Genetic Deletion of mPGES-1 Suppresses Intestinal Tumorigenesis

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Abstract

Elevated levels of prostaglandin E2 (PGE2) are often found in colorectal cancers. Thus, nonsteroidal anti-inflammatory drugs, including selective cyclooxygenase-2 (COX-2) inhibitors, are among the most promising chemopreventive agents for colorectal cancer. However, their long-term use is restricted by the occurrence of adverse events believed to be associated with a global reduction in prostaglandin production. In the present study, we evaluated the chemopreventive efficacy of targeting the terminal synthase microsomal PGE2 synthase 1 (mPGES-1), which is responsible for generating PGE2, in two murine models of intestinal cancer. We report for the first time that genetic deletion of mPGES-1 in Apc-mutant mice results in marked and persistent suppression of intestinal cancer growth by 66%, whereas suppression of large adenomas (>3 mm) was almost 95%. This effect occurred despite loss of Apc heterozygosity and β-catenin activation. However, we found that mPGES-1 deficiency was associated with a disorganized vascular pattern within primary adenomas as determined by CD31 immunostaining. We also examined the effect of mPGES-1 deletion on carcinogen-induced colon cancer. The absence of mPGES-1 reduced the size and number of preneoplastic aberrant crypt foci (ACF). Importantly, mPGES-1 deletion also blocked the nuclear accumulation of β-catenin in ACF, confirming that β-catenin is a critical target of PGE2 procarcinogenic signaling in the colon. Our data show the feasibility of targeting mPGES-1 for cancer chemoprevention with the potential for improved tolerability over traditional nonsteroidal anti-inflammatory drugs and selective COX-2 inhibitors. [Cancer Res 2008;68(9):3251-9]

Introduction

Prostaglandin E2 (PGE2) is a bioactive lipid that can elicit a wide range of biological effects associated with inflammation and cancer. PGE2 and other prostaglandins (PG) are derived from arachidonic acid incorporated within membrane phospholipids, which is rapidly metabolized by cyclooxygenase (COX) enzymes and subsequently converted into a panel of PGs by specific terminal PG synthases. The physiologic roles of PGE2 are diverse, as it has been shown to stimulate key downstream signaling cascades that regulate cell proliferation, apoptosis, angiogenesis, inflammation, and immune surveillance (1). Elevated levels of COX-2 and concomitant overproduction of PGE2 are often found in human colon adenomas and adenocarcinomas (2, 3). These and other observations have led to the use of non-steroidal anti-inflammatory drugs (NSAIDs) as chemopreventive agents for colorectal cancer, including most recently the selective COX-2 inhibitors (e.g., celecoxib, rofecoxib). Regular use of NSAIDs has been shown in clinical trials to markedly reduce the relative risk of developing colorectal cancer by up to 40% to 50% (4). However, long-term clinical use of these agents is not without risk because they have been associated with gastrointestinal toxicity and an increased risk of cardiovascular events associated with the nonspecific reduction of key protective PGs (5–7).

An alternative approach for reducing the levels of PGE2 without affecting the production of other key PGs has focused on the inducible form of terminal PGE2 synthase (PGES), microsomal PGES-1 (mPGES-1; ref. 8). Three closely related PGES isoforms are expressed in the intestine: cytosolic PGES (cPGES), mPGES-1, and mPGES-2. Whereas cPGES and mPGES-2 are constitutively expressed at relatively low levels, mPGES-1 is highly inducible by growth factors and proinflammatory stimuli (9). Furthermore, in vitro studies have shown that mPGES-1 is functionally coupled with COX-2, thereby efficiently generating PGE2 during inflammation (10, 11). This cooperative action of COX-2 and mPGES-1 has also been shown in a variety of human cancers including colon cancer (12–18). The tumorigenic potential of mPGES-1 has also been shown in cell culture systems where cotransfection of COX-2 and mPGES-1 induced rapid proliferation in HEK293 cells (11). Moreover, HEK293 cells cotransfected with COX-2 and mPGES-1 formed large, well-vascularized tumors when transplanted into nude mice (18). These cell culture studies have provided the rationale for conducting a comprehensive analysis of the potential role of mPGES-1 in cancer pathogenesis with the use of mouse genetic models.

