Advances in Brief

Genetic Disruption of Ptgs-1, as well as of Ptgs-2, Reduces Intestinal Tumorigenesis in Min Mice

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Abstract

Two isoforms of cyclooxygenase (COX) are known, and to date most studies have implicated COX-2, rather than COX-1, as the isoform involved in colon carcinogenesis. In the present study, we show that homologous disruption of either Ptgs-1 or Ptgs-2 (genes coding for COX-1 or COX-2, respectively) reduced polyp formation in Min/+ mice by ≈80%. Only COX-1 protein was immunohistochemically detected in normal intestinal tissue, whereas both COX-1 and variable levels of COX-2 protein were detected in polyps. Prostaglandin E2 was increased in polyps compared with normal tissue, and both COX-1 and COX-2 contributed to the PGE2 produced. The results indicate that COX-1, as well as COX-2, plays a key role in intestinal tumorigenesis and that COX-1 may also be a chemotherapeutic target for nonsteroidal anti-inflammatory drugs.

Introduction

Colorectal cancer is the most common cancer in North America and the second leading cause of cancer deaths in the United States. Several independent lines of research support a chemopreventive association between NSAIDs2 and a reduced risk for colorectal cancer (1–6). Although the mechanism(s) by which NSAIDs reduce intestinal tumors are not precisely known, their inhibition of COX activity (7) is thought to be important. Two isoforms of COX, COX-1 and COX-2, have been characterized (8), and studies with Ptgs-1 and Ptgs-2 (genes coding for COX-1 and COX-2, respectively) knockout mice indicate that the isoforms have different physiological roles (9–11). Several types of studies have suggested that COX-2, rather than COX-1, is the isoform contributing to colorectal cancer development (12–15).

To determine whether COX-1 may also contribute to the development of intestinal cancer, we bred mice disrupted for the Ptgs-1 (9) or Ptgs-2 (10) genes to the Min/+ mouse (16). The Min/+ mouse contains a truncating mutation in the Apc gene and spontaneously develops intestinal adenomas. In the present study, we used the Min/+ mouse to demonstrate that the deficiency of COX-1, as well as of COX-2, reduces intestinal polyp formation.

Materials and Methods

Development of Mice. In an attempt to have all mice on a homogeneous background, the Ptgs-1 (9) and Ptgs-2 (10) mutations were transferred from the 129 Ola/C57Bl/6 background onto the C57Bl/6 background prior to crossing with the Min/+ mouse (Jackson Laboratories) already on the C57Bl/6 background. The Ptgs-1 knockout was transferred without difficulty. However, backcrossing the Ptgs-2 knockout into the C57Bl/6 strain was detrimental to the generation of Ptgs-2(-/-) mice. Therefore, Ptgs-1(-/-) or Ptgs-2(-/-) mice at three generations into the C57Bl/6 background were used to cross with the Min/+ line. All mice used in the study were four to five generations into the C57Bl/6 background. Because COX(+/-), COX(+/-), and COX(-/-) Min mice were obtained by similar breeding strategies, differences in modifying genes, other than Ptgs-1 and Ptgs-2, were not considered to be factors when the tumorigenic responses of wild-type and COX-deficient mice were compared.

Determination of Tumor Numbers and Histological Analysis. To determine the number of intestinal tumors, the entire intestinal tract was removed, opened longitudinally, and washed with cold saline, and the proximal, mid, and distal sections of the small intestine, along with the colon, were spread flat, mucosal surface up, on filter paper for counting of nodules. Macroscopic nodules were scored with a limit of detectability of 1 mm. Selected sections of the tract were fixed in 10% neutral buffered formalin (NBF), paraffin embedded and histologically sectioned for immunohistochemistry. Swiss rolls (17) of intestinal sections from mice of each genotype were likewise fixed in 10% neutral buffered formalin and sectioned for microscopic examination to assess correlations between genotype and the incidence of preneoplastic lesions, as well as the histomorphology of adenomas. Microscopic examination of Swiss rolls also confirmed that nodules corresponding to gut-associated lymphoid tissue were few in number relative to adenoma nodules and did not affect tumor numbers obtained by macroscopic counting.

