Colon Cancer Chemopreventive Drugs Modulate Integrin-mediated Signaling Pathways

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INTRODUCTION

Epidemiological studies of colorectal cancer incidence suggest that the development of this disease can be modulated by dietary factors. Among the micronutrients showing significant efficacy in tumor prevention are polyphenolic antioxidants found in fruits and vegetables. Epidemiological studies also indicate that nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the incidence of colorectal cancer. Integrin-mediated cell-matrix contact provides critical signaling that regulates cellular proliferation, migration, and apoptosis. A signaling mediator for this system is focal adhesion kinase (FAK). Thus far, FAK has not been identified as a target for the inhibitory action of any chemopreventive drug in vivo or in vitro. However, the loss of integrin-mediated cell-matrix contact can induce apoptosis (anoiikis), and effective chemopreventive agents typically increase the rate of enterocyte apoptosis. Therefore, we asked whether the NSAID, sulindac sulfide, and the phenolic antioxidant, caffeic acid phenethyl ester (CAPE), affected FAK expression or tyrosine phosphorylation in human colon carcinoma cells. We show that subapoptotic doses of both sulindac sulfide and CAPE caused a rearrangement of the actin cytoskeleton and consequently the loss of focal adhesion plaques. These drugs also reduced the tyrosine phosphorylation of FAK and an associated factor, p130<sup>SH2</sup>. Steady-state levels of these proteins, together with other relevant signaling molecules, remained unchanged after treatments. Finally, we show that both CAPE and sulindac reduced cell invasion, a functional assay for the inhibition of signaling downstream of FAK.

These data strongly suggest that chemopreventive drugs can regulate FAK activity. In conclusion, these novel studies add modulation of integrin-mediated signaling to the spectrum of activity of NSAIDs and plant phenolics.

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3 The abbreviations used are: ECM, extracellular matrix; FAK, focal adhesion kinase; SH-2, Src homology domain 2; Grb2, growth factor receptor binding protein 2; CAPE, caffeic acid phenethyl ester; FAP, familial adenomatous polyposis; Min<sup>−/−</sup>; c57Bl/6J-Min<sup>−/−</sup>; Apc or APC, adenomatous polyposis coli gene or its product, respectively; Cox, cyclooxygenase; NSAID, nonsteroidal anti-inflammatory drug; FBS, fetal bovine serum; IB, immunoblot; IP, immunoprecipitation.
Previously, we demonstrated the in vivo efficacy of sulindac (26), its metabolite, sulindac sulfide (27), and the phenolic antioxidant, CAPE (28), in preventing intestinal tumors in an animal model for FAP, the Min/+ mouse. The mechanisms by which these agents prevent adenoma formation are not well understood. Several observations, however, suggest that chemopreventive drugs may modulate integrin-mediated signaling. For instance, the loss of integrin-mediated cell-matrix contact can induce anoikis (anoikis; reviewed in Ref. 29). To varying degrees, all of the compounds that are effective in preventing intestinal tumors induce anoikis of intestinal cells, both in cell culture and in vivo (30–33). Furthermore, studies of the Min/+ mouse suggest that some chemopreventive compounds alter intestinal cell migration. For example, when Min/+ mice were treated with chemopreventive doses of sulindac, the migration rate of cells in the tumor-prone small intestine increased by 20–25% (34). Finally, intestinal epithelial cells that overexpress Cox-2 exhibit decreased anoikis and enhanced adhesion to the ECM. These properties were reversed upon culture with sulindac sulfide, which is a potent nonselective Cox inhibitor (35, 36).

As yet, FAK has not been identified as a target for the inhibitory action of any chemopreventive drug in vivo or in vitro. Given that the loss of integrin-mediated cell-matrix contact can induce anoikis (29), we asked whether sulindac sulfide and/or CAPE affected FAK expression or tyrosine phosphorylation in human colon carcinoma cells (DLD-1 and HT-29). We selected these two cell lines because of their APC-null phenotype, the typical situation in both sporadic and heritable forms of colon cancer. We show that subapoptotic doses of both sulindac sulfide and CAPE caused a rearrangement of the actin cytoskeleton with the attendant loss of focal adhesion plaques. Importantly, these drugs also reduced the tyrosine phosphorylation of both FAK and p130Cas, although the steady-state expression of these proteins, together with other relevant signaling molecules, remained unchanged. Finally, we show that treatments with the chemopreventive drugs reduced cell invasion, a functional assay for the inhibition of signaling downstream of FAK. These data strongly suggest that chemopreventive drugs can regulate FAK activity and provide a possible link between inhibition of prostaglandin synthesis and integrin-ECM signaling.

