD), supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin, 100 µg/mL streptomycin and maintained at 37°C in a humidified atmosphere of 95% air and 5% CO<sub>2</sub>. HCA-7 cells are known to produce significant quantities of prostaglandin E<sub>2</sub> (33). Cells were seeded at a density of 3 × 10<sup>6</sup> cells/100-mm dish and incubated in serum-free medium with 1 mg bovine serum albumin/mL for 24 hours before drug treatment. Cells were treated with sulindac sulfide (Sigma, St. Louis, MO) at the specified times and dosages (0–160 µmol/L). The drug was dissolved in 100% DMSO and then diluted for experiments with the final concentration of DMSO maintained at 0.1%. Cells were also treated with NS398 (Cayman Chemical, Ann Arbor, MI) for 3 hours. After 24 hours, fresh medium was added containing drug(s) for analysis of cell viability or apoptosis. For experiments using the p38 MAPK/ERK kinase (MEK) inhibitor SB203580 (Calbiochem, La Jolla, CA) or the MEK1 inhibitor, PD098059 (Calbiochem), HCA-7 cells were maintained in serum-free media for 24 hours, then treated with either inhibitor for the indicated dosages and times alone or before the addition of sulindac sulfide.

### 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay

Cells were seeded in 96-well plates at a density of 2 × 10<sup>4</sup> and allowed to attach overnight. The cells were then treated with sulindac sulfide (120 µmol/L) for 48 hours alone or after preincubation for 20 minutes with PD98059 or SB203580 (both at 2.5–10 µmol/L). Medium was then replaced with 100 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (Sigma) at a final concentration of 1 mg per mL per well. After incubation at 37°C for 4 hours, the supernatant was aspirated, and 100 µL of 10% SDS was added to each well and incubated for 8 hours at 37°C. Absorbance was measured at wavelength of 75 nm using a microplate reader.

### Terminal Deoxynucleotidyl Transferase – Mediated Nick End Labeling Assay

Cultured cells were treated for 48 hours with sulindac sulfide (0–160 µmol/L). DNA strand breaks, consistent with apoptosis, were detected by the terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay (*In situ* Cell Death Detection kit, Roche Diagnostics, Mannheim, Germany). In separate experiments, cells were incubated with sulindac sulfide (120 µmol/L) in the presence or absence of PD98059 (0–10 µmol/L) or SB203580 (0–10 µmol/L). Both floating and attached cells were harvested and then fixed using 4% paraformaldehyde in PBS (pH 7.4) for 1 hour at room temperature. Cells were resuspended in 100 µL of 0.1% Triton X-100 in 0.1% sodium citrate for 2 minutes on ice for permeabilization and then labeled with fluorescein dUTP and stained with propidium iodide, according to the manufacturer's instructions. Thereafter, the cells were analyzed for apoptosis using a FACScan flow cytometer (Epics Profile; Coulter Co., Hialeah, FL).

### Immunoblot of MAPK Phosphorylation

Cells were washed with cold PBS and suspended in a lysis buffer [20 mmol/L HEPES (pH 7.5), 100 mmol/L NaCl, 10% glycerol, 1% Triton X-100, 1 mmol/L EGTA (pH 7.5), 0.1 mmol/L Na<sub>3</sub>VO<sub>4</sub>, 1 µg/mL aprotinin, 1 µg/mL leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride] on ice for 20 minutes, then cell debris was removed by centrifugation at 16,000 rpm for 15 minutes. The protein concentration in cell lysates was determined using a Bradford protein assay (Bio-Rad, Richmond, CA). Samples containing 50 µg of protein were added to SDS-PAGE loading buffer with 5% β-mercaptoethanol, heated to 95°C for 5 minutes, and loaded onto 10% polyacrylamide gels. Proteins were then transferred to nitrocellulose membranes. After blocking the blot with 5% milk in PBS containing 0.1% Tween 20, the membrane was incubated for 1 hour at room temperature with an anti-COX-2 (1:1,000; Cayman Chemical) and anti-β-actin (1:3,000; Sigma) monoclonal antibodies. Membranes were incubated with highly specific antibodies against total MEK (1:1,000), ERK<sup>P44/42</sup> (1:2,000) or p38 (1:1,000). For analysis of phosphorylated MAPKs, membranes were blocked with 5% nonfat milk in TBST [10 mmol/L Tris (pH 7.4), 100 mmol/L NaCl, 0.5% Tween 20] and then probed for 1 hour with phosphospecific antibodies against p-MEK (1:1,000), phospho-ERK Thr202/Tyr204 (1:1,000), phospho-p38

(1:1,000) for 1 hour (all MAPK antibodies from Cell Signaling, Beverly, MA). Blots were then incubated with secondary antibodies conjugated with peroxidase (Bio-Rad) for 1 hour at room temperature. The signal was detected by chemiluminescence using the enhanced chemiluminescence detection kit (Amersham, Arlington Heights, IL).

#### COX-2 Antisense Oligonucleotides

HCA-7 cells ( $3 \times 10^5$ ) in 2 mL of RPMI 1640 were plated in six-well tissue culture plates (Corning Costar Co., Cambridge, MA). Twenty-four hours later, cells were cotransfected with 20  $\mu$ g COX-2 antisense oligodeoxynucleotide [(GGAAACATCGACAGT); Sigma Genosys; TX] and 10  $\mu$ g of pcDNA1 vector containing a neomycin-resistant marker (Invitrogen Co., Carlsbad, CA) using 100  $\mu$ L LipofectAMINE reagent according to the vendor's instructions. Sixteen hours posttransfection, the cells were selected in RPMI 1640 containing 500  $\mu$ g/mL geneticin (Life Technologies) and 1 mg bovine serum albumin/mL for 24 hours. Cells were incubated in the presence or absence of sulindac sulfide (80  $\mu$ mol/L) for 48 hours. Cells were then harvested and analyzed for COX-2 expression by immunoblotting or apoptosis by the TUNEL assay.

