Genotype-Phenotype Correlation in Murine Apc Mutation: Differences in Enterocyte Migration and Response to Sulindac

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ABSTRACT

The adenomatous polyposis coli (APC) gene product mediates coordinated cell growth in the intestinal mucosa. In humans, germ-line mutations of APC are associated with colorectal carcinogenesis, a process that varies in severity depending on the length of the protein resulting from the mutant allele. In a previous study of the C57BL/6J-Min+/+ (Min/+ mouse), we found that the protein fragment resulting from truncation at codon 850 of murine Apc was associated with changes in enterocyte migration, proliferation, apoptosis, and β-catenin expression. This effect was reversed upon treatment of Min/+ mice with the chemopreventive drug sulindac sulfide. In this study, we measured enterocyte migration in the Apc1638N mouse, an animal with an Apc mutation that yields no detectable APC protein. We found no difference in enterocyte migration, proliferation, apoptosis, or β-catenin levels in the Apc1638N mouse when compared to wild-type littermates bearing two normal Apc alleles. Furthermore, administration of sulindac sulfide to Apc1638N mice did not alter enterocyte migration. These observations suggest that a dominant negative effect altering cell migration is exerted by the truncated APC protein present in the Min/+ mouse. These data also suggest that the effectiveness of chemopreventive agents in preventing APC-related tumor formation may depend on which type of mutation is present.

INTRODUCTION

Both human and animal studies suggest that mutation of the Apc gene is a powerful inducer of intestinal carcinogenesis. The Apc mutation is one of the earliest events in the adenoma-carcinoma sequence, occurring coincident with the development of aberrant crypt foci (1). APC mutations are present in more than 85% of sporadic colorectal cancers (2); therefore, understanding the function of APC is crucial to studies of early carcinogenesis and tumor prevention.

FAP is an autosomal dominant disorder of humans caused by a germ-line mutation in APC. This disease is characterized by the development of multiple adenomas of the colon and duodenum with progression to colorectal carcinoma in the third to fourth decade of life in an untreated individual. Tumors arising in patients with FAP show loss of the wild-type APC allele. The location of APC gene mutation correlates with the severity of disease in these individuals. Truncations in the extreme 5′ end of the coding sequence (before codon 169) produce an attenuated form of FAP with fewer intestinal tumors and later progression to colorectal cancer (3–6). The great majority of mutations found in patients with FAP result in stable truncated proteins of greater than Mr 80,000 (6–9). The “profuse” FAP phenotype, which is characterized by extremely numerous lower intestinal tumors, is associated with mutations from codon 1250 to codon 1464 (6). There are few germ-line mutations after codon 1700 (8), suggesting that mutations in the 3′ end of the coding sequence produce little or no phenotype.

The Min/+ mouse is an animal model of FAP produced by a truncation mutation at codon 850 of the murine Apc gene. This results in a Mr 95,000 protein that retains the homodimerization domain of APC (10–12). The Min mutation results in a phenotype with a severity comparable to classical FAP in humans. These animals develop 30–50 intestinal tumors by 20 weeks of age and die from tumor-associated anemia or intestinal obstruction by 21–22 weeks of age (12). Unlike tumors in humans with FAP, which arise in the large intestine and duodenum, tumors in Min/+ mice are predominantly located in the small intestine. In a previous study of preneoplastic small intestinal epithelium from the Min/+ mouse, we found that this codon 850 mutation is associated with changes in the growth characteristics of preneoplastic tissue, including decreased apoptosis and proliferation and decreased enterocyte migration. The result of this is an increased residence time for enterocytes in the small intestine of Min/+ animals. These growth changes suggest that the truncated APC protein resulting from the Min mutation exerts a dominant negative effect (13).

The Apc1638N mouse is an animal model of FAP that was developed by altering the Apc gene of embryonic stem cells at codon 1638 by the insertion of a neomycin expression cassette (14). This mutational event results in a frameshift predicted to produce a truncated Apc protein of Mr 185,000. However, Western blot analysis fails to detect a truncated protein in these animals, and the product of this mutation is therefore presumed to be unstable. Like humans with extreme 5′ APC mutations, Apc1638N mice have an attenuated phenotype. Apc1638N heterozygotes develop an average of three to four intestinal tumors by 20 weeks of age, and 30% of these animals are alive at 1 year (14).