Immunohistochemistry Protocol. Paraffin-embedded sections of intestinal tissue were stained according to the protocol from the Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA). The polyclonal antibodies used were goat-antimouse COX-1 (1:4000; Santa Cruz Biotechnology, Santa Cruz, CA) or rabbit-antimouse COX-2 (1:4000; Cayman Chemical, Ann Arbor, MI). Immunoreactivity was detected with 3,3-diaminobenzidine (Sigma Chemical Co., St. Louis, MO), and slides were counterstained with Mayer’s hematoxylin (Sigma). Intestinal tissues from COX-1(-/-) or COX-2(-/-) were run as negative controls to check the specificities of the respective antibodies.

Prostaglandin E2 Analyses. For PGE2 analysis, normal or tumor tissue was excised and snap frozen in liquid N2. The frozen tissues were thawed, weighed, and homogenized in 0.5–1.5 ml of 50 mM Tris-HCl (pH 7.4) containing 5 μg/ml indomethacin. Often, it was necessary to pool two to three polyps from a particular mouse to obtain adequate tissue prior to homogenization. Homogenates were centrifuged at 1700 × g, 4°C, and the supernatant was analyzed for PGE2 levels using the Amersham-Pharmacia Biotech (Piscataway, NJ) 125I-labeled PGE2 RIA.

Statistical Analyses. For tumor counts and PGE2 levels, ANOVA procedures were used to assess sex and genotype differences. No significant differences were observed between males and females. Therefore, the data were pooled from the two sexes. The Freeman-Tukey Transformation for Poisson data (18) was used as a variance stabilizing logarithmic transformation. Although some degree of extra Poisson variability was present in the tumor counts, the Freeman-Tukey transformation was successful in eliminating the heterogeneity of variances across groups. If overall differences among genotypes were detected, pairwise comparisons were made by Fisher’s LSD test (19).

Received 5/19/00; accepted 7/19/00.

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2 The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase; Min, multiple intestinal neoplasia; Apc, adenomatous polyposis coli gene; PGE2, prostaglandin E2; LSD, least significant difference.
Results

Genetic Disruption of \( \text{Ptgs-1} \) or \( \text{Ptgs-2} \) Reduces the Number of Polyps in \( \text{Min}^+/+ \) Mice. As shown in Fig. 1A, a statistically significant gene dosage-dependent reduction (43 and 77%, respectively) in the number of intestinal tumors was observed when \( \text{Ptgs-I}(+/+) \) and \( \text{Ptgs-I}(+/-) \) \( \text{Min}^+/+ \) mice were compared with \( \text{Ptgs-I}(+/-) \) \( \text{Min}^+/+ \) mice at 6 months of age. Similar results were obtained from \( \text{Ptgs-I}(+/-) \) \( \text{Min}^+/+ \), \( \text{Ptgs-I}(+/-) \) \( \text{Min}^+/+ \), and \( \text{Ptgs-I}(+/-) \) \( \text{Min}^+/+ \) mice at 8 months of age (data not shown). Survival studies showed that \( \text{Ptgs-I}(+/-) \) \( \text{Min}^+/+ \) mice lived \( \sim \)10 months, and \( \text{Ptgs-I}(+/-) \) \( \text{Min}^+/+ \) mice lived 12 months or longer compared with the 7–8-month life span of \( \text{Ptgs-I}(+/-) \) \( \text{Min}^+/+ \) mice. In 1-year-old \( \text{Ptgs-I}(+/-) \) \( \text{Min}^+/+ \) mice, the numbers of tumors (2.5 ± 0.8 in proximal; 5.5 ± 2.0 in mid; 8.8 ± 3.0 in distal small intestines; and 0.5 ± 0.5 in colon) were only slightly increased over those in 6-month-old mice with equivalent genotypes.