## Results

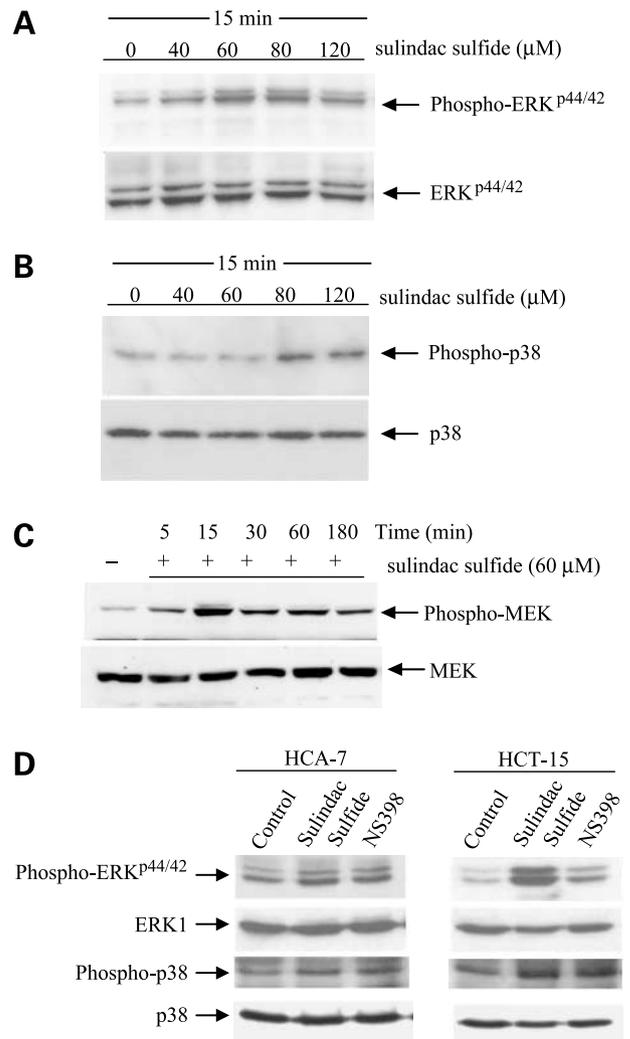
### Sulindac Sulfide Activates MEK, ERK<sup>P44/42</sup>, and p38 MAPKs

Using antibodies that recognize their phosphorylated forms, we detected a low level of MEK, ERK<sup>P44/42</sup>, and p38 MAPK activation in serum starved HCA-7 human colon cancer cells, consistent with their constitutive activation (Fig. 1A–C). Treatment of these cells with sulindac sulfide (0–120  $\mu$ mol/L) for up to 24 hours produced early and sustained activation of ERK (p42 and p44) that was detected at 15 minutes (Fig. 1A and B) and persisted at 3 and 24 hours following incubation with drug (Fig. 2). Activation occurred at all doses of sulindac sulfide tested and at all time points. Total ERK<sup>P44/42</sup> levels (unphosphorylated and phosphorylated) were essentially unchanged at 15 minutes, 3, and 24 hours time points (Figs. 1A and 2). Activation of p38 MAPK by sulindac sulfide occurred at 15 minutes at a threshold dose of 80  $\mu$ mol/L, whereas 40  $\mu$ mol/L was effective at longer incubations (3 and 24 hours; Figs. 1B and 2). Total p38 levels were unchanged to slightly increased at 24 hours. Similar to sulindac sulfide, the selective COX-2 inhibitor NS398 activated phospho-ERK<sup>P44/42</sup> and p38 in HCA-7 cells (Fig. 1D). In a separate experiment, we determined whether sulindac sulfide could alter MEK phosphorylation, the upstream activator of ERK<sup>P44/42</sup>. Sulindac sulfide treatment produced an early activation of MEK that was maximal at a 15 minutes and sustained at 3 hours (Fig. 1C). Total MEK levels were unaffected by drug treatment. This result indicates that sulindac sulfide activates MEK upstream of ERK<sup>P44/42</sup>. To exclude the possibility that the effects upon MAPKs were cell line dependent, HCT-15 colon cancer cells were treated with sulindac sulfide and the selective COX-2 inhibitor NS398.

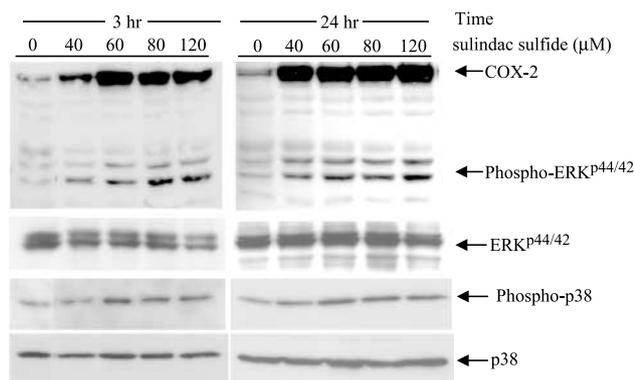
In HCT-15 cells, both NSAIDs activated ERK<sup>P44/42</sup> and the increase in phosphorylation was greater with sulindac sulfide than with NS398, although similar levels of p38 MAPK activation were observed (Fig. 1D). NSAID treatment did not alter the levels of ERK1 or total p38 proteins.

### Sulindac Sulfide Induces COX-2 Expression by Activation of ERK<sup>P44/42</sup> and p38 MAPKs

Treatment of serum starved HCA-7 cells with sulindac sulfide (0–120  $\mu$ mol/L) was found to induce COX-2



**Figure 1.** Sulindac sulfide activates MEK, ERK<sup>P44/42</sup>, and p38 MAPK in human colon carcinoma cells. Cultured HCA-7 cells were treated with sulindac sulfide at the indicated concentrations and times. Cells were then harvested and lysed, and equal aliquots of extracted protein were analyzed for phospho-ERK<sup>P44/42</sup> and total ERK (A), phospho-p38 and total p38 (B), and phospho-MEK and total MEK (C) expression by immunoblotting. Incubation of cells with sulindac sulfide induces an early, dose-dependent phosphorylation of ERK<sup>P44/42</sup> and p38 MAPK without affecting total ERK<sup>P44/42</sup> or p38 levels. Phosphorylation of upstream MEK by sulindac sulfide was time dependent and maximal at 15 min. after incubation with drug. Total MEK levels were unchanged by treatment. D, sulindac sulfide (80  $\mu$ mol/L) and the selective COX-2 inhibitor NS398 (200  $\mu$ mol/L) activate ERK<sup>P44/42</sup> and p38 MAPK in HCA-7 cells and in the COX-2-deficient HCT-15 colon cancer cell line at 3 h.