**MATERIALS AND METHODS**

**Materials.** Sulindac sulfide was a generous gift from Merck. CAPE was obtained by esterification of caffeic acid with phenethyl alcohol in the presence of p-toluenesulfonic acid as described previously (37). Genistein was purchased from Calbiochem-Novabiochem Corp. (La Jolla, CA). DMEM/F-12, FBS and calf serum were purchased from Life Technologies, Inc. (Burlingame, CA), and streptavidin-horseradish peroxidase was obtained from PharMingen (San Diego, CA). The micro BCA protein assay reagent kit was purchased from Pierce Corp. (Rockford, IL). Immunoprecipitations used the Protein A kit of Boehringer Mannheim (Roche Molecular Biochemicals, Indianapolis, IN). Western blot analysis used OptiGel nitrocellulose (Schleicher & Schuell, Keene, NH). Electrophoresis of proteins used electroblot buffers of Owl Separation Systems (Woburn, MA). Biocat Matrigel invasion chambers were purchased from Becton Dickinson Labware (Bedford, MA), and hematotoxin 2 was obtained from Richard-Allan Scientific (Kalamazoo, MI). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

**Cell Culture and Treatment Conditions.** Human colon carcinoma cell lines DLD-1 and HT-29 were obtained from the American Type Culture Collection (Manassas, VA). Both cell lines carry APC and p53 mutations; the genotypes of these cell lines have been reported (38–43). NIH3T3 cells were kindly provided by Dr. Lonny R. Levin (Weill Medical College, Cornell University). Cultures were maintained in DMEM/F-12 medium supplemented with 10% FBS and cultured at 37°C in a humidified 5% CO2 incubator. Cells in log-phase growth (~70% confluent) were treated with CAPE, sulindac sulfide, or genistein in fresh medium containing 0.1% FBS for 24 h.

**Immunohistochemistry.** Approximately 5000 DLD-1 cells were seeded into four-well Lab-Tek chamber slides (Nunc, Inc., Naperville, IL) and incubated for 48 h prior to treatments. The medium was aspirated and replaced with DMEM/F-12 containing 0.1% FBS with or without a range of CAPE (2.5, 5.0, and 7.5 μg/ml) or sulindac sulfide (5, 10, and 15 μM) concentrations. Treated cultures were incubated an additional 24 h and then fixed in 4% PBS-buffered formaldehyde for 20 min. Upon repeated washings in PBS, slides were placed in 50 mM NH4Cl for 15 min and then with 0.2% Triton X-100 for 10 min. Blocking was for 30 min in PBS containing 3% BSA. Rhodamine-labeled phallolidin in PBS containing 1% BSA was applied, and the slides were placed in a humidified chamber at room temperature for 1 h. After further washing, slides were mounted with coverslips, and imaging was performed using a Zeiss LSM510 laser scanning microscope.

**Immunoblot Analysis and Immunoprecipitation.** Cells that were washed twice in cold PBS were scraped from culture dishes in lysis buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM EGTA, MgCl2 2 mM, 1% (v/v) Triton X-100, 10% glycerol, 10 mM DT T, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 5 μg/ml pepstatin A, 50 mM NaF, 10 mM Na3P2O7, 1 mM NaVO4, and 3 mM H2O2]. Lysates were centrifuged for 10 min at 4°C at 12,000 × g. Protein concentrations of lysates were determined by micro-BCA assay. For IB analyses, the same concentration of proteins (30 or 50 μg) were subjected to 7.5% SDS-PAGE and were electrotransferred to nitrocellulose membranes using electroblot buffers. Membranes were blocked in PBS containing 1% Triton X-100, 0.1% SDS, and 3% BSA for 1 h. Reactions with the primary antibodies in buffer containing 1% BSA were carried out at 4°C overnight. After extensive washing, membranes were placed on a shaker for 1 h with biotinylated secondary IgG. Upon further washing, membranes were reacted with streptavidin-horseradish peroxidase for 45 min and ECL detection re-
agents immediately prior to autoradiography. IP used 300 or 500 µg of total protein, together with 5 or 7 µg of the clone 4G10 antibody and agarose A beads following, the manufacturer’s protocol. The entire final extract from each immunoprecipitated sample was resolved by 7.5% SDS-PAGE. All IB and IP experiments were repeated at least three separate times.