If the intestinal growth changes observed in Min/+ mice are related to a dominant negative effect exerted by the truncated APC protein, we would expect these changes to be absent in the Apc1638N mouse. We therefore studied enterocyte apoptosis, proliferation, and migration in the preneoplastic epithelium of Apc1638N mice. We found that these mice exhibit normal enterocyte growth characteristics, including a normal migration pattern. Sulindac sulfide, a drug that alters enterocyte growth and migration in the Min/+ mouse, did not alter the migration of enterocytes in Apc1638N mice. These data support a dominant negative effect of the truncated APC resulting from the Min mutation. These data may also partly explain the genotype-phenotype correlations seen in humans with FAP.

MATERIALS AND METHODS

Determination of Small Intestine Morphology. To compare the morphology of the small intestinal mucosa from Apc1638N and their wild-type littermates (+/+), we measured crypt-villus length, nuclear density, and the total...
number of cells/crypt-villus unit using a Feulgen stain. Serial sections of formalin-fixed mid-small intestine were deparaffinized in xylene for 10 min. After alcohol rehydration, slides were rinsed in PBS and hydrolyzed in 5 N HCl for 1 h. The slides were then placed in Feulgen stain, covered with parafilm, and allowed to stain for 1 h before rinsing in Feulgen rinse solution. After a wash in tap water for 5 min, slides were rinsed with 1% acid alcohol for 5 min, placed in 100% methanol for 6 min, and cleared in xylene before coverslipping. Analysis of crypt-villus length was accomplished using the micrometer function of the CAS 200 software. Nuclear density was analyzed using the quantitative DNA program of the CAS 200. Five animals in each group were examined. For each animal, 10 sections of mid-small intestinal mucosa and 10 intact crypt-villus units from each section were measured.

Identification of Intact APC Protein in Intestinal Mucosa. Serial sections of intestinal mucosa from Apc1638N and wild-type animals were deparaffinized and rehydrated as described above. Endogenous peroxidases were quenched in 3% H2O2, and the slides were rinsed in PBS. The sections were then incubated at 25°C for 1 h with a polyclonal rabbit antihuman APC antibody that identifies the COOH terminus of APC protein (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). After rinsing, slides were incubated with goat antirabbit IgG for 30 min at 25°C, followed by a 30-min treatment using the Vector Elite ABC kit (Vector Laboratories, Inc., Burlingame, CA). The slides were then stained with DAB chromatogen solution and counterstained with methyl green. As a control, the anti-APC antibody was absorbed using 10 times the amount of intact APC protein. This mixture was then used as a primary antibody in the method described above. As expected, this resulted in no staining of the Apc1638N and wild-type tissue sections, thereby demonstrating the specificity of the antibody staining. As an additional control, a polyclonal rabbit antianhuman APC antibody that identifies the NH2 terminus of APC protein (Santa Cruz Biotechnology, Inc.) was also used as the primary antibody in the method outlined above. This last control resulted in the ubiquitous staining of both the tumor and the adjacent normal mucosa in Apc1638N tissue. Absorption of this antibody with 10 times the amount of APC protein revealed no staining and was thus used as a negative control.

Determination of β-Catenin Expression. Slides were deparaffinized in xylene for 10 min, followed by alcohol rehydration. After quenching endogenous peroxidases with 0.45% H2O2 in methanol, the slides were rinsed in PBS, and an antigen retrieval step was carried out in a 700-W microwave oven placed in 100% methanol for 6 min, and cleared in xylene for 10 min, followed by alcohol rehydration. After quenching endogenous peroxidases with 0.45% H2O2 in methanol, the slides were rinsed in PBS. The sections were then incubated at 25°C for 1 h with a polyclonal rabbit antihuman APC antibody that identifies the COOH terminus of APC protein (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). After rinsing, slides were incubated with goat antirabbit IgG for 30 min at 25°C, followed by a 30-min treatment using the Vector Elite ABC kit (Vector Laboratories, Inc., Burlingame, CA). The slides were then stained with DAB chromatogen solution and counterstained with methyl green. As a control, the anti-APC antibody was absorbed using 10 times the amount of intact APC protein. This mixture was then used as a primary antibody in the method described above. As expected, this resulted in no staining of the Apc1638N and wild-type tissue sections, thereby demonstrating the specificity of the antibody staining. As an additional control, a polyclonal rabbit antianhuman APC antibody that identifies the NH2 terminus of APC protein (Santa Cruz Biotechnology, Inc.) was also used as the primary antibody in the method outlined above. This last control resulted in the ubiquitous staining of both the tumor and the adjacent normal mucosa in Apc1638N tissue. Absorption of this antibody with 10 times the amount of APC protein revealed no staining and was thus used as a negative control.