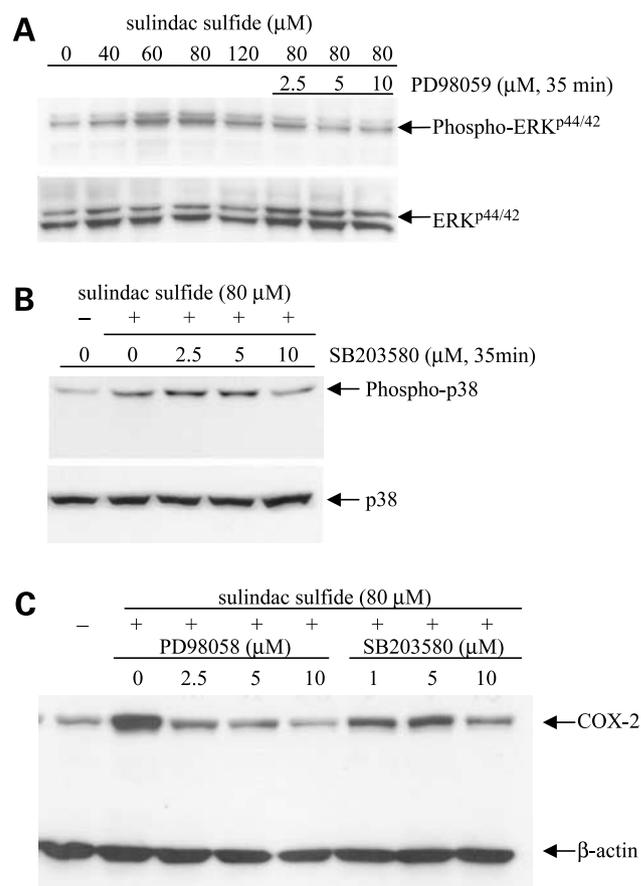


**Figure 2.** COX-2 induction accompanies the activation of ERK<sup>p44/42</sup> and p38 MAPK by sulindac sulfide. A marked induction of COX-2 (72 kDa) protein expression is seen at 3 and 24 h postincubation with sulindac sulfide. Whereas COX-2 induction is modulated by drug dose at 3 h, a high level of sustained induction is observed at 24 h, irrespective of dose. ERK<sup>p44/42</sup> and p38 MAPK activation accompany COX-2 induction at both time points without appreciable changes in total ERK<sup>p44/42</sup> or p38. Phospho-p38 MAPK activation is dose dependent at both time points.

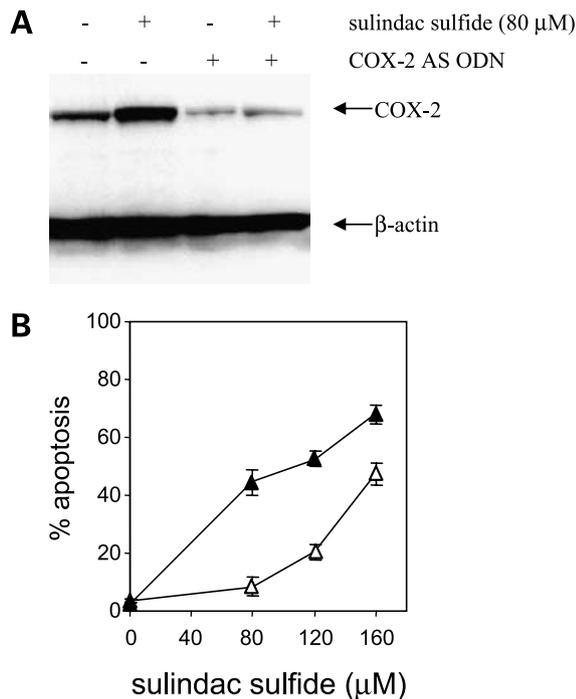
protein expression in a dosage- and time-dependent manner (Fig. 2). COX-2 induction occurred at 3 hours postincubation with drug and was sustained at 24 hours for all doses relative to untreated cells (Fig. 2). In contrast to sulindac sulfide, treatment of HCA-7 cells with 5-fluorouracil or cisplatin failed to induce COX-2 expression (data not shown). On these same blots, phosphorylated and total ERK<sup>p44/42</sup> and p38 MAPK expression were analyzed. As shown in Fig. 2, activation of these MAPKs accompanied COX-2 induction by sulindac sulfide. To confirm that COX-2 induction was indeed mediated by MAPKs, we used the MEK1 inhibitor PD98059 and the p38 MAPK inhibitor SB203580. Initially, we verified that these MAPK inhibitors could block sulindac sulfide-induced ERK<sup>p44/42</sup> and p38 activation. As shown by immunoblotting, pretreatment of HCA-7 cells with PD98059 (2.5–10 μmol/L) or SB203580 (2.5–10 μmol/L) resulted in a dose-dependent inhibition of sulindac sulfide-induced ERK<sup>p44/42</sup> or p38 activation, respectively (Fig. 3A and B). Moreover, both PD98059 and SB203580 were found to inhibit sulindac sulfide-induced COX-2 expression (3 hours, Fig. 3C). PD98059 was a more potent inhibitor of sulindac sulfide-induced COX-2 than was SB203580, yet both inhibitors at 10 μmol/L restored COX-2 to its level of constitutive expression. Together, these data show that ERK<sup>p44/42</sup> and p38 MAPK activation mediate the induction of COX-2 expression by sulindac sulfide in HCA-7 cells.

As shown above, treatment of HCA-7 cells with sulindac sulfide induced COX-2 protein expression via activation of MAPKs. Forced expression of COX-2 has been associated with apoptosis resistance *in vitro* (25, 27, 34). Therefore, we determined the effect of COX-2 suppression upon the apoptotic susceptibility of HCA-7 cells to sulindac sulfide. HCA-7 cells were transfected with COX-2 antisense oligonucleotides (COX-2 AS ODN).

COX-2 AS ODN blocked both constitutive and sulindac sulfide-induced COX-2 expression, as shown by immunoblotting (Fig. 4A). Furthermore, the dose-dependent induction of apoptosis (TUNEL assay) by sulindac sulfide was significantly augmented in HCA-7 cells transfected with the COX-2 AS ODN, compared with untransfected cells (Fig. 4B). These data suggest that constitutive COX-2 expression in HCA-7 cells confers resistance to sulindac sulfide-induced apoptosis. This finding is consistent with our previous data demonstrating that ectopic COX-2 expression in the COX-2-deficient HCT-15 cell line attenuates mitochondrial and death receptor-mediated apoptotic signaling triggered by sulindac sulfide (25, 27). Given that sulindac sulfide is a potent inhibitor of COX



**Figure 3.** Pharmacologic inhibitors of MEK/ERK (PD98059) and p38 MAPK (SB203580) inhibit sulindac sulfide-induced ERK<sup>p44/42</sup> and p38 activation, as well as COX-2 expression. **A** and **B**, HCA-7 cells were serum starved for 24 h, then treated with the indicated concentrations of PD98059 or SB203580 for 35 min. Cells were then incubated with sulindac sulfide at the indicated concentrations for 15 min before harvesting. Cell lysates were analyzed by immunoblotting using ERK<sup>p44/42</sup> or p38 antibodies to detect phosphorylated and unphosphorylated proteins. PD98059 (**A**) and SB203580 (**B**) dose-dependently inhibited sulindac sulfide-induced ERK<sup>p44/42</sup> and p38 phosphorylation, respectively. **C**, both PD98059 and SB203580 were found to inhibit sulindac sulfide-induced COX-2 expression in a dose-dependent manner.  $\beta$ -actin, control for protein loading.