**Invasion Assay.** The invasion assay was performed as detailed previously (36). Briefly, HT-29 cells were subjected to the standard treatment conditions (see above), incubated for 24 h, and then trypsinized. The number of viable cells was determined by trypsin blue exclusion and counted using a hemacytometer. Cells (5 × 10^6) in low serum DMEM/F-12 medium were placed on the Matrigel-coated polycarbonate filters of each well of invasion assay plates (8-mm wells, 8-µm pores). Conditioned medium from NIH3T3 cells, supplemented with 10% calf serum, was placed in the bottom chambers as a chemoattractant. Plates were incubated at 37°C for 24 h. Cells that migrated to the bottom compartment were stained with hematoxylin and counted under a light microscope. A blinded viewer counted the number of cells present in four fields per chamber, and the experiment was separately repeated. Statistical analysis of the data from this assay was computed by Student’s *t* test.

**RESULTS**

Chemopreventive Drugs Disrupt Actin Stress Fibers in Colon Carcinoma Cells. When integrins bind to the ECM, they cluster at focal adhesions and associate into a signaling complex that promotes the assembly of actin filaments into large units known as stress fibers. In response to the withdrawal of survival factors, epithelial cells sever their contacts with neighboring cells or the ECM and reorganize their actin cytoskeleton. This process, resulting in the retraction and loss of stress fibers, was associated with the perinuclear accumulation of actin (44).

To learn the possible effects of treatments with chemopreventive agents on the architecture of the actin cytoskeleton and focal adhesion complexes, DLD-1 cells were treated with and without CAPE and sulindac sulfide. These cells were placed in low serum medium containing a range of drug concentrations and incubated for 24 h. As shown in Fig. 1, the actin cytoskeleton was visualized by immunofluorescence staining with phalloidin. Stress fibers that are anchored to focal adhesions in a fan-shaped array are apparent in the untreated control cells. This result is typical of cells with intact cell-ECM contacts. However, exposure of DLD-1 cells to relatively low concentrations of CAPE (2.5–7.5 µg/ml, where the lowest dose is equivalent to 8.8 µM) and sulindac sulfide (5–15 µM) induced a dramatic reorganization of the cytoskeleton with a dose-dependent retraction and loss of the actin stress fibers. Both treatment conditions inhibit cell growth but do not induce apoptosis in these p53 mutant cell lines (data not shown). At the highest dose of CAPE, there is a circumferential aggregation of actin at the cell periphery. In contrast upon treatment with sulindac sulfide, the overall level of actin staining appears markedly reduced. These structural changes suggest that CAPE and sulindac sulfide modulated cellular adhesion and integrin-mediated signaling events.

Chemopreventive Compounds Decrease the Phosphorylation of FAK and p130cas. Activation of the tyrosine kinase, FAK, is coupled to the assembly of focal adhesion plaques and the maintenance of actin stress fibers (45, 46). At the focal adhesion complex, FAK interacts with the cytoplasmic tail of integrin β1, either directly or through association with cytoskeletal proteins, talin and paxillin (9). After integrin binding, FAK is autophosphorylated at Tyr577, creating a binding site for the SH-2 domain of Src (11, 12). p130cas is a signal transduction protein that binds directly to FAK and is phosphorylated upon cell adhesion to ECM proteins in a FAK- and Src-dependent manner (18, 19).