In the present study, we generated Apc<sup>14/14</sup> mice deficient in mPGES-1 (Apc<sup>14/14;mPGES-1<sup>−/−</sup></sup>) to evaluate the potential chemopreventive efficacy of targeting mPGES-1 in colorectal cancer. We report for the first time that genetic deletion of mPGES-1 results in a marked suppression of intestinal cancer growth, an effect that is directly related to the loss of sustained PGE2 production within the intestinal mucosa. We also examined the effect of mPGES-1 deletion on carcinogen-induced colon cancer and found that mPGES-1 deletion suppresses the growth of preneoplastic aberrant crypt foci (ACF). This effect may be associated with attenuated nuclear translocation of β-catenin in azoxymethane-induced colonic lesions, showing a direct association between PGE2 and β-catenin in vivo. Our data show the feasibility of targeting mPGES-1 for cancer chemoprevention and further illustrate that β-catenin is a critical target of PGE2 procarcinogenic signaling in the colon.
Materials and Methods

Generation of Apc\(^{D14/+}\):mPGES-1\(^{-/-}\) compound mutant mice. Apc\(^{D14/+}\) mice on the C57BL/6 background were kindly provided by Dr. Christine Perret at Universite Paris V (Paris, France) and the generation of this mouse line has previously been described (19). mPGES-1\(^{-/-}\)/C0/C0 mice on a C57BL/6 background were provided by Merck Frosst Canada, Ltd. and the generation of the mouse lines has previously been described (20). To generate the compound mutant mice, male Apc\(^{D14/+}\) mice were crossed with female mPGES-1\(^{-/-}\)/C0/C0 mice. Genotyping was done by tail biopsy. Mice were maintained in a temperature-controlled, light-cycled room and allowed free access to drinking water and standard diet (LM-485, Harlan Teklad). Animals and food were weighed twice weekly and mice were checked daily for signs of weight loss or lethargy indicating intestinal obstruction or anemia associated with tumors. Animal experiments were conducted with approval from the Center for Laboratory Animal Care committee, University of Connecticut Health Center.

Tumor incidence and multiplicity. Both male and female Apc\(^{D14/+}\):mPGES-1\(^{-/-}\) and Apc\(^{D14/+}\):mPGES-1\(^{-/-}\) mice were sacrificed at 16 wk of age for polyp scoring and histologic analyses. The entire small intestine and colon were harvested and flushed with ice-cold PBS and excised longitudinally. Specimens were fixed flat in 10% neutral buffered formalin solution for 4 h and stored in 70% ethanol. Tissues were stained with 0.2% methylene blue and the number and size of tumors were scored under a dissecting microscope.

Azoxymethane treatment. Four-week-old male and female mPGES-1\(^{+/+}\) and mPGES-1\(^{-/-}\) mice were placed on a semipurified diet (AIN93M, Harlan Teklad). At 5 wk of age, mice received either six weekly injections of azoxymethane (Sigma-Aldrich Co.; 10 mg/kg body weight, i.p.) or 0.9% NaCl as a vehicle control. Nine weeks after the last injection, mice were sacrificed and the number and size of ACF were scored as described in our previous studies (21–23).

Quantitative real-time PCR. Polyps and normal-appearing intestinal mucosa were harvested from the small intestine and colon of 16- to 20-wk-old Apc\(^{D14/+}\) compound mutant mice. Tissues were homogenized and total RNA was extracted with TRizol reagent (Invitrogen Corp.). cDNA was synthesized using iScript according to the manufacturer's protocol (Bio-Rad Laboratories). mRNA expression levels were examined with SYBR green (Bio-Rad Laboratories) using the following primers; mPGES-1, forward 5'-ccagtattacaggagtgacccagat-3' and reverse 5'-ggaaaggatagattgtctccatgtc-3'; mPGES-2, forward 5'-tggtgtgctgcgagtgatg-3' and reverse 5'-caggtaccaaggcttgagtg-3'; cPGES, forward 5'-tgtttgcgaaaaggagaatccg-3' and reverse 5'-ccatgtgatccatcatctcagag-3'; cPLA\(_2\), forward 5'-cattttgggttcaggtgggg-3' and reverse 5'-ccagcaatgtatgtagcacagtc-3'; COX-1, forward 5'-agtgcggtcacaaccttatcc-3' and reverse 5'-gcagaatgcgagtatagtagctc-3'; and COX-2, forward 5'-gcagaatgcgagtatagtagctc-3' and reverse 5'-gcagaatgcgagtatagctc-3'.