**Figure 4.** COX-2 antisense oligonucleotides inhibit endogenous and stimulated COX-2 expression and increase the apoptotic susceptibility of HCA-7 cells to sulindac sulfide. **A**, transfection of HCA-7 cells with COX-2 antisense oligodeoxynucleotide (COX-2 AS ODN) was shown to suppress constitutive and sulindac sulfide-induced COX-2 protein expression. **B**, HCA-7 cells transfected with COX-2 AS ODN show a significant increase in their apoptotic susceptibility to sulindac sulfide relative to untransfected cells. Apoptosis was determined by the TUNEL assay. Points, means of triplicate observations; bars,  $\pm$ SD.

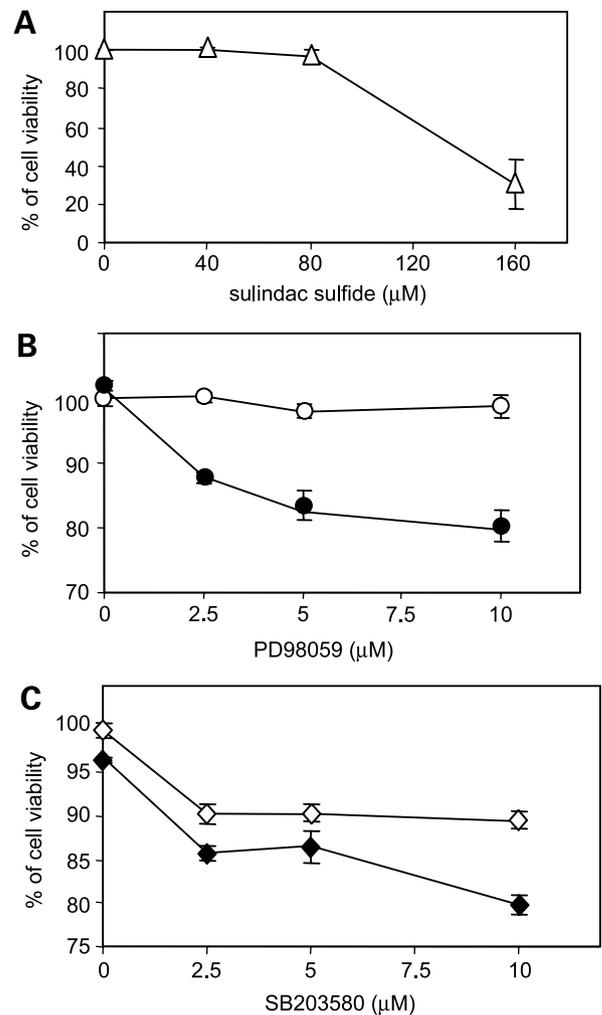
enzymatic activity and prostaglandin synthesis, the inhibitory effect of COX-2 upon apoptotic susceptibility is prostaglandin independent. These *in vitro* data suggest that COX-2 protein expression may contribute to tumor cell resistance to apoptotic doses of NSAIDs and accordingly, may reduce the effectiveness of these drugs for colorectal cancer prevention and treatment.

#### Pharmacologic Inhibitors of ERK<sup>P44/42</sup> and p38 Activation Potentiate Sulindac Sulfide-Induced Growth Inhibition and Apoptosis

NSAIDs, including sulindac sulfide, inhibit the growth of cultured human colon cancer cells mainly through induction of apoptosis (4, 5). However, the signaling pathways involved in NSAID-induced apoptosis remain unclear. We determined the role of ERK<sup>P44/42</sup> and p38 MAPK activation in mediating sulindac sulfide-induced growth inhibition and apoptosis induction in HCA-7 cells. The effect of sulindac sulfide (0–160  $\mu$ mol/L) upon HCA-7 cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. Next, cells were incubated with the MEK1/ERK<sup>P44/42</sup> inhibitor PD098059 or the p38 inhibitor SB203580 alone or in combination with sulindac sulfide (80  $\mu$ mol/L) for 48 hours (Fig. 5). Whereas PD98059 (2.5–10  $\mu$ mol/L) did not

reduce cell viability, SB203580 (2.5–10  $\mu$ mol/L) reduced the viable cell population by 10% and this effect was maximal at the lowest dose studied. At a dose up to 80  $\mu$ mol/L, sulindac sulfide alone did not appreciably alter cell viability; however, its combination with PD98059 or SB203580 resulted in a significant dose-dependent reduction in cell viability compared with either drug alone (Fig. 5).

Evidence indicates that both ERK<sup>P44/42</sup> and p38 can mediate extracellular stimulus-induced apoptosis (24, 35, 36), although these MAPKs have been shown to exert both antiapoptotic and proapoptotic effects depending



**Figure 5.** Inhibition of MAPKs potentiates the reduction in cell viability induced by sulindac sulfide. **A**, sulindac sulfide ( $\Delta$ ) treatment (48 h) reduced HCA-7 cell viability in a dose-dependent manner, as shown using the MTT assay. **B** and **C**, cells were pretreated (20 min) with PD98059 ( $\circ$ ) or SB203580 ( $\diamond$ ) at the indicated concentrations alone and in combination with sulindac sulfide (80  $\mu$ mol/L;  $\bullet$ ,  $\blacklozenge$ ) for 48 h. Neither PD98059 nor sulindac sulfide reduced cell viability alone (**B**); however, their coadministration produced a significant reduction in cell viability. SB203580 reduced cell viability as a single agent and this effect was significantly augmented by the addition of sulindac sulfide (**C**). Points, means for triplicate experiments; bars, SD.