Oshima et al. (15) had shown previously that \( \text{Ptgs-2} \) deficiency decreased intestinal tumor formation in an Apc knockout mouse. Therefore, to determine whether \( \text{Ptgs-2} \) deficiency could also reduce tumorigenesis in the \( \text{Min}^+/+ \) mouse, the tumor numbers in \( \text{Ptgs-2}(+/-) \) \( \text{Min}^+/+ \) mice were compared with \( \text{Ptgs-2}(+/-) \) and \( \text{Ptgs-2}(+/-) \) \( \text{Min}^+/+ \) mice. The data in Fig. 2B show that a significant reduction (84%) in polyps was also observed in \( \text{Ptgs-2}(+/-) \) \( \text{Min}^+/+ \) mice. For \( \text{Ptgs-2}(+/-) \) \( \text{Min}^+/+ \), the decrease in polyps was \( \sim \)10% compared with \( \text{Ptgs-2}(+/-) \) \( \text{Min}^+/+ \) mice (Fig. 1B). Like the \( \text{Ptgs-I}(+/-) \) \( \text{Min}^+/+ \) mice, the survival of \( \text{Ptgs-2}(+/-) \) \( \text{Min}^+/+ \) mice \((n = 2)\) was also increased to \( \sim \)1 year. Therefore, our observation that \( \text{COX-2} \) deficiency causes effects in the \( \text{Min}^+/+ \) mouse similar to those seen by Oshima et al. (15) in the Apc\(^{-/-}\) knockout mouse suggests that the \( \text{Ptgs-2} \), as well as the \( \text{Ptgs-1} \), effects observed in the present study are not limited to the \( \text{Min}^+/+ \) mouse.

The distribution of tumors was similar in all \( \text{Ptgs} \) genotypes of \( \text{Min}^+/+ \) mice, with a proximal to distal increase in tumor burden in the small intestine and with few tumors in the colon. In \( \text{Min}^+/+ \) mice with wild-type \( \text{Ptgs} \) alleles, tumors ranged from 1 mm (the limit of macroscopic detectability) to 7 mm in size; the larger tumors were typically broad based with depressed centers. Those in the colon were fewer in number and more polypoid in shape. Intestinal tumors in \( \text{Ptgs-I}(+/-) \) and \( \text{Ptgs-2}(+/-) \) \( \text{Min}^+/+ \) mice ranged from 1 to 4 mm. Analysis of Swiss rolls (17) prepared from the intestinal tracts from mice of all genotypes indicated that most tumors were adenomas, based on irregular glands that were raised above the mucosal surface and were lined with atypical epithelial cells. The numbers of adenomas determined by analysis of the Swiss roll sections (data not shown) correlated with those determined by gross counts shown in Fig. 1.

**COX-1 Is Immunologically Detected in Normal Tissue Whereas Both COX-1 and COX-2 Are Detected in Polyps.** Normal and neoplastic tissues from all mouse genotypes were immunostained with antibodies specific for COX-1 or COX-2. Only COX-1 was detected in normal tissue of all mouse genotypes except from mice lacking a functional \( \text{Ptgs-I} \) gene. COX-1 was localized to the inner muscular layer, cells in the lamina propria, and a few rare villous epithelial cells.
in the mucosa (Fig. 2, a and b). This pattern of COX-1 immunoreactivity was similar in both normal and neoplastic tissue (Fig. 2, c and d). Whereas COX-2 protein was generally not detectable in normal intestinal tissue, localized areas of COX-2 immunostaining were detected in cells of the lamina propria in many adenomas (except those from Ptgs-2(−/−) mice; Fig. 2, e and f). However, the size of the positive regions and intensity of COX-2 immunostaining varied from polyp to polyp, with smaller polyps generally showing less detectable COX-2 protein.