Figure 1. mPGES-1 deletion suppresses intestinal tumorigenesis in Apc\(^{D14/+}\) mice. A, representative H&E staining of small intestinal polyps from 16-wk-old Apc\(^{D14/+}\):mPGES-1\(^{+/+}\) compound mutant and wild-type mice. Bar, 100 \(\mu\)m. B and C, total number and size of polyps per mouse in the small intestine (B) and colon (C) from Apc\(^{D14/+}\):mPGES-1\(^{+/+}\) (\(n = 29\)) and Apc\(^{D14/+}\):mPGES-1\(^{-/-}\) (\(n = 46\)) mice, respectively. Polyps are classified by size as indicated. Each data point represents an individual mouse and the numbers indicate the mean value for each group. *, \(P < 0.05\), compared with Apc\(^{D14/+}\):mPGES-1\(^{+/+}\) mice (Student's t test).
forward 5’-gctttagaatggtgctag-3’ and reverse 5’-attttcctagacaccccttt-tag-3’. PCR amplification was carried out by denaturing cDNA at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 55°C for 45 s. mRNA expression levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

**Immunohistochemistry.** Small intestine and colon tissues were paraffin embedded and sectioned at 7-μm thickness. Tissue sections were deparaffinized and incubated with 1 to 3 % hydrogen peroxide for 10 min at room temperature. Sections were subjected to antigen retrieval and blocked with 10% normal goat or horse serum in PBS. Sections were then incubated overnight at 4°C with anti–proliferating cell nuclear antigen (PCNA; 1:250; Abcam, Inc.), anti–adenomatous polyposis coli (Apc; 1:250; Abcam, Inc.), anti–β-catenin (1:2,000; Sigma-Aldrich), anti-cleaved caspase-3 (1:200; Cell Signaling Technology, Inc.), goat anti-CD31 (M20; 1:500; Santa Cruz Biotechnologies, Inc.). Sections were washed and incubated with biotinylated anti-rabbit, anti-mouse, or anti-goat secondary antibody for 30 min at room temperature. Sections were washed and then incubated with avidin–biotin complex reagent (Vector Laboratories, Inc.) for 30 min at room temperature, followed by signal detection with 3,3’-diaminobenzidine solution (Vector Laboratories). Tissues were counterstained with hematoxylin. For PCNA and cleaved caspase-3 staining, the number of positive cells per 50 μm² of polyp section was scored for quantification. For CD31 staining, the total number of cells positive for the protein and the number of vessels with clear lumen per 114 mm² of polyp section were scored for quantification.

**Measurements of prostanooids by liquid chromatography-mass spectrometry.** The distal portions (~5 cm) of small intestine and entire colons were harvested from 10-wk-old mice and snap-frozen in liquid nitrogen. Frozen tissues were homogenized at 4°C in PBS supplemented with 10 μM/L indomethacin and 1× Complete Protease Inhibitor Tablet (Roche Diagnostics) using a tissue homogenizer (Polytron PRO 200). Following homogenization, the samples were centrifuged at 10,000 × g for 10 min at 4°C. Supernatants were isolated and stored at −80°C until further analysis. PG standards and deuterated PGs were purchased from Cayman Chemical. The levels of PGF₂α, 6-keto-prostaglandin F₁α (6-keto-PGF₁α, stable breakdown product of PGI₂), PGD₂, thromboxane B₂, and prostaglandin F₃α (PGF₃α) were quantify by liquid chromatography-mass spectrometry as previously described (24). Briefly, samples (50 μL) containing deuterated PGs as internal standards were injected onto a 4.6 × 150-mm YMC ODS-A column using a Shimadzu SIL-HTc autosampler and LC-10ADVP pumps. Prostaglandins were eluted at 1 mL/min using a linear gradient from 10% to 90% acetonitrile versus 0.1% formic acid over 10 min. Detection of PGs was achieved using a Scion API-4000 triple quadruple mass spectrometer. Analysis was carried out using negative ion electrospray with 1 mL/min entering the source. The limit of detection was determined as 16 pg/mL by a standard curve.

**Statistical analyses.** For the comparison of size and number of polyps and ACF, statistical analyses were done with Student’s t test. Statistical analysis for PG levels was done with one-way ANOVA with a Bonferroni comparison test. Differences were considered statistically significant at P < 0.05.

**Results**

**Homoygous deletion of mPGES-1 suppresses intestinal tumorigenesis in Apc<sup>+/-</sup> mice.** To determine the effect of mPGES-1 status on intestinal tumorigenesis, we compared the number and size of small intestinal and colonic polyps in Apc<sup>+/+</sup>:mPGES-1<sup>+/+</sup> and Apc<sup>+/+</sup>:mPGES-1<sup>-/-</sup> mice at 16 weeks of age. Analysis of methylene blue–stained whole mounts revealed a marked reduction in polyp size in the small intestine of Apc<sup>+/+</sup>:mPGES-1<sup>-/-</sup> mice. Sections were further evaluated by a board-certified veterinary pathologist (P.R.N.). As shown in Fig. 1A, histologic examination of the small intestine revealed the presence of large, polypoid or sessile, noninvasive adenomas in the Apc<sup>+/+</sup>:mPGES-1<sup>-/-</sup> mice, consistent with earlier findings in Apc<sup>+/+</sup>:mPGES-1<sup>-/-</sup> mice (19). Although adenomas in the Apc<sup>+/+</sup>:mPGES-1<sup>-/-</sup> mice shared similar histologic features, they were considerably smaller and appeared more like microadenomas (Fig. 1A). Cytologically, there were no differences noted between the two groups. More importantly, there were no histologic differences observed between adjacent normal tissues from the two groups. Furthermore, there was no evidence of infiltrative nests of cancer cells occurring within the local lymphoid nodules, nor evidence of transmural spread. In addition, polyps were located primarily within the distal small intestine, a location that was unaffected by mPGES-1 genotype.