Measurement of Enterocyte Proliferation. Small bowel sections were deparaffinized and rehydrated, and endogenous peroxidase activity was blocked by incubating the slides with 0.45% methanol/H2O2. Antigen retrieval was achieved by microwaving. PCNA (Dako, Carpinteria, CA) antibody was applied and incubated for 1 h at room temperature. Indirect detection was performed by incubating with secondary biotinylated horse-antimouse IgG followed by Vector Elite ABC for 30 min at room temperature. Incubation for 5 min in DAB was used for color development. The specimens were counterstained with methyl green. For each specimen, eight crypt-villus units were chosen randomly from serial sections of small bowel mucosa by an individual blinded to the animal’s genetic status. The percentage of staining of enterocytes in these crypt-villus units was measured using the CAS 200.

Measurement of Enterocyte Apoptosis. Apoptosis was identified using the ApopTag kit (Oncon, Gaithersburg, MD). Small bowel sections were processed according to the manufacturer’s directions. For each specimen, eight crypt-villus units were chosen randomly from serial sections of small bowel mucosa by an individual blinded to the animal’s treatment group and genetic status. The percentage of staining of enterocytes in these crypt-villus units was measured using the CAS 200. To confirm that uniform sampling was achieved, nuclear density was measured and confirmed to be equivalent in all three study groups.

Measurement of Enterocyte Migration. After 1 month of the AIN-76A diet with or without sulindac sulfide as described above, 10 mice from each experimental group (Min/+, Apc1638N, wild-type, Min/sulindac, and Apc1638N/sulindac sulfide) were injected with 0.3 μg/kg of BrdUrd (Sigma, St. Louis, MO). One animal from each of the three groups was euthanized by CO2 inhalation 1 h after injection and after several 18-h intervals of time. At harvest, the intestinal tracts were immediately removed from the stomach to the distal rectum, opened, flushed with PBS, and examined under ×3 magnification. Multiple samples of grossly normal full-thickness bowel were obtained from the mid-small intestine. Samples were fixed in 10% formalin for histological examination and immunohistochemistry.

To detect BrdUrd incorporation into the newly synthesized DNA of epithelial cells, mid-small bowel specimens of approximately 5 mm in length were formalin fixed, embedded in paraffin, and sectioned at 5 μm. Several serial sections were obtained. Before staining, the sections were deparaffinized in Hematoxylin and rehydrated through an alcohol series. The first section was stained with H&E to document normal mucosal histology. The sections were then stained with anti-BrdUrd antibody as follows. Endogenous peroxidase activity was blocked by incubating the specimens in 3% H2O2 for 10 min at 37°C. DNA was then denatured by immersing the specimens in 2 N HCl for 30 min, followed by enzymatic pretreatment by immersing the specimens in trypsin for 20 min. Subsequently, 5% goat serum was then applied to the slides for 15 min, followed by a 2-h incubation with clone Bu33 anti-BrdUrd antibody (Sigma) at 37°C. Speciments were incubated with biotinylated horse-antimouse IgG and ABC for 30 min at room temperature, followed by color development by incubation in DAB for 5 min. Finally, the specimens were counterstained using the Vector alkaline phosphatase substrate kit (Vector Laboratories, Inc.) and coverslipped. Eight intact histologically normal crypt-villus units from each animal were examined.

Measurement of PGE2. PGE2 measurements were performed as described previously (13). Intact small intestine, rather than intestinal mucosa only, was used for this study due to the relatively large volume of material that was required for analysis. Samples were homogenized, transferred to microcentrifuge tubes, and centrifuged at 4°C for 10 min at 10,000 × g. The supernatants were decanted, and a 10-μl aliquot of supernatant was used to determine protein concentration with a bicinchoninic acid protein assay kit (Sigma). Determination of PGE2 levels by EIA was accomplished using a PGE2-monoclonal enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Plates were read at 410 nm with a UV max kinetic plate reader (Molecular Devices, San Jose, CA). Data were computerized with DeltaSoft 3, and statistical analyses were performed with InStat 2.00 software.