**PGE2 Production in Normal Intestinal Tissue and in Polyps.** To determine the relative contribution of the COX isoforms to intestinal prostaglandin production, PGE2 was used as an indicator of prostaglandin synthesis because it is a prostaglandin that is increased in adenomas. Fishers LSD test was used to determine statistical significance of normal tissue from 6-month-old mice; Fig. 1). Furthermore, it was observed that both COX-1 and COX-2 contribute to PGE2 production in normal tissue, because PGE2 levels are reduced by 99% in Ptgs-1(−/−) and Ptgs-2 Min/+ mice. The data show that COX-1 is the major isoform responsible for basal PGE2 production in normal tissue, because PGE2 levels are reduced by 99% in Ptgs-1(−/−) mice. PGE2 levels were increased in polyps compared with normal tissue in the distal intestine (Fig. 3) in wild-type mice and the data from the COX-1 and COX-2 deficient mice indicate that both COX-1 and COX-2 contribute to PGE2 production in the polyp. Similar results were obtained when colonic normal tissue and polyps were compared (data not shown). In summary, the data show that COX-1 is the major source of PGE2 in normal tissue and that both COX-1 and COX-2 contribute to PGE2 production in polyps.

**Discussion**

The data demonstrate that the deficiency of either COX-1 or COX-2 reduces intestinal tumorigenesis in the Min/+ mouse. However, the deficiency of either isoform still allowed some tumor formation (Fig. 1) and thus raises the question as to whether the isoforms can partially substitute for one another, or if COX independent mechanisms are involved in the development of these tumors. Notwithstanding, the data show that the functional presence of both COX-1 and COX-2 is required to produce the expected level of polyp formation in the Min/+ mouse.

The deficiency of either COX-1 or COX-2 caused similar decreases in intestinal tumorigenesis in the Min/+ mouse (i.e., 77 and 84%, respectively; Fig. 1). Furthermore, it was observed that both COX-1 and COX-2 contributed to PGE2 production in polyps (Fig. 3). Simplistically, one possible interpretation of the data may be that it is the total prostaglandin level in the incipient polyp that is important for adenoma development, and that decreased prostaglandin production attributable to the loss of either isoform significantly reduces tumor formation. In support of this possibility, it has recently been reported that mice deficient in the PGE2 receptor, EP1, show about a 40% decrease in aberrant crypt foci after azoxymethane treatment (21). Furthermore, an EP1 antagonist decreased the number and size of polyps formed in the Min/+ mouse (21). Both COX-1 and COX-2 could contribute to the production of PGE2, which interacts with the EP1 receptor. Alternatively, the COX isoforms could lead to the production of different prostaglandins and thereby influence tumorigenesis through different receptor-mediated pathways. As discussed below, it is also possible that the individual COX isoforms contribute to polyp formation at different stages of the tumorigenesis process.

Recent studies have provided some insight into possible roles of COX-2 in intestinal tumorigenesis (22). Oshima et al. (15) demonstrated previously that COX-2 deficiency decreased intestinal tumorigenesis in an Apc knockout mouse. Additionally, these authors observed that in Ptgs-2(+/-) mice COX-2 protein was detectable in the intestinal polyps after they reached a size of ~2 mm. Prescott and White (22), in their discussion of the work of Oshima et al. (15), postulated that COX-2 was up-regulated after the loss of the wild-type Apc allele and that COX-2-derived prostaglandins contributed to tumor promotion. The data we obtained with the COX-2(−/−) Min/+ mouse are essentially the same as those reported by Oshima et al. (15), and therefore COX-2-derived prostaglandins could also contribute to tumor promotion in the Min/+ mouse. Similar to Oshima et al. (15), we observed that COX-2 expression was in the interstitial cells rather than the epithelial cells of the polyp. It has been reported recently that macrophages in the lamina propria of the polyps of the Min/+ mouse were responsible for the increased COX-2 expression (23). Therefore, our data, and those of Oshima et al. (15), Hull et al. (23), and Shattuck-Brandt et al. (24) raise the question as to whether COX-2 expression is required in the epithelial tumor cells or whether COX-2-derived prostaglandins from the interstitial cells at this stage of tumor development can act by a paracrine mechanism on the neoplastic epithelial cells.