As shown in Fig. 1B, deletion of mPGES-1 significantly reduced the total number of polyps in the small intestine of Apc<sup>+/+</sup> mice by 66% (92.6 ± 5.4 versus 31.1 ± 1.8 in Apc<sup>+/+</sup>-mPGES-1<sup>-/-</sup> mice, respectively; P < 0.01). However, elimination of mPGES-1 resulted in 83% (70.2 ± 4.5 versus 12.3 ± 1.3; P < 0.01) and 94% (12.2 ± 1.5 versus 0.7 ± 0.2; P < 0.01) reductions in medium-sized (1–2 mm) and large-sized (>2 mm) polyps, respectively (Fig. 1B). Interestingly, there was a shift in the ratio of adenomas according to their size, with mPGES-1 deficiency resulting in smaller adenomas (<1.0 mm in diameter; 10.2 ± 0.9 versus 18.1 ± 1.1 in Apc<sup>+/+</sup>-mPGES-1<sup>-/-</sup> wild-type and null, respectively; P < 0.01; Fig. 1B). These results suggest that mPGES-1 does not directly affect tumor initiation but plays an important role in tumor promotion.

**Figure 2.** Genetic deletion of mPGES-1 reduces PGF₂α without metabolic shunting. Levels of prostaglandins were determined in the small intestine and colon of 10-wk-old Apc<sup>+/+</sup> compound mutant and wild-type mice by liquid chromatography-mass spectrometry as described in Materials and Methods. Levels of PGF₂α, 6-keto-PGF₁α, thromboxane B₂ (TxB₂), PGD₂, and PGF₂α, in the small intestine (A) and colon (B). Concentrations are expressed as nanograms of PG per milligram of protein. Columns, mean of 10 samples per group; bars, SE. *P < 0.05, compared with Apc<sup>+/+</sup>:mPGES-1<sup>-/-</sup> (one-way ANOVA with a Bonferroni comparison test).
In the colon, mPGES-1 deletion resulted in a 51% overall reduction in the number of polyps per mouse (4.1 ± 0.5 versus 2.0 ± 0.3 in wild-type and null, respectively; P < 0.01; Fig. 1C). Although the number of small-sized (≤1.0 mm) and medium-sized (1.0–3.0 mm) adenomas was not significantly altered by mPGES-1 status, there was a marked reduction in large (≥3.0 mm) adenomas (2.6 ± 0.3 versus 0.8 ± 0.1, respectively; P < 0.01; Fig. 1C). These findings were consistent with the growth-suppressive effects of mPGES-1 deletion observed in the small intestine.

Genetic deletion of mPGES-1 reduces PGE₂ without metabolic shunting. There are five major prostanoid species (PGE₂, PGI₂, PGD₂, thromboxane A₂, and PGF₂α), all produced from COX-derived prostaglandin H₂ (PGH₂; ref. 1). The relative levels of these metabolites are controlled by the activities of their respective synthases. Recent studies have shown that PGH₂ can be metabolically shunted into alternative prostanoid synthetic pathways in the gastric mucosa by genetic deletion of mPGES-1 (25). To determine whether prostanoid profiles might be similarly altered in the intestine and colon by mPGES-1 deletion, we performed a liquid chromatography-mass spectrometry analysis of a panel of PGs under basal conditions in normal-appearing intestinal epithelium. As predicted, minimum production of PGE₂ was detected in the small intestine (P < 0.003; Fig. 2A) and colon (P < 0.00002; Fig. 2B) of ApcΔ14+/mPGES-1/− mice. mPGES-1 deletion, however, did not significantly alter the tissue levels of 6-keto-PGF₁α (stable breakdown product of PGI₂), PGD₂, thromboxane B₂ (stable breakdown product of thromboxane A₂), and PGF₂α, providing direct evidence for the absence of PGH₂ metabolite shunting in the intestine under basal, nonstimulated conditions (Fig. 2A and B).