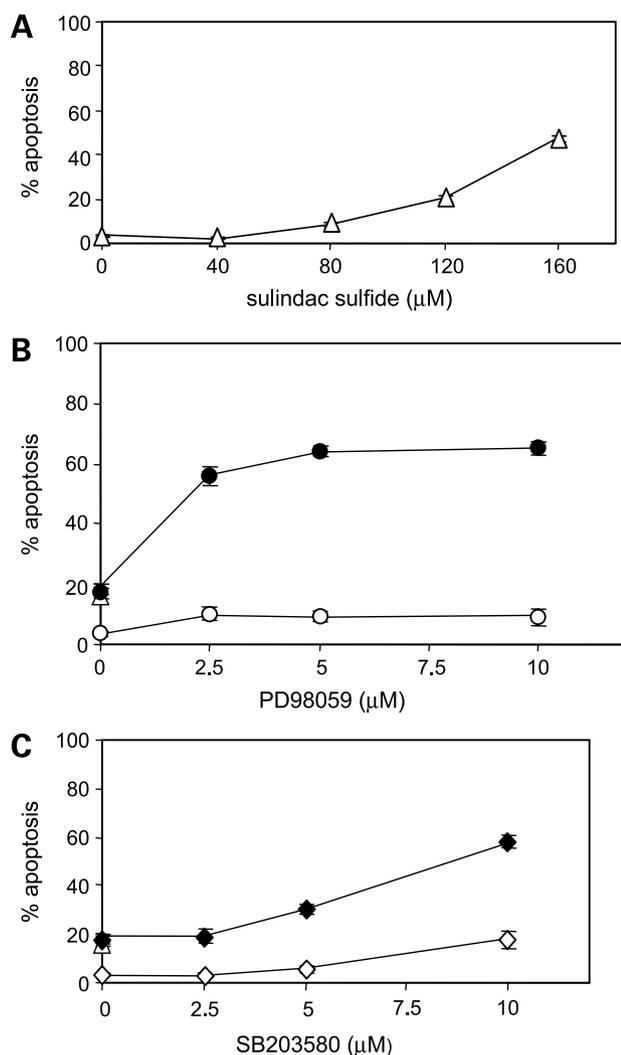
upon the cellular context (19, 37–40). HCA-7 cells were pretreated with increasing concentrations of PD98059 or SB203580 alone or in combination with sulindac sulfide (120  $\mu\text{mol/L}$ , 48 hours). By themselves, these inhibitors exerted only a modest proapoptotic effect as shown by the TUNEL assay (Fig. 6). Pretreatment of cells with PD98059 or SB203580 augmented sulindac sulfide-induced apoptosis  $\sim 3$ -fold compared with that produced by sulindac sulfide alone (Fig. 6). PD98059 was a more potent modulator of sulindac sulfide-induced apoptosis than was SB203580. These data indicate that sulindac sulfide-

induced activation of ERK<sup>P44/42</sup> and p38 MAPKs promote the survival of HCA-7 cells. As shown here, selective inhibitors of these MAPKs potentiate sulindac sulfide-induced growth inhibition and apoptosis induction.

## Discussion

We examined the role of the MAPK pathway in the regulation of COX-2 expression and apoptosis induction by sulindac sulfide. HCA-7 cells were chosen for study as they constitutively express the inducible COX-2 isoform and produce abundant PGE<sub>2</sub> (33). We found that sulindac sulfide induced COX-2 protein expression and that this effect was mediated by the early and sustained activation of MEK/ERK<sup>P44/42</sup> and p38 MAPKs without changes in total MAPK levels. Additionally, the selective COX-2 inhibitor NS398 similarly activated ERK<sup>P44/42</sup> and p38 in HCA-7 cells, and both NSAIDs also activated these MAPKs in COX-2 deficient HCT-15 (25) colon cancer cells. Pharmacologic inhibitors of MEK/ERK<sup>P44/42</sup> (PD98059) and p38 (SB203580) were found to prevent activation of these MAPKs and to block COX-2 induction by sulindac sulfide in a dose-dependent manner in HCA-7 cells. Together, our data suggest that MAPK activation is sufficient to induce COX-2 expression in human colon cancer cells. Activation of the ERK pathway has been shown to be involved in the transcriptional and post-transcriptional regulation of COX-2 expression (41). Specifically, ERK<sup>P44/42</sup> inhibition reduced COX-2 promoter activity and the stability of the COX-2 3'-untranslated region (6). Activated ERK<sup>P44/42</sup> can directly phosphorylate transcription factors including AP-1 complexes that have been shown to transactivate the COX-2 promoter (42). Our results are supported by data showing that COX-2 induction by transforming growth factor  $\alpha$  or IFN  $\gamma$  require activation of both the ERK<sup>P44/42</sup> and p38 MAPK pathways in human epidermal keratinocytes and squamous carcinoma cells (43). Furthermore, Yoon et al. (44) showed that bile acid stimulation of human cholangiocarcinoma cells induces COX-2 expression and involves both ERK<sup>P44/42</sup> and p38 MAPK pathways, but not JNK. Consistent with our *in vitro* results, exposure of *Apc* mutant min mice to NSAIDs for a 3- to 12-week period was shown to increase COX-2 and PGE<sub>2</sub> levels in colorectal epithelia.<sup>3</sup>

There have been conflicting reports as to the effect of NSAIDs on ERK<sup>P44/42</sup> activation. Whereas some studies have also shown that NSAIDs can activate ERK<sup>P44/42</sup> (11, 12, 45) in epithelial cancer cells, another (46) study found that sulindac sulfide inhibits ERK<sup>P44/42</sup> activation in COX-2-deficient HCT116 colon cancer cells. Experiments in the latter study were conducted in the presence of serum and resultant high basal levels of ERK activation (46), in contrast to our data in serum-starved cells. Sulindac sulfide has been reported to inhibit Ras/Raf signaling (47);



**Figure 6.** PD98059 and SB203580 potentiate sulindac sulfide-induced apoptosis in HCA-7 cells. **A**, sulindac sulfide ( $\Delta$ ) induced apoptosis in a dose-dependent manner in HCA-7 cells, as shown using the TUNEL assay (see Materials and Methods). **B**, **C**, cells were pretreated with PD98059 ( $\circ$ ) or SB203580 ( $\diamond$ ) at the indicated concentrations for 20 min and grown in culture or incubated with sulindac sulfide (120  $\mu\text{mol/L}$ ) for 48 h. Cells were then harvested and analyzed for apoptosis. **B**, the combination ( $\bullet$ ) of PD98059 and sulindac sulfide markedly increased apoptosis induction relative to either drug alone. **C**, SB203580 in combination ( $\blacklozenge$ ) with sulindac sulfide augmented apoptosis in a dose-dependent manner. Points, means triplicate experiments; bars, SD.

<sup>3</sup> M. Bertagnolli, unpublished data.

however, its relationship to downstream ERK<sup>P44/42</sup> is complex. Activated *Ki-Ras*-mediated signals were shown to be involved in the PKC-dependent SEK1-JNK pathway, but not in the MEK1/2/ERK pathway in human colon cancer cells (48). Other studies also indicate that ERK<sup>P44/42</sup> activation can occur independently of *ras* (49, 50). Effects of NSAIDs upon MAPK activation may also reflect the cellular context as well as cell type-specific differences. NSAIDs have also been shown to activate peroxisome proliferator-activated receptors (PPAR; ref. 51). Acting as PPAR activators, various NSAIDs were found to induce COX-2 expression in epithelial cells through interaction with a peroxisome proliferator response element in the COX-2 promoter (52). PPARs use a wide variety of protein-protein interactions to regulate transcription of target genes. PPAR- $\alpha$  and PPAR- $\gamma$  ligands have recently been shown to induce activation of ERK<sup>P44/42</sup> and p38 MAPKs, which in turn phosphorylate PPARs, thereby affecting transcriptional activity (53). In this regard, Gardner et al. (53) showed that various PPAR- $\alpha$  and PPAR- $\gamma$  agonists rapidly induced ERK<sup>P44/42</sup> and/or p38 phosphorylation in rat liver epithelial cells. MAPK activation by these PPAR agonists was associated with epidermal growth factor receptor transactivation, which is a known regulator of COX-2 (43, 44).