To investigate the effect of chemopreventive drugs upon focal adhesion-associated signaling proteins, we examined the tyrosine phosphorylation of FAK and p130cas. The human colorectal cancer cell lines, HT-29 and DLD-1, were treated with the same subapoptotic doses of sulindac sulfide (5–15 µM) and CAPE (2.5–7.5 µg/ml), after which lysates were prepared. IP was performed using an anti-phosphotyrosine antibody, followed by immunoblotting with an anti-FAK antibody. As demonstrated in Fig. 2A, a dose-dependent reduction in phosphotyrosine was observed in the M125,000 FAK protein in both cell lines treated with sulindac sulfide and CAPE. Moreover as a positive control, Fig. 2B shows that a similar inhibition of the tyrosine phosphorylation of FAK was observed after treatment of DLD-1 cells with a known tyrosine kinase inhibitor, genistein (100 µM). Previously, genistein was found to block the tyrosine phosphorylation of FAK and the assembly of focal adhesions and stress fibers in Swiss 3T3 fibroblasts (47). Thus, the magnitude of the effect of sulindac sulfide and CAPE on the tyrosine phosphorylation status of FAK in these colon carcinoma cells appears to be comparable with that inducible by genistein.

To examine events downstream of FAK, we determined the effect of treatments with the chemopreventive agents on the tyrosine phosphorylation state of p130cas. As expected, exposure to sulindac sulfide and CAPE also produced a decrease in p130cas tyrosine phosphorylation (Fig. 3). This result suggests that chemopreventive drugs may suppress the activity of Src kinase, as well as FAK. We next examined by immunoblot analysis the effects of the treatments on the steady-state levels of FAK and other proteins that associate with this kinase. As shown in Fig. 4, the overall amounts of FAK, p130cas, paxillin, integrin β1, and Grb2 were unchanged by treatment with sulindac sulfide or CAPE. This result implies that these chemopreventive drugs are not likely to alter the synthesis or degradation pathways for proteins associated with the focal adhesion complex.

Sulindac Sulfide and CAPE Inhibit Invasion of Colon Cancer Cells. Migration is a process carried out by normal intestinal epithelial cells as they travel from the proliferative zone of crypts to the luminal surface of villi tips. Cancer cells lose the controls restricting migration beyond the confines of the basement membrane and acquire the ability to invade into the submucosa. Migration requires the serial assembly and disassembly of focal adhesion complexes that provide traction for the filopodia and lamellipodia. These events, in turn, propel the cell
forward. Increases in the tyrosine phosphorylation of FAK and p130Cas have been directly implicated in the up-regulation of cell migration (16).

To further characterize the effects of chemopreventive drugs upon the effector function of FAK, we measured the migration of HT-29 cells through Matrigel. HT-29 cells treated for 24 h with CAPE (7.5 μg/ml) or sulindac sulfide (15 μM) were placed on top of Matrigel-coated polycarbonate filters, with conditioned medium from NIH3T3 cells (supplemented with 10% calf serum) in the chambers below. After a further incubation of 24 h, cells that succeeded in migrating to the bottom compartment were stained with hematoxylin and counted under a light microscope. As illustrated in Fig. 5, both CAPE and sulindac sulfide significantly reduced cell invasion ($P < 0.00001$, SD $\pm 2.5$ and $P < 0.0001$, SD $\pm 1.6$, respectively).

Fig. 1  Chemopreventive drugs disrupt actin stress fibers in colon carcinoma cells. Relatively low concentrations of CAPE (2.5 μg/ml-7.5 μg/ml) and sulindac sulfide (5–15 μM) were used to treat DLD-1 cells for 24 h. Details of the immunohistochemistry and image analysis are described in “Materials and Methods.”
same manner as above (a positive control (65). Lysates were prepared and processes in the risk of colon carcinogenesis. By which NSAIDs and other chemopreventive agents reduce the efficacy of many potential chemopreventive agents (reviewed in Ref. 50). Despite these clues, we still do not understand the mechanism of activity of many chemopreventive agents.

Fig. 2 Chemopreventive drugs induce a dose-dependent inhibition of FAK tyrosine phosphorylation in human colon carcinoma cells. HT-29 and DLD-1 cell lines were subjected to treatments with a range of sulindac sulfide and CAPE concentrations in low serum medium for 24 h. Lysates were prepared, and the same amount of protein (500 μg) from each treatment group was immunoprecipitated using the mouse anti-phosphotyrosine monoclonal antibody, 4G10. Subsequently, the eluted proteins were resolved by 7.5% SDS-PAGE for IB analysis to determine the levels of FAK protein present in each of the treatment samples. The size of the marker band appearing in the last lane on the left of each gel is M, 116,000 (A). DLD-1 cells were treated with an arbitrary concentration of genistein (100 μM) as a positive control (65). Lysates were prepared and processes in the same manner as above (B).