Genetic deletion of mPGES-1 alters the expression of PGES isoforms and COXs. RNA was isolated from the small intestine and colon of 16-wk-old ApcΔ14+/− compound mutant and wild-type mice and subjected to quantitative PCR as described in Materials and Methods. A, mRNA expression of mPGES-1, mPGES-2, and cPGES in normal-appearing tissue (N) and polyps (P) in small intestine and colon. B, mRNA expression of cPLA₂, COX-1, and COX-2 in small intestine and colon. Expression levels were normalized to GAPDH as a loading control. Columns, mean of five samples per group; bars, SE. *, P < 0.05, between genotypes (Student’s t test).
of mPGES-2 and cGES (P = 0.1 and P = 0.3, respectively) in normal-appearing small intestinal mucosa. In adjacent polyps, however, there was no compensatory increase in the expression of either PGES isoform. In the colon, cGES expression was slightly increased (P = 0.1) in polyps compared with the normal-appearing tissues from both mPGES-1 wild-type and null mice, suggesting a tumor-specific response. Next, we examined the expression of several key arachidonic acid–metabolizing enzymes (i.e., cPLA2, COX-1, and COX-2), which are often found to be up-regulated under inflammatory conditions or during tumorigenesis (12, 16, 27). As shown in Fig. 3B, COX-2 levels were significantly elevated (3-fold; P < 0.001) in small intestinal polyps. The increase in COX-2 expression in the colon was even more pronounced (up to 30-fold; P < 0.0001). mPGES-1 deletion also resulted in a modest increase in cPLA2 (2-fold; P = 0.2) and COX-2 (3-fold; P = 0.1) expression in normal-appearing small intestinal mucosa, although there were further elevations observed in these end points in polyp tissue. As expected, COX-1 expression was unaffected by mPGES-1 status in either organ (Fig. 3B).

mPGES-1 deficiency does not influence cell turnover in intestinal polyps but affects the formation of organized vasculature. Similar to the Apc<sup>14/+</sup> model, loss of heterozygosity of Apc occurs in all intestinal polyps from Apc<sup>14/+</sup> mice (19, 28). To determine whether mPGES-1 deletion may affect Apc status, immunohistochemistry analysis was done with a COOH terminus–specific antibody. As shown in Fig. 4A, loss of Apc protein was consistently observed in intestinal adenomas regardless of mPGES-1 status, an effect that was independent of polyp size. We then examined serial sections for β-catenin staining and found increased cytoplasmic and nuclear accumulation in polyps derived from either mPGES-1 genotype (Fig. 4A).

Although mPGES-1 status did not affect loss of Apc, its functional elimination resulted in a significant reduction in polyp size, suggesting that mPGES-1 may influence cell turnover. To determine the effects of mPGES-1 deficiency on proliferation and apoptosis, we measured the levels of PCNA (proliferation) and cleaved caspase-3 (apoptosis) in size-matched adenomas. As shown in Fig. 4B, abundant PCNA nuclear staining was evident, distributed equally across the tumor tissue regardless of mPGES-1 status in either genotype (Fig. 4B).

Figure 4. Vascular tube formation is altered by mPGES-1 deficiency in the intestinal polyps of Apc<sup>14/+</sup> mice. Tissue sections were prepared from the small intestine and colon of 16-wk-old Apc<sup>14/+</sup> compound mutant mice (n = 10; Student’s t test) and processed for immunohistochemical analyses as described in Materials and Methods. A, loss of Apc staining is evident in polyps from both genotypes (arrowheads) regardless of size. β-Catenin staining was shifted from the lateral membrane (normal) to the cytoplasm and nucleus (polyps). Bar, 100 μm. Higher magnification of β-catenin staining showing cytoplasmic and nuclear accumulation in both genotypes. Bar, 50 μm. B, PCNA immunostaining shows increased cellular proliferation in polyps relative to adjacent normal crypts in polyps from both mPGES-1 genotypes. Cleaved caspase-3 immunostaining showed minimal positive cells in polyps from both genotypes. Bar, 100 μm. C, CD31 staining showing a number of vessels within the polyps (arrows). CD31-positive cells without obvious tube formation are also present (arrowheads). Bar, 50 μm. Higher magnification of interrupted and developed vessels in mPGES-1−null polyps. Bar, 15 μm. D, total number of CD31-positive cells as well as number of vessels present in the Apc<sup>14/+</sup>:mPGES-1−/− (n = 20) and Apc<sup>14/+</sup>:mPGES-1+/+ (n = 25) polyps. The ratio between CD31-positive cells and vessels indicates the presence of cells that are not participating in the organization of neovessels. Each point represents a polyp section. Bars and numbers indicate the mean from each group. *, P < 0.05, compared with mPGES-1<sup>+/+</sup> (Student’s t test).
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genotype. Similarly, the total number of tumor cells staining positive for cleaved caspase-3 failed to discriminate between genotypes, indicating that tumor cell turnover was unaffected by mPGES-1 status.