As shown here and in other reports (4, 5), sulindac sulfide reduces cell viability and induces apoptosis which may be critical to its antitumor effects. To date, the mechanisms underlying NSAID-induced apoptosis remain unclear. We determined the role of the MAPK pathway in modulating the effects of sulindac sulfide on cell viability and apoptosis in HCA-7 cells. Whereas treatment of HCA-7 cells with PD098059 had no effect upon cell viability or apoptosis, the combination of PD098059 and sulindac sulfide was found to significantly reduce cell viability and to augment apoptosis induction compared with sulindac sulfide alone. These data indicate that inhibition of ERK<sup>P44/42</sup> increases apoptotic susceptibility in these cells (24, 54). The mechanism by which ERK inhibits apoptosis is poorly understood. Studies have shown that ERK<sup>P44/42</sup> acts downstream of B-Raf to inhibit caspase activation following mitochondrial cytochrome *c* release (55). In this manner, ERK<sup>P44/42</sup> could block the effects of sulindac sulfide on the mitochondrial apoptotic pathway. ERK<sup>P44/42</sup> may also phosphorylate and inactivate the proapoptotic Bcl-2 family member Bad (56). Furthermore, ERK<sup>P44/42</sup>-mediated induction of COX-2 expression, as shown here, may contribute to apoptosis resistance as discussed below. We found that the p38 MAPK inhibitor, SB203580, exerted a modest effect upon cell viability and apoptosis in HCA-7 cells. However, pretreatment with SB203580 significantly reduced cell viability and augmented apoptosis induction by sulindac sulfide. Similarly, Roulston et al. (57) found that inhibition of tumor necrosis factor-induced p38 and JNK kinases, using SB203580 or MKK4/MKK6 mutants, increased tumor necrosis factor-induced apoptosis (57). p38 MAPK activation has generally been associated with cellular stress and enhanced apoptosis (24, 58, 59); however, the role of MAPK

casades in the regulation of apoptosis can depend upon the cell type, its state of activation, and the cellular context. Accordingly, MAPKs have been shown to function in either a proapoptotic or antiapoptotic capacity.

To further define the role of COX-2 in apoptotic susceptibility, we transfected HCA-7 cells with COX-2 antisense oligonucleotides. Antisense oligos were found to suppress both constitutive and sulindac sulfide-induced COX-2 protein expression. Moreover, cells transfected with COX-2 antisense oligos showed a significant and dose-dependent increase in sulindac sulfide-induced apoptosis relative to untransfected cells. Since sulindac sulfide is a potent inhibitor of COX enzymatic activity (3), the antiapoptotic effect of COX-2 seems to be prostaglandin-independent as was previously reported by our laboratory (25, 27), and others (34, 60). Our results are consistent with studies showing that forced COX-2 expression (25, 27, 34) or its induction by growth factors (61) can confer apoptosis resistance. Studies indicate that COX-2 mediated apoptosis resistance is related to Bcl-2 induction (25, 34, 62), survivin expression (63), and transcriptional repression of membrane death receptor 5 that can be restored by sulindac sulfide (25, 64). COX-2 inhibition has been shown to down-regulate c-IAP-2 (an inhibitor of caspase enzymatic activity) and to promote apoptosis, both of which were reversed by exogenous PGE<sub>2</sub> (65). Whereas further studies are needed to determine *in vivo* relevance of these findings, it is plausible that such mechanisms contribute to the frequent resistance of COX-2 overexpressing colorectal tumors from familial adenomatous polyposis patients to NSAID treatment (2, 66). Concentrations of sulindac sulfide required to induce apoptosis in short-term cell culture experiments exceed those required to inhibit its known biochemical target (i.e., COX-1 and COX-2 enzymatic activity). However, we used doses that have been shown by multiple laboratories to exert relevant biological effects on colon cancer cell growth and apoptosis. Measurements of the sulfide metabolite in the plasma of humans indicate that up to 50  $\mu\text{mol/L}$  is achievable depending upon dose and schedule (67). However, sulindac sulfide is concentrated in the colonic epithelium to levels that are at least 20-fold higher than those achieved in serum (68).

In summary, we found that sulindac sulfide can activate the MAPK pathway to induce COX-2 expression. Selective inhibitors of the MEK1/ERK<sup>P44/42</sup> and p38 MAPK signaling pathways inhibit sulindac sulfide-induced COX-2 expression and significantly augment apoptosis induction by this NSAID. These findings establish a mechanistic link between the MAPK pathway and NSAID-induced COX-2 expression and apoptosis. Furthermore, the combination of MAPK inhibitor and a NSAID may represent a novel strategy for the prevention and treatment of colon cancer.

## References

1. Sinicrope FA, Gill S. Role of cyclooxygenase-2 in colorectal cancer. *Cancer Metastasis Rev* 2004;23:63–75.
2. Giardiello FM, Hamilton SR, Krush AJ, et al. Treatment of colonic and