DISCUSSION

Colorectal cancer is one of the leading causes of death in Western countries. Epidemiological studies of colorectal cancer incidence suggest that the development of this disease can be modulated by dietary factors, with a high intake of fruits and vegetables providing a protective effect (48). Many diet-derived substances, such as plant polyphenolic compounds, calcium, antioxidant vitamins, and ω-3 fatty acids have demonstrated significant efficacy in tumor prevention. Epidemiological studies also indicate that the routine use of NSAIDs decreases the incidence of colorectal cancer (49). Rodent models, including carcinogen-induced tumor studies and treatment of adenomas produced by germ-line Apc mutations, have provided useful data concerning the relative efficacy of many potential chemopreventive agents (reviewed in Ref. 50). Despite these clues, we still do not understand the mechanism by which NSAIDs and other chemopreventive agents reduce the risk of colon carcinogenesis.

Here we show that inhibition of FAK-mediated signaling is a characteristic of two chemopreventive compounds, representing the broader classes of NSAIDs and natural polyphenolic antioxidants. Although this observation is novel, the antitumor effects of these drugs are known to be associated with inhibition of tyrosine kinases. Sulindac inhibited HER-2 tyrosine kinase expression in the rectal mucosa of FAP patients (51), and sulindac sulfide inhibited ras-mediated cell transformation, a process that involves the activation of tyrosine kinases (52). Furthermore, in keratinocytes, CAPE was found to inhibit the autophosphorylation of the epidermal growth factor receptor (53).

The isoflavonoid, genistein, is a tyrosine kinase inhibitor that blocks the tyrosine phosphorylation of FAK and the assembly of focal adhesions and stress fibers in Swiss 3T3 fibroblasts (47). Genistein is a component of soy, and epidemiological evidence has linked high soy intake to lower rates of breast and prostate cancer (54). Despite these studies supporting a role for genistein in cancer prevention, some measurements of the effect of genistein on FAK activity and tumor formation provide conflicting data. In contrast to its effects in fibroblasts, genistein increased the adhesion of cultured prostate carcinoma cells, which was accompanied by the transient activation of FAK activity and the enhanced association between integrin β1 and FAK (52). Consistent with these observations, the administration of genistein to rats in an azoxymethane-induced colon cancer model produced a significant increase in both invasive and noninvasive intestinal tumors (55). Also, the administration of diets rich in soy isoflavones to Min/+ mice failed to reduce tumor numbers (56). Nonetheless, the activity of genistein in the colon cancer cells studied here is consistent with its role as a chemopreventive agent.

A growing body of evidence suggests that inhibition of prostaglandin synthesis through blockade of Cox activity may be the mechanism of activity of many chemopreventive agents. In particular, Cox-2, the inducible form of Cox, has been identified as a significant potentiator of carcinogenesis (56). Compounds that prevent tumors in Apc-deficient animals and carcinogen-induced rodent studies, such as sulindac, aspirin, NS-398, SC58635, curcumin, and CAPE, all reduce Cox-2 expression, prostaglandin production, and the proliferation of cultured carcinoma cells (57–59). Both sulindac sulfide and CAPE are nonselective inhibitors of Coxs. Sulindac sulfide has been well characterized as an inhibitor of tumor formation in animal models and in humans with FAP. Sulindac produces apoptosis in the rectal mucosa of patients with FAP (60), in cultured human colon cancer cells (61), and in the enterocytes of Min/+ mice (26). Intestinal epithelial cells that overexpress Cox-2 are resistant to apoptosis, although treatment with sulindac sulfide rescued the apoptotic mechanism (34, 35). This drug-induced reversal of the Cox-2 effect was accompanied by decreased adhesion of the treated epithelial cells to ECM elements. CAPE is an antioxidant compound that also induces apoptosis of premalignant intestinal cells, an activity associated with tumor prevention in vivo (28, 62). In a human oral epithelial cell line that inducibly expresses Cox-1 and Cox-2, CAPE-mediated suppression of prostaglandin production reduced Cox enzyme activities, arachidonic acid release, COX2 gene transcription, and inflammation in vivo (59). It is presently unclear whether the effect on cell growth is directly attributable to the inhibition of Cox-2 activity or whether it is a secondary consequence of decreased prostaglandin synthesis (26, 61, 63).
The data presented here suggest that sulindac and CAPE may also inhibit tumors in a Cox-2-independent manner. The cancer cell lines used in these studies do not inducibly express Cox-2, and yet treatments with sulindac sulfide produced changes in apoptosis and invasion (30). Thus, these agents may cause phenotypic alterations in tumor cells that render them less aggressively metastatic in vivo.