Next, to determine the effect of mPGES-1 deficiency on angiogenesis, we examined the expression of CD31 [platelet endothelial cell adhesion molecule 1 (PECAM-1)] within intestinal polyps. As shown in Fig. 4C, CD31 staining in intestinal polyps from Apc<sup>14/+</sup>:mPGES-1<sup>+/+</sup> mice was associated with small blood vessels containing clear lumens. In the Apc<sup>14/+</sup>:mPGES-1<sup>−/−</sup> polyps, blood vessels with small or incompletely formed lumens were present within the tumor stroma (Fig. 4C). Interestingly, there was an abundance of CD31-positive cells scattered within the stroma that were not integrated into the neovessels. Quantification of these structures showed that Apc<sup>14/+</sup>:mPGES-1<sup>−/−</sup> polyps had a significant increase in the overall total number of CD31-positive cells compared with Apc<sup>14/+</sup>:mPGES-1<sup>+/+</sup> CD31 staining (16.4 ± 1.5 versus 35.6 ± 4.6, mPGES-1 wild-type and null, respectively; P < 0.0008; Fig. 4D). Whereas the number of vessels defined by CD31-positive luminal structures remained unchanged between genotypes (8.9 ± 1.0 versus 10.8 ± 1.4; P < 0.3; Fig. 4D), the ratio between total CD31-positive cells and luminal vessels was significantly higher in the mPGES-1 deficient polyps (2.4 ± 0.4 versus 4.1 ± 0.3; P < 0.01; Fig. 4D). These data suggest that mPGES-1 may function to promote endothelial cell and vascular morphogenesis in primary lesions that are needed to support tumor progression.

**mPGES-1 deletion suppresses carcinogen-induced ACF formation by blocking β-catenin nuclear translocation.** To further evaluate the effect of mPGES-1 status on colon carcinogenesis, mPGES-1<sup>−/−</sup> and mPGES-1<sup>−/−</sup> mice received six weekly injections of the organospecific colon carcinogen azoxymethane, and the animals were sacrificed 9 weeks after the last injection. As shown in Fig. 5A, deletion of mPGES-1 resulted in a 51% reduction in the average number of total ACF (20.4 ± 2.0 versus 10.2 ± 1.9; P < 0.01). The frequency of intermediate-sized ACF consisting of three to four crypts showed the greatest suppression (up to 52%; 13.9 ± 1.5 versus 6.8 ± 1.5; P < 0.01; Fig. 5B).

PGE<sub>2</sub> has been shown to play a role in the indirect activation of β-catenin via inactivation of Axin and glycogen synthase kinase 3β (GSK-3β), proteins that form part of the β-catenin degradation complex (29). Unlike the complete loss of Apc function that occurs in Apc<sup>14/+</sup>-derived adenomas, the loss of Apc protein is a gradual process following azoxymethane treatment, accompanied by increased cytoplasmic and nuclear staining of β-catenin (21, 30–32). To examine the effect of mPGES-1 on β-catenin localization, immunostaining was done on serial sections of ACF. As shown in Fig. 6, size-matched dysplastic ACF had similar histologic characteristics regardless of mPGES-1 status, showing a preponderance of hyperchromatic nuclei with pseudostatification and loss of goblet cells. Furthermore, colonic ACF retained Apc expression regardless of mPGES-1 genotype (Fig. 6). In mPGES-1 wild-type colons, extensive cytoplasmic and nuclear accumulation of β-catenin was observed in serial sections of ACF. However, mPGES-1 deletion blocked the nuclear translocation of β-catenin while preserving lateral membrane staining with only minimal cytoplasmic accumulation observed in all five ACF examined. We next analyzed the serial sections for the expression of the β-catenin targets, c-myc and cyclin D1. Although c-myc staining was extensive in the largest colonic lesions and was unaffected by mPGES-1 genotype, cyclin D1 staining was higher in the mPGES-1 wild-type lesions (data not shown). These analyses were done in a small number of samples that it needs to be further examined in future studies.