- rectal adenomas with sulindac in familial adenomatous polyposis. *N Engl J Med* 1993;328:1313–16.
3. Duggan DE, Hooke KF, Rislely EA, Shen TY, Arman CG. Identification of the biologically active form of sulindac. *J Pharmacol Exp Ther* 1977;201:8–13.
  4. Piazza GA, Rahm AL, Krutzsch M, et al. Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis. *Cancer Res* 1995;55:3110–6.
  5. Shiff SJ, Qiao L, Tsai LL, Rigas B. Sulindac sulfide, an aspirin-like compound, inhibits proliferation, causes cell cycle quiescence, and induces apoptosis in HT-29 colon adenocarcinoma cells. *J Clin Invest* 1995;96:491–503.
  6. Shao J, Sheng H, Inoue H, Morrow JD, DuBois RN. Regulation of constitutive cyclooxygenase-2 expression in colon carcinoma cells. *J Biol Chem* 2000;275:3395–6.
  7. Ridley SH, Sarsfield SJ, Lee JC, et al. Actions of IL-1 are selectively controlled by p38 mitogen-activated protein kinase: regulation of prostaglandin H synthase-2, metalloproteinases, and IL-6 at different levels. *J Immunol* 1997;158:3165–73.
  8. Pouliot M, Baillargeon J, Lee JC, Cleland LG, James MJ. Inhibition of prostaglandin endoperoxide synthase-2 expression in stimulated human monocytes by inhibitors of p38 mitogen-activated protein kinase. *J Immunol* 1997;158:4930–7.
  9. Niiro H, Otsuka T, Ogami E, et al. MAP kinase pathways as a route for regulatory mechanisms of IL-10 and IL-4 which inhibit COX-2 expression in human monocytes. *Biochem Biophys Res Commun* 1998;250:200–5.
  10. Wikstrom K, Ohd JF, Sjolander A. Regulation of leukotriene-dependent induction of cyclooxygenase-2 and Bcl-2. *Biochem Biophys Res Commun* 2003;302:330–5.
  11. Elder DJE, Halton DE, Playle LC, Paraskeva C. The MEK/ERK pathway mediates COX-2-selective NSAID-induced apoptosis and induced COX-2 protein expression in colorectal carcinoma cells. *Int J Cancer* 2002;99:323–7.
  12. Kim TI, Jin SH, Kim WH, et al. Prolonged activation of mitogen-activated protein kinases during NSAID-induced apoptosis in HT-29 colon cancer cells. *Int J Colorectal Dis* 2001;16:167–73.
  13. Xu XM, Sansores-Garcia L, Chen XM, Matijevic-Aleksic N, Du M. Suppression of inducible cyclooxygenase 2 gene transcription by aspirin and sodium salicylate. *Proc Natl Acad Sci U S A* 1999;96:5292–7.
  14. Paik JH, Ju JH, Lee JY, Boudreau MD, Hwang DH. Two opposing effects of non-steroidal anti-inflammatory drugs on the expression of the inducible cyclooxygenase. *J Biol Chem* 2000;275:28173–9.
  15. Reddy KB, Nabha SM, Atanaskova N. Role of MAP kinase in tumor progression and invasion. *Cancer Metastasis Rev* 2003;22:395–403.
  16. Fan M, Chambers TC. Role of mitogen-activated protein kinases in the response of tumor cells to chemotherapy. *Drug Resist Updat* 2001;5:253–67.
  17. McCawley LJ, Li S, Wattenberg EV, Hudson LG. Sustained activation of the mitogen-activated protein kinase pathway. *J Biol Chem* 1999;274:4347–53.
  18. Foey AD, Parry SL, Williams LM, Feldmann M, Foxwell BM, Brennan FM. Regulation of monocyte IL-10 synthesis by endogenous IL-1 and TNF- $\alpha$ : role of the p38 and p42/44 mitogen-activated protein kinases. *J Immunol* 1998;160:920–8.
  19. Persons DL, Yazlovitskaya EM, Pelling JC. Effect of extracellular signal-regulated kinase on p53 accumulation in response to cisplatin. *J Biol Chem* 2000;275:35778–85.
  20. Lewis TS, Shapiro PS, Ahn NG. Signal transduction through MAP kinase cascades. *Adv Cancer Res* 1998;74:49–139.
  21. Subbaramaiah K, Hart JC, Norton L, Dannenberg AJ. Microtubule-interfering agents stimulate the transcription of cyclooxygenase-2. *J Biol Chem* 2000;275:14838–45.
  22. Graves JD, Draves KE, Craxton A, Saklatvala J, Krebs EG, Clark EA. Involvement of stress-activated protein kinase and p38 mitogen-activated protein kinase in mlgM-induced apoptosis of human B lymphocytes. *Proc Natl Acad Sci U S A* 1996;93:13814–8.
  23. Raingeaud J, Gupta S, Rogers JS, et al. Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J Biol Chem* 1995;270:7420–6.
  24. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* 1995;270:1326–31.
  25. Tang X, Sun YJ, Half E, Kuo MT, Sinicrope F. Cyclooxygenase-2 overexpression inhibits death receptor 5 expression and confers resistance to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in human colon cancer cells. *Cancer Res* 2002;62:4903–8.
  26. He Q, Luo X, Huang Y, Sheikh MS. Apo2L/TRAIL differentially modulates the apoptotic effects of sulindac and a COX-2 selective non-steroidal anti-inflammatory agent in Bax-deficient cells. *Oncogene* 2002;21:6032–40.
  27. Sun Y, Tang XM, Half E, Kuo MT, Sinicrope FA. Cyclooxygenase-2 overexpression reduces apoptotic susceptibility by inhibiting the cytochrome c-dependent apoptotic pathway in human colon cancer cells. *Cancer Res* 2002;62:6323–8.
  28. Samaha HS, Kelloff GJ, Steele V, Rao CV, Reddy BS. Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate, and 6-phenylhexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. *Cancer Res* 1997;57:1301–5.
  29. Mahmoud NN, Boolbol SK, Dannenberg AJ, et al. The sulfide metabolite of sulindac prevents tumors and restores enterocyte apoptosis in a murine model of familial adenomatous polyposis. *Carcinogenesis* 1998;19:87–91.
  30. Keller JJ, Offerhaus GJ, Polak M, et al. Rectal epithelial apoptosis in familial adenomatous polyposis patients treated with sulindac. *Gut* 1999;45:822–8.
  31. Sinicrope FA, Half E, Morris JS, et al. Cell proliferation and apoptotic indices predict adenoma regression in a placebo-controlled trial of celecoxib in familial adenomatous polyposis (FAP) patients. *Cancer Epidemiol Biomarkers Prev* 2004;13:920–7.
  32. Grosch S, Tegeder I, Niederberger E, Brautigam I, Geisslinger G. COX-2 independent induction of cell cycle arrest and apoptosis by the selective COX-2 inhibitor celecoxib. *FASEB J* 2001;15:2742–4.
  33. Sheng H, Shao J, Kirkland SC, et al. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J Clin Invest* 1997;99:2254–9.
  34. Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 1995;83:493–501.
  35. Qiao D, Stratagouleas ED, Martinez JD. Activation and role of mitogen-activated protein kinases in deoxycholic acid-induced apoptosis. *Carcinogenesis* 2001;22:35–41.
  36. Petrache I, Choi ME, Otterbein LE, et al. Mitogen-activated protein kinase pathway mediates hyperoxia-induced apoptosis in cultured macrophage cells. *Am J Physiol* 1999;277:L589–95.
  37. Bhat NR, Zhang P. Hydrogen peroxide activation of multiple mitogen-activated protein kinases in an oligodendrocyte cell line: role of extracellular signal-regulated kinase in hydrogen peroxide-induced cell death. *J Neurochem* 1999;72:112–9.
  38. Bueno OF, De Windt LJ, Tymitz KM, et al. The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. *EMBO J* 2000;19:6341–50.
  39. Kurokawa H, Lenferink AE, Simpson JF, et al. Inhibition of HER2/*neu* (erbB-2) and mitogen-activated protein kinases enhances tamoxifen action against HER2-overexpressing, tamoxifen-resistant breast cancer cells. *Cancer Res* 2000;60:5887–94.
  40. Remacle-Bonnet MM, Garrouste FL, Heller S, Andre F, Marvaldi JL, Pommier GJ. Insulin-like growth factor-I protects colon cancer cells from death factor-induced apoptosis by potentiating tumor necrosis factor  $\alpha$ -induced mitogen-activated protein kinase and nuclear factor  $\kappa$ B signaling pathways. *Cancer Res* 2000;60:2007–17.
  41. Dean JL, Brook M, Clark AR, Saklatvala J. p38 mitogen activated protein kinase regulates COX-2 mRNA stability and transcription in lipopolysaccharide-treated human monocytes. *J Biol Chem* 1999;274:264–9.
  42. Guo YS, Hellmich MR, Wen XD, Townsend CMJ. Activator protein-1 transcription factor mediates bombesin-stimulated cyclooxygenase-2 expression in intestinal epithelial cells. *J Biol Chem* 2001;276:22941–7.
  43. Matsuura H, Sakaue M, Subbaramaiah K, et al. Regulation of