The possible role(s) of COX-1 in intestinal tumorigenesis have received less attention than those for COX-2. Our data show that COX-1 is constitutively expressed in normal intestinal tissue (Fig. 2) and that although it is the primary source of prostaglandins as measured by PGE2 production in intestinal tissue (Fig. 3), no pathology of intestinal tissue was detected in COX-1(−/−) mice (9). However, both COX-1 and COX-2 contribute to PGE2 levels in the adenoma (Fig. 3). Therefore, COX-1 could exert its effects in the tumorigenesis process both at an early stage and at later stages in tumor development. In support of an early role for COX-1, studies have indicated that COX-1 can metabolically activate procarcinogens to mutagenic intermediates (3), and that aspirin can inhibit this metabolic activation. Craven and DeRubertis (4) demonstrated that aspirin, a more effective inhibitor of COX-1 than COX-2 (7), administered at the time of 1,2-dimethylhydrazine treatment reduced intestinal tumorigenesis by 60%, whereas starting aspirin administration after the 1,2-dimethylhydrazine had little effect on tumorigenesis. In addition to the possible activation of dietary procarcinogens, normal COX-1 metabolism of endogenous arachidonic acid can lead to the generation of a known mutagen, malondialdehyde (3). Because the Min/+ mouse is already genetically mutated at one Apc allele, a second mutagenic event according to the two-hit mechanism of Knudson (25) is required. It has been shown that 100% of the spontaneous polyps in the C57 Bl/6-Min/+ mouse lose the wild-type Apc allele (16). It is possible that in COX-1(−/−) mice, malondialdehyde production decreases, and that a second mutagenic event, possibly leading to the loss of the wild-type allele, is less frequent, and therefore, fewer tumors result. Alternatively, independent of contributing to mutagen production, COX-1 has been shown to protect colonic stem cells after gamma irradiation. Cohn et al. (26) demonstrated that after in vivo
gamma irradiation, COX-1 produced prostaglandins that significantly enhanced stem cell survival and growth ex vivo. In the absence of COX-1 in the Min/+ mouse, genetically damaged cells and/or cells undergoing the loss of the wild-type Apc allele may have impaired survival and thus lead to less adenoma formation. However, this possible early role for COX-1 in intestinal tumorigenesis does not preclude COX-1-derived prostaglandins from also contributing to tumor promotion.

In the present study, we have demonstrated that both COX-1 and COX-2 deficiencies reduce the tumorigenic responses in the Min/+ mouse. Although our data do not allow us to define separate roles for COX-1 and COX-2, based on our observations and data from previous studies, we have speculated that the two isoforms function via different mechanisms and/or during different stages of the tumorigenesis process. The possibility that the COX isoforms act at different stages during tumor development suggests that COX dual inhibitors may be effective at both early and late stages, whereas selective inhibitors might be more effective when administered either early (COX-1 specific) or late (COX-2 specific). In summary, our data show that genetic ablation of either COX isoform can significantly impact the course of intestinal tumorigenesis in the Min/+ mouse.

Acknowledgments

We thank Dr. Joseph Haseman for performing all statistical analyses in this report and Drs. Amy Moser, Barbara Davis, Thomas Eling, and Carl Barrett for helpful comments during preparation of the manuscript, and Norris Flagler for the photographic work. This report is dedicated to the memory of Patricia Chuладa’s husband and co-author, Morrow B. Thompson, who died last year from prostate cancer. His input and support of these studies were invaluable.

References

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*Cancer Res* 2000;60:4705-4708.

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