RESULTS

Enterocyte Migration Is Normal in Apc1638N Mice. In a previous study of the Min/+ mouse, we found that enterocyte migration in the preneoplastic small intestinal mucosa was decreased by 25%, a result interpreted to be a consequence of a dominant negative effect exerted by truncated APC protein (13). In the present study, we used
a similar approach to track enterocyte migration in the small intestine of Apc1638N mice, an animal with what is effectively a null mutation in one Apc allele.

To confirm the absence of minor histological changes in the mucosa of Apc1638N mice, Feulgen stain was used to compare the small intestines of 65 day-old Apc1638N mice to those of their littermates with two normal Apc alleles (+/+). No differences in crypt length, nuclear density, or the number of cells/crypt-villus unit were observed (Table 1). We then injected Apc1638N mice with the thymidine analogue BrdUrd to label all actively proliferating cells. At the indicated intervals after injection, the mid-small intestines from these animals were examined by immunohistochemistry using an anti-BrdUrd antibody to detect the location of proliferating cells at the time of BrdUrd injection. As expected, by 72 h after BrdUrd injection, the tips of the villi were labeled in the wild-type animals (+/+) In spite of the germ-line mutation, the Apc1638N mice also showed a progression of the labeled cells to the tip of the villi by 72 h after injection (Fig. 1). A timed comparison of the location of the labeled cell highest along the crypt-villus axis revealed no differences between the Apc1638N and +/+ mice (Fig. 1, ANOVA with time as a covariate; P > 0.05). These data show that in contrast to Min/+ mice, in which enterocyte migration is slowed by 25%, the enterocytes in Apc1638N mice follow a normal migration time course.

Normal APC protein binds to intracellular β-catenin and targets β-catenin for degradation via the ubiquitin pathway (15). Therefore, abnormal APC function is associated with increased intracellular β-catenin levels. Because of its association with the actin cytoskeleton and its ability to bind nuclear transcription factors, β-catenin may be an important modulator of epithelial cell migration (16, 17). We therefore studied the differences in β-catenin expression between Min/+ and Apc1638N animals and their wild-type littermates. By immunohistochemistry using a monoclonal antibody against β-catenin, we found a 3-fold increase in intracellular β-catenin in the histologically normal mucosa of the Min/+ mouse, but not the Apc1638N mice (Fig. 2). This result suggests that the migration defect observed in Min/+ animals may result from an effect of the truncated APC protein on β-catenin expression.

Preneoplastic Mucosal Growth Kinetics Are Unaffected by the Apc1638N Mutation. Tissue homeostasis in the intestinal tract is achieved by a properly coordinated balance between enterocyte proliferation and apoptosis, a balance that is disrupted in animals with the Min mutation (13). Moreover, this truncation mutation of Apc is associated with a decrease in both proliferation and apoptosis, an observation that is consistent with a decreased enterocyte migration rate. To compare the effect of a null mutation of Apc with the Min truncation mutation, we measured proliferation and apoptosis in the small intestines of Min/+ and Apc1638N animals.

Min/+ and Apc1638N animals were fed a standardized AIN-76A diet, and at 65 days of age, enterocyte proliferation and apoptosis were measured in the mid-small intestine. When measured by immunohistochemistry using an antibody to PCNA, the level of proliferation observed in the small intestine of Apc1638N mice was no different from that of the wild-type (+/+) animals (Fig. 3). This is in contrast to the Min/+ mouse, in which proliferation was 41% of that of the wild-type littermates (+/+; Ref. 13). Serial sections of small intestine were also evaluated by TUNEL, an in situ measurement of apoptosis. Whereas Min/+ animals showed a 71% decrease in apoptosis (13), the percentage of positive cells by TUNEL analysis was not altered by the Apc1638N mutation (Fig. 4).

These data suggest that unlike the Min mutation, which produces a decrease in enterocyte proliferation and apoptosis, the Apc1638N mutation does not alter these in vivo measurements of intestinal cell kinetics.

Tumors in Apc1638N Mice Show a Loss of Wild-Type Apc Function. Although APC-related changes are found in the intestine of Min/+ mice, tumor formation generally requires inactivation of the
null was measured in sulindac sulfide-treated mice, no difference was seen between the treated and control animals (Fig. 2).

