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 polyprop 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). 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 effect 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(−/−) mice 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 Analysis. 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).

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1 To 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 Ptgs-1 or Ptgs-2 Reduces the Number of Polyps in 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 Ptgs-I(+/-) and Ptgs-I(-/-) Min/+ mice were compared with Ptgs-I(+/+) Min/+ mice at 6 months of age. Similar results were obtained from Ptgs-I(+/-) Min/+ Ptgs-I(+/-) Min/+ and Ptgs-I(-/-) Min/+ mice at 8 months of age (data not shown). Survival studies showed that Ptgs-I(-/-) Min/+ mice lived ~10 months, and Ptgs-I(-/-) Min/+ mice lived 12 months or longer compared with the 7–8-month life span of Ptgs-I(-/-) Min/+ mice. In 1-year-old Ptgs-I(-/-) 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 Ptgs-2 deficiency decreased intestinal tumor formation in an Apc knockout mouse. Therefore, to determine whether Ptgs-2 deficiency could also reduce tumorigenesis in the Min/+ mouse, the tumor numbers in Ptgs-2(+/+) Min/+ mice were compared with Ptgs-2(+/-) and Ptgs-2(-/-) Min/+ mice. The data in Fig. 2B show that a significant reduction (84%) in polyps was also observed in Ptgs-2(-/-) Min/+ mice. For Ptgs-2(+/+) Min/+, the decrease in polyps was ~10% compared with Ptgs-2(+/-) Min/+ mice (Fig. 1B). Like the Ptgs-I(-/-) Min/+ mice, the survival of Ptgs-2(-/-) Min/+ mice (n = 2) was also increased to ~1 year. Therefore, our observation that COX-2 deficiency causes effects in the Min/+ mouse similar to those seen by Oshima et al. (15) in the Apc(+/−) knockout mouse suggests that the Ptgs-2, as well as the Ptgs-I, effects observed in the present study are not limited to the Min/+ mouse.

The distribution of tumors was similar in all Ptgs genotypes of Min/+ mice, with a proximal to distal increase in tumor burden in the small intestine and with few tumors in the colon. In Min/+ mice with wild-type Ptgs alleles, tumors ranged from 1 mm (the limit of microscopic 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 polyoid in shape. Intestinal tumors in Ptgs-I(-/-) and Ptgs-2(-/-) 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 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 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.

**PGE_2 Production in Normal Intestinal Tissue and in Polyps.** To determine the relative contribution of the COX isoforms to intestinal prostaglandin production, PGE_2 was used as an indicator of prostaglandin synthesis because it is a prostaglandin that is increased in adenomas. The data show that COX-1 is the major isoform responsible for basal PGE_2 production in normal tissue, because PGE_2 levels are reduced by 99% in Ptgs-1(−/−) mice. PGE_2 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 PGE_2 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 PGE_2 in normal tissue and that both COX-1 and COX-2 contribute to PGE_2 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 poly 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 PGE_2 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 PGE_2 receptor, EP_1, show about a 40% decrease in aberrant crypt foci after azoxymethane treatment (21). Furthermore, an EP_1 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 PGE_2, which interacts with the EP_1 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 that mice deficient in the PGE_2 receptor, EP_1, show about a 40% decrease in aberrant crypt foci after azoxymethane treatment (21). Furthermore, an EP_1 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 PGE_2, which interacts with the EP_1 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.

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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.

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