**Discussion**

Elevated levels of COX-2 are consistently associated with a variety of epithelial tumors (33). COX-2 is responsible for the production of a wide range of PG metabolites, including PGE<sub>2</sub>,
which has long been implicated in tumorigenesis. Recently, a mouse model has been developed in which mPGES-1, a PGE$_2$ terminal synthase, has been genetically deleted (20). Although the involvement of mPGES-1 in cancer promotion has been suggested (11, 12, 18, 34, 35), the present study is the first to directly test the role of mPGES-1 and its metabolic product, PGE$_2$, in the pathogenesis of intestinal cancer. This study may also have important implications for the feasibility of mPGES-1 targeting for colorectal cancer chemoprevention.

Our results show that deletion of mPGES-1 markedly reduces both the number and size of intestinal polyps in Apc$^{+/+}$ mice. Importantly, the absence of mPGES-1 primarily affects the growth of larger polyps, unequivocally establishing the role of PGE$_2$ as a tumor promoter. In addition, the effect of mPGES-1 deletion is long-lasting, in some cases persisting up to 30 weeks (data not shown). Such extended survival and sustained suppression of polyp growth have also been reported in other mouse genetic models targeting PG production. For example, Oshima et al. (36) showed an 86% reduction in small intestinal polyps by deletion of COX-2 in Apc$^{-/-}$ mice. Chulada et al. (37) found an ~80% reduction in polyps in Apc$^{Min/+}$ mice by genetic deletion of either COX-1 or COX-2. Seno et al. (38) showed a lack of COX-2 expression in polyps smaller than 1 mm in Apc$^{-/-}$ mice, postulating that COX-2 induction is necessary for polyp expansion. In fact, we found that COX-2 expression was somewhat less pronounced in the absence of mPGES-1 in colon, suggesting a positive feedback between mPGES-1, PGE$_2$, and COX-2 during polyp expansion. Our results are consistent with those of Takeda et al. (39) suggesting that polyp growth requires sustained and coordinate induction of COX-2 and mPGES-1 to maintain elevated PGE$_2$. Interestingly, COX-2 expression was found to be modestly elevated (~2-fold) in normal-appearing small intestinal mucosa in the mPGES-1 null versus wild-type mice (Fig. 3). This may support the previous finding that PGE$_2$ can negatively regulate COX-2 expression (40).

Despite the modestly elevated COX-2 levels within the normal-appearing small intestine of the mPGES-1 null mice, PG levels did not show a significant metabolic redirection of PGH$_2$ under basal conditions. However, alterations of PG profiles may be tissue specific. Boulet et al. (25) reported that genetic deletion of mPGES-1 resulted in metabolic shunting of PGH$_2$ into alternative prostanoid pathways within the gastric mucosa. A similar metabolic shunting has also been shown in mouse embryonic fibroblasts (41) and in lipopolysaccharide-stimulated macrophages from mPGES-1 null mice (42). Regardless, the absence of PGE$_2$ shunting in normal-appearing intestinal tissue suggests that the toxicity associated with prolonged COX-2 inhibition may be minimized by mPGES-1 targeting. Accordingly, the recent study by Cheng et al. (43) showed that genetic deletion of mPGES-1 does not result in elevated blood pressure or thrombogenesis, effects that underlie the cardiovascular toxicity associated with selective COX-2 inhibition and are largely dependent on imbalances between PGI$_2$ and thromboxane A$_2$ (44). Thus, targeting mPGES-1 may not only provide an effective strategy for chemoprevention of colorectal cancer but may also reduce the risks associated with long-term nonsteroidal anti-inflammatory drug treatment. We cannot yet exclude the possibility that comparable metabolic shunting may have occurred within intestinal polyps as well. However, functional inactivation of mPGES-1 was so effective in the present study that it was not possible to harvest sufficient amounts of polyp tissue to determine PG profiles.

Establishing a mechanism by which reduced PGE$_2$ production suppresses intestinal tumorigenesis is complicated by extensive cross talk occurring between multiple downstream signaling pathways (reviewed in ref. 33). Recently, using colon cancer cells, Castellone et al. (29) showed that PGE$_2$-EP2 signaling results in subsequent inactivation of Axin and GSK-3β. These observations, however, cannot fully explain the suppression of polyp growth that occurs as a result of mPGES-1 deletion in Apc$^{+/+}$ mice because

**Figure 6.** Nuclear translocation of β-catenin is blocked by loss of mPGES-1 in azoxymethane-induced ACF. Representative, size-matched dysplastic ACF were stained with H&E and serial sections were prepared for Apc and β-catenin immunostaining. Apc expression is retained in both mPGES-1 genotypes, whereas cytoplasmic and nuclear β-catenin staining is evident only in wild-type mice. Bar, 100 μm. Higher magnification of boxed area on β-catenin staining, showing intense cytoplasmic and nuclear accumulation of β-catenin in the wild-type and membrane staining in the null mice. Bar, 10 μm.
every polyp sustains loss of the wild-type Apc allele (19, 28) with a consequent activation of β-catenin that may be independent of PGE2-mediated Axin-GSK-3β degradation. To more fully explain the inhibitory effects of mPGES-1 deletion on β-catenin nuclear localization, further analyses of adenomas at the earliest stage may be informative.