- cyclooxygenase-2 by interferon  $\gamma$  and transforming growth factor  $\alpha$  in normal human epidermal keratinocytes and squamous carcinoma cells. Role of mitogen-activated protein kinases. *J Biol Chem* 1999;274:29138–48.
44. Yoon J-H, Higuchi H, Werneburg NW, Kaufmann SH, Gores GJ. Bile acids induce cyclooxygenase-2 expression via epidermal growth factor receptor in a human cholangiocarcinoma cell line. *Gastroenterology* 2002;122:985–93.
45. Yip-Schneider MT, Schmidt CM. MEK inhibition of pancreatic carcinoma cells by UO126 and its effects in combination with sulindac. *Pancreas* 2003;27:337–44.
46. Rice PL, Goldberg RJ, Ray EC, Driggers LJ, Ahnen DJ. Inhibition of extracellular signal-regulated kinase 1/2 phosphorylation and induction of apoptosis by sulindac metabolites. *Cancer Res* 2001;61:1541–7.
47. Herrmann C, Block C, Geisen C, et al. Sulindac sulfide inhibits Ras signaling. *Oncogene* 1998;17:1769–76.
48. Okumura K, Shirasawa S, Nishioka M, Sasazuki T. Activated Ki-Ras suppresses 12-*O*-tetradecanoylphorbol-13-acetate-induced activation of the c-Jun NH2-terminal kinase pathway in human colon cancer cells. *Cancer Res* 1999;59:2445–50.
49. Saito T, Okada S, Ohshima K, et al. Differential activation of epidermal growth factor receptor (ErbB1/EGFR) downstream signaling pathways by betacellulin and EGF. *Endocrinology* 2004;145:4232–43.
50. Schmidt EK, Fichelson S, Feller SM. PI3 kinase is important for Ras, MEK and Erk activation of Epo-stimulated human erythroid progenitors. *BMC Biol* 2004;2:7.
51. He TC, Chan TA, Vogelstein B, Kinzler KW. PPAR $\delta$  is an APC-regulated target of nonsteroidal anti-inflammatory drugs. *Cell* 1999;99:335–45.
52. Meade EA, McIntyre TM, Zimmerman GA, Prescott SM. Peroxisome proliferators enhance cyclooxygenase-2 expression in epithelial cells. *J Biol Chem* 1999;274:8328–34.
53. Gardner OS, Dewar BJ, Earp HS, Samet JM, Graves LM. Dependence of peroxisome proliferator-activated receptor ligand-induced mitogen-activated protein kinase signaling on epidermal growth factor receptor transactivation. *J Biol Chem* 2003;278:46261–9.
54. Anderson CN, Tolkovsky AM. A role for MAPK/ERK in sympathetic neuron survival: protection against p53-dependent, JNK-independent induction of apoptosis by cytosine arabinoside. *J Neurosci* 1999;19:664–73.
55. Erhardt P, Schremser EJ, Cooper GM. B-Raf inhibits programmed cell death downstream of cytochrome *c* release from mitochondria by activating the MEK/Erk pathway. *Mol Cell Biol* 1999;19:5308–15.
56. Scheid MP, Duronio V. Dissociation of cytokine-induced phosphorylation of Bad and activation of PKB/akt: involvement of MEK upstream of Bad phosphorylation. *Proc Natl Acad Sci U S A* 1998;95:7439–44.
57. Roulston A, Reinhard C, Amiri P, Williams LT. Early activation of c-Jun N-terminal kinase and p38 kinase regulate cell survival in response to tumor necrosis factor  $\alpha$ . *J Biol Chem* 1998;273:10232–9.
58. Stadheim TA, Xiao H, Eastman A. Inhibition of extracellular signal-regulated kinase (ERK) mediates cell cycle phase independent apoptosis in vinblastine-treated ML-1 cells. *Cancer Res* 2001;61:1533–40.
59. Bacus SS, Gudkov AV, Lowe M, et al. Taxol-induced apoptosis depends on MAP kinase pathways (ERK and p38) and is independent of p53. *Oncogene* 2001;20:147–55.
60. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci U S A* 1997;94:3336–40.
61. Zeng Q, McCauley LK, Wang CY. Hepatocyte growth factor inhibits anoikis by induction of activator protein 1-dependent cyclooxygenase-2. Implication in head and neck squamous cell carcinoma progression. *J Biol Chem* 2002;277:50137–42.
62. Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Res* 1998;58:362–6.
63. Krysan K, Dalwadi H, Sharma S, Pold M, Dubinett S. Cyclooxygenase 2-dependent expression of survivin is critical for apoptosis resistance in non-small cell lung cancer. *Cancer Res* 2004;64:6359–62.
64. Huang Y, He Q, Hillman MH, Rong R, Sheikh MS. Sulindac sulfide-induced apoptosis involves death receptor 5 and the caspase-8-dependent pathway in human colon and prostate cancer cells. *Cancer Res* 2001;61:6918–24.
65. Nishihara H, Kizaka-Kondoh S, Insel PA, Eckmann L. Inhibition of apoptosis in normal and transformed intestinal epithelial cells by cAMP through induction of inhibitor of apoptosis protein (IAP)-2. *Proc Natl Acad Sci U S A* 2003;100:8921–6.
66. Steinbach G, Lynch PM, Phillips RK, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 2000;342:1946–52.
67. Davies NM, Watson MS. Clinical pharmacodynamics of sulindac: a dynamic old drug. *Drug Dispos* 1997;32:437–59.
68. Duggan DE, Hooke KF, Hwang SS. Kinetics of the tissue distributions of sulindac and metabolites. Relevance to sites and rates of bioactivation. *Drug Met Dispos* 1980;8:241–6.

# Molecular Cancer Therapeutics

## Selective inhibitors of MEK1/ERK<sup>44/42</sup> and p38 mitogen-activated protein kinases potentiate apoptosis induction by sulindac sulfide in human colon carcinoma cells

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