Because both sulindac sulfide and CAPE are nonselective inhibitors of Coxs, it is conceivable that inhibition of Cox-1 activity is responsible for their antitumor effect. It is also possible that sulindac sulfide and CAPE inhibit FAK activation through a mechanism that is independent of both Cox-1 and Cox-2. If this is the case, identification of this mechanism may provide an opportunity to develop agents capable of suppressing the formation of tumors that are refractory to Cox-2 inhibition.

For a chemopreventive agent to be effective, the ability to inhibit tumor formation during both initiation and postinitiation phases of carcinogenesis may be required. Previous work in our laboratory showed that enterocyte migration in the crypts of Min/+ mice is slower than in wild-type animals and that sulindac sulfide normalized the migration rate (33). Correction of this defect by a chemopreventive drug is consistent with the idea that effective agents may modulate certain stroma-enterocyte interactions early in the promotion or progression phases of tumorigenesis. We recently obtained further evidence for this view by examining FAK protein levels and the relative tyrosine phosphorylation of this kinase in enterocytes from the small bowel of both Min/+ and wild-type mice. 

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\(^{5}\) K. Subbaramaiah, unpublished data.

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**Fig. 3** Chemopreventive drugs reduce the tyrosine phosphorylation of the FAK-associated protein, p130cas, in human colon carcinoma cell lines. HT-29 and DLD-1 cells were treated with and without CAPE (7.5 μg/ml) or sulindac sulfide (15 μM) for 24 h. Five hundred μg of the prepared lysates were used for IP using the 4G10 antibody, and immunoblotting was performed on the resolved proteins using a mouse antihuman p130cas monoclonal antibody.

**Fig. 4** Chemopreventive drugs do not alter cellular levels of FAK and its associated proteins in colon epithelial cells. Immunoblot analysis of proteins isolated from HT-29 and DLD-1 cells treated with and without CAPE or sulindac sulfide for 24 h is shown. For blots of FAK, p130cas, and paxillin, 50 μg of protein were applied to gels; for blots of integrin β1 and Grb2, 30 μg of protein were used. FAK, p130cas, and integrin β1 were resolved by 7.5% SDS-PAGE; 10% gels were used in the case of blots probed for paxillin and Grb2.

**Fig. 5** Chemopreventive drugs significantly inhibit Matrigel invasion by HT-29 cells after a 24-h treatment of HT-29 cells with CAPE (7.5 μg/ml) or sulindac sulfide (15 μM). The assay procedure is described in “Materials and Methods.” The number of fields per treatment group evaluated was eight. Both CAPE and sulindac sulfide significantly reduced cell invasion (P < 0.00001, SD ± 2.5 and P < 0.0001, SD ± 1.6, respectively). Bars, SD.
mice and in adenomas from Min/+ . Specifically, we showed that FAK protein expression was increased in Min/+ tumors compared with Min/+ mucosa. Moreover, levels of tyrosine phosphorylated FAK were increased in nonadenomatous Min/+ small bowel enterocytes compared with those cells obtained from wild-type mice. Further support for the hypothesis that ECM-enterocyte interactions play an important role in early stages of intestinal carcinogenesis was demonstrated when elimination of the matrix metalloproteinase gene, MMP-7, in a Min/+ mouse background yielded 60% fewer intestinal adenomas than in Min/+ controls (64). The tumor cell line studies presented here, though, also indicate that the antitumor effects of sulindac sulfide andCAPE are maintained in cells displaying a fully malignant phenotype. In summary, these novel studies add modulation of integrin-mediated signaling to the spectrum of activities of NSAIDs and plant phenolics. Further work is needed to understand the relationship between Cox-2 expression and FAK activity and to learn whether modulation of FAK activity is a useful target for comparing chemopreventive agents.

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REFERENCES


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