DISCUSSION

The Apc gene encodes a M, 310,000 protein that regulates intracellular levels of β-catenin (24) in association with GSK-3β, a serine threonine glycogen synthase kinase. The oncogenic effects of deficient Apc function seem to involve an alteration of β-catenin expression. The small percentage of colorectal cancers lacking the Apc mutation may have mutations in the β-catenin gene (25, 26). The exact function of β-catenin is unknown. β-Catenin is an intracellular protein associated with the actin cytoskeleton. Because it is also a component of the adherens junction of epithelial cells, β-catenin may mediate contact-associated growth regulation. β-Catenin also plays a role in the nucleus, where, in combination with the protein Tcf, it provides a DNA binding domain. The Tcf/β-catenin complex is thought to activate the transcription of genes modulating cell proliferation and apoptosis (27–29).

The Apc protein has several functional domains. The first 171 amino acids mediate homodimerization through the formation of α-helical rods (10, 30). Truncated APC proteins can associate with wild-type protein through the homodimerization domain (10), thus providing a mechanism for dominant negative effects by proteins retaining the first 171 amino acids of Apc. APC also contains a series of armadillo repeats between amino acids 435 and 766 (31–33). It is possible that this region mediates protein-protein interactions, providing an additional mechanism for dominant negative effects (34).

Constitutive binding of β-catenin occurs at three 15-amino acid repeats located between amino acids 1020 and 1169 (35, 36). A second β-catenin binding region regulated by the activation of GSK-3β is located between residues 1342 and 2075 (15). This site contains seven repeated segments of 20 amino acids in length that constitute the β-catenin binding sites (15). The MCR region of APC encompasses the β-catenin binding sites. Mutations in this region produce the most severe phenotype in humans (37, 38). An analysis of the effect of stepwise deletion mutations of Apc shows that binding of APC to β-catenin requires only a single 20-amino acid sequence, whereas effective down-regulation of β-catenin requires the presence

null was measured in sulindac sulfide-treated mice, no difference was seen between the treated and control animals (Fig. 2).

Figure 3. Comparison of proliferation in Min/+ and Apc1638N small intestine. Specimens of small intestine from 65-day-old animals were formalin fixed, embedded in paraffin, and sectioned at 5 μm. Where indicated, animals were treated with sulindac sulfide (0.05 mg/kcal/day) as described in “Materials and Methods.” Sections of small intestine were stained with an antibody to PCNA. The percentage of staining of enterocytes in these crypt-villus units was measured by an observer blinded to the animal’s genetic status using the CAS 200. Values expressed are the percentage of positively stained cells ± SE, with 100% equal to the entire crypt-villus population. A, Min/+ animals; *, P < 0.0001 compared to /+ animals; **, P = 0.008 compared to /+ animals; †, P < 0.0001 compared to Min/+; B, Apc1638N animals: †, P = 0.45 compared to /+ animals; ‡, P = 0.41 compared to /+ animals; ‡, P = 0.97 compared to Apc1638N.

Figure 4. Comparison of apoptosis in Min/+ and Apc1638N small intestine. Specimens of small intestine from 65-day-old animals were formalin fixed, embedded in paraffin, and sectioned at 5 μm. Where indicated, animals were treated with sulindac sulfide (0.05 mg/kcal/day) as described in “Materials and Methods.” Sections of small intestine were stained with an antibody to BrdUrd as described above. The percentage of positively stained enterocytes was measured by an observer blinded to the animal’s genetic status using the CAS 200. Values expressed are the percentage of positively stained cells ± SE, with 100% equal to the entire crypt-villus population. A, Min/+ animals: *, P < 0.0001 compared to /+ animals; **, P = 0.01 compared to /+ animals; †, P < 0.0001 compared to Min/+; B, Apc1638N animals: †, P = 0.13 compared to /+ animals; ‡, P = 0.0003 compared to /+ animals; ‡, P = 0.0001 compared to Apc1638N.
of at least three of these regions (39). The high frequency and more severe phenotype of MCR mutations suggest that mutations altering the β-catenin binding sites are more effective in inducing carcinogenesis. The COOH terminus of APC mediates interactions with the cytoskeleton (34). The final residues of APC bind microtubules as well as the human homologue of the Drosophila discs large (DLG) gene product (40–42). Amino acids 2560–2843 of APC also bind Eβ1, a protein of unknown function (43). Amino acid substitutions in the COOH terminus of APC apparently do not yield a phenotype.