Mechanisms by which PGE2 affects cellular proliferation within the intestinal epithelium may be supported by several alternative hypotheses. First, it is possible that PGE2 may be required for full and sustained activation of β-catenin signaling. In fact, Wang et al. (45) showed that promotion of adenoma growth associated with PGE2 results from its ability to transactivate a key β-catenin target, peroxisome proliferator–activated receptor δ (PPARδ; ref. 45). In support of this, Gupta et al. (46) showed that treatment of Apc<sup>Min/+</sup> mice with a PPARδ agonist resulted in a 5-fold increase in polyp size. Thus, it is possible that elevated levels of PGE2 may stabilize PPARδ and accelerate growth of adenomas. Second, it is possible that PGE2 blocks the activity (or expression) of a negative regulator of the Wnt pathway, and its absence releases this proliferative restraint. Lastly, the possibility cannot be ruled out that there are additional factors that may suppress proliferation through Wnt-independent mechanisms, such as the ability of PGE2 to trans-activate the epidermal growth factor receptor, which can trigger a potent mitogenic signaling (47).

As an alternative mechanism for the tumor suppressive effects observed in Apc<sup>14/14</sup>/mPGES-1<sup>−/−</sup> mice, we found significant alterations in vascular organization and structure within mPGES-1<sup>−/−</sup> null polyps. Histologic staining for CD31 (PECAM-1), a cell adhesion molecule expressed on endothelial cells, significantly showed more positive cells within primary adenomas of the mPGES-1<sup>−/−</sup> mice. Interestingly, PGE2 has been shown to promote endothelial cell migration and capillary formation in aortic ring assays (48). Moreover, PGE2 has previously been shown to stimulate tumor angiogenesis by increasing vascular endothelial growth factor (VEGF) via EP2 receptor in Apc<sup>S716+</sup> mice (49). Consistent with these findings, our study showed that the majority of the CD31-positive cells in the mPGES-1 null polyps did not seem to participate in the formation of neovessels, showing that mPGES-1 itself or PGE2 may have a direct role in regulating vascular tube formation. The abnormal vascular structures observed in the Apc<sup>14/14</sup>/mPGES-1<sup>−/−</sup> mice likely contribute to the suppression of tumor growth. Future studies will attempt to determine whether PGE2 drives the vascular organization alone or through regulation of secondary angiogenic factors such as VEGF and fibroblast growth factor 2 within the lesion.

To gain additional insight into the potential role of mPGES-1-derived PGE2 in colon tumorigenesis, we used the chemical carcinogen azoxymethane to induce colon lesions in both mPGES-1<sup>−/−</sup> and mPGES-1<sup>+/−</sup> mice. Azoxymethane induces large numbers of ACF and adenocarcinomas that are confined to the distal colon. In addition, because there is a gradual loss of Apc, the role of PGE2 in β-catenin signaling can be evaluated during ACF formation (21, 30–32). Our results showed that loss of mPGES-1 significantly reduced ACF size, suggesting that promotion associated with PGE2 can also occur early during tumorigenesis. Notably, a striking redistribution of β-catenin within the cytoplasm and nucleus was observed within dysplastic lesions from wild-type mice, an effect that was absent in the colons of mPGES-1 null mice. Although the analyses on downstream targets of β-catenin, such as c-myc and cyclin D1, need to be further examined, this result not only supports an earlier observation in cell culture (29, 50) but also shows that PGE2 may exert direct control over β-catenin translocation in vivo.

In summary, we have shown that genetic deletion of mPGES-1 suppresses intestinal tumorigenesis in Apc<sup>14/14</sup> mice. These studies provide strong evidence that PGE2 plays a key role in tumor promotion. In addition, we report that mPGES-1 deficiency is associated with a disorganized vascular pattern within the primary adenomas, perhaps contributing to the observed suppression in tumor growth. In addition, we show only minimal metabolic shunting of PGH<sub>2</sub> into alternative PGs without a compensatory induction of alternative terminal synthases, including mPGES-2 and cPGES. Finally, deletion of mPGES-1 blocked β-catenin translocation in carcinogen-induced ACF, establishing a direct association between PGE2 and Wnt signaling in vivo. Taken together, our data provide new and convincing evidence to suggest that mPGES-1 may represent a novel target for suppressing tumor growth while minimizing the cardiovascular risk associated with long-term use of coxibs and NSAIDs.

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