In humans, attenuated FAP is a phenotype characterized by presentation with fewer than 100 colonic polyps and by the development of colorectal cancer at an average of 15 years later than patients with classical FAP (3–5). Genotype-phenotype correlations in humans with FAP show that mutations at either the extreme 5' end or the extreme 3' end of the APC gene are found in patients with attenuated FAP. Germ-line analysis of these individuals reveals mutations before codon 540 (3–5), resulting in a very small protein fragment, or after codon 1600, for which no truncated protein is detected (44, 45). The latter form of mutation is similar to that of the Apc1638N mouse.

Comparisons between the APC proteins present in Min/+ and Apc1638N mice suggest that the difference between the attenuated phenotype and classical phenotype of FAP may be due to the presence of a dominant negative effect in classical FAP. Both of these mice arise from the C57BL/6J background, thus minimizing the differences in genetic background that can contribute to phenotypic variability. Similar to MCR mutations in humans with classical FAP, the Min mutation produces an APC fragment containing the homodimerization domain and the armadillo repeat sequences but lacking the constitutive and kinase-regulated β-catenin binding sites. This truncated protein can therefore form a homodimer with wild-type APC. It is possible that this interaction produces an abnormal APC function, resulting in a dominant negative effect. In contrast, in the Apc1638N mouse, as in humans with attenuated FAP resulting from mutations after codon 1600, the extreme 5' or null mutation does not produce a heterodimer between wild-type and truncated APC. In this case, Apc acts as a classic tumor suppressor gene, requiring random loss of the second Apc allele for tumor development.

Analysis of the growth characteristics of preneoplastic tissue from Min/+ and Apc1638N mice also supports the presence of a dominant negative effect by truncated APC. In the Apc1638N mouse bearing only wild-type APC, tumors are less frequent, the progression phase to cancer is longer, and the growth characteristics of preneoplastic tissue are indistinguishable from those of wild-type animals. In contrast, in the Min/+ mouse, the APC fragment retaining the homodimerization domain and a portion of the armadillo repeat sequences is associated with a decrease in the enterocyte migration rate and with a 25% increase in the time that an enterocyte resides in the mucosa. This increased enterocyte residence time provides an enhanced opportunity for the cell to accumulate the additional genetic and epigenetic alterations required for tumor development. The preneoplastic intestinal epithelium of Min/+ also exhibits increased β-catenin expression, a factor that may contribute to accelerated carcinogenesis.

The truncated protein resulting from Min/+ mutation lacks the region necessary for APC-facilitated degradation of β-catenin. Therefore, it is possible that the elevated β-catenin expression and altered migration observed in Min/+ animals result from a gene dosing effect, rather than a dominant negative effect. In this study, we found an absence of β-catenin elevation or migration defect in the Apc1638N mouse, thus making a gene dosing effect less likely.

Substantial data from murine and human studies indicate that the effect of an Apc mutation can be modulated by antiprostaglandin agents. In the Min/+ mouse, sulindac (19), sulindac sulfide (20), piroxicam (46), and aspirin (47) all inhibit tumor formation to varying degrees. The most effective inhibition is by sulindac, a drug that has also been shown to decrease the size and number of rectal polyps in
humans with FAP (21–23). However, many patients with FAP who have established adenomatous polyps respond to sulindac partially or not at all (22, 48–50). Our data suggest that sulindac modulates the effects of the Min mutation upon enterocyte migration, but that the migration rate is unaffected when the mutation lacks the homodimerization domain or armadillo repeat sequences. It is unclear how this information from preneoplastic tissue relates to the treatment or regression of established polyps that have lost both APC alleles. In this study, we found that sulindac slightly increased apoptosis in the enterocytes of Apc1638N mice. Intestinal cell apoptosis in response to sulindac has been observed in humans with FAP (51, 52), in colorectal cancer cell lines (53–55), and in animal models (19, 20). Nonsteroidal anti-inflammatory-related induction of apoptosis is therefore a possible mechanism of the antitumor effect of sulindac. Studies are underway to determine whether sulindac will prevent or treat tumors in Apc1638N mice.

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