Intestinal tumor suppression in Apc<sup>Min/+</sup> mice by prostaglandin D<sub>2</sub> receptor PTGDR

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Introduction

Prostaglandin studies in intestinal neoplasia usually focus on prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), a pro-tumorigenic compound [1–4]. In an opposite effect, knockout of the gene for hematopoietic prostaglandin D synthase (HPGDS) caused more adenomas in Apc<sup>Min/+</sup> mice. Moreover, high HPGDS production from transgenes allowed fewer [5]. Prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) and PGE<sub>2</sub> are both made from PGH<sub>2</sub>, so Hpgds knockouts could have shunted conversion of PGH<sub>2</sub> to PGE<sub>2</sub>. Likewise, HPGDS transgenes could have drawn prostaglandin synthesis away from PGE<sub>2</sub>.

Lewis lung cancer cells implanted onto the backs of mice lacking the PGD<sub>2</sub> receptor (PTGDR, also known as DP1), grew faster than tumors implanted onto wild-type mice [6]. Furthermore, the PTGDR agonist, BW245C, reduced tumor growth. These results support a role for PGD<sub>2</sub> itself.

Tumor suppression by PGD<sub>2</sub> might also occur through inhibition of inflammatory genes by molecules that bind PGD<sub>2</sub> metabolites. For example, PGD<sub>2</sub> metabolites bind...
Prostaglandin D₂ and Intestinal Tumors in Mice

Prostaglandin D₂ (PGD₂) and intestinal tumors have been studied extensively. Here, we show that knockouts of the Apc gene increase tumor numbers in Apc<sup>Min/+</sup> mice, indicating that PGD₂ and PTGDR act to suppress tumors. PPARG had smaller effects in our experiments.

**Material and Methods**

**Mice**

The protocol and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Los Angeles Biomedical Research Institute. C57BL/6, FVB/N, and Apc<sup>Min/+</sup> (C57BL/6; no. 002020) mice came from Jackson (Bar Harbor, ME), as did mice carrying the Cre transgene controlled by the adenovirus EIIa promoter [Tg(EIIa-Cre) C5379Lmgd/J; FVB/N strain; no. 003314]. Mice in which exon 2 of the Pparg gene is flanked by loxP sites were from F. Gonzalez (line B20; FVB/N mouse).

To produce Apc<sup>Min/+</sup> mice with heterozygous Ptgdr knockout mice, we crossed male Apc<sup>Min/+</sup> mice with female homozygous Ptgdr knockout mice. Male Apc<sup>Min/+</sup> mice with heterozygous or homozygous Ptgdr knockout mice were then bred with female homozygous Ptgdr knockout mice to produce Apc<sup>Min/+</sup> mice with homozygous Ptgdr knockout mice (100% C57BL/6).

Our PTGDS transgenic mice (line B20; FVB/N) overexpress human PTGDS in all tissues. Reported basal brain levels of PGD₂ were 1.5-fold higher than wild-type levels and rose fivefold upon stimulation. PGE₂ levels did not change. The mice had more eosinophilia in a bronchial asthma model, compared to HPGDS transgenic mice.

To generate heterozygotic Parg knockout mice, we crossed Ppar<sup>lox/lox</sup> mice with Tg(EIIa-Cre) mice and identified heterozygotes lacking exon 2 of the Ppar gene (Ppar<sup>+/-</sup> mice; all FVB/N). We then crossed female Ppar<sup>+/-</sup> FVB/N mice with male Apc<sup>Min/+</sup> C57BL/6 mice to produce Apc<sup>Min/+</sup> Ppar<sup>+/-</sup>, Apc<sup>Min/+</sup>, and Ppar<sup>+/-</sup> mice, all on an F<sub>1</sub> mixed background. Similarly, we bred PTGDS transgenic FVB/N males with C57BL/6 females to produce transgenic mice on an F<sub>1</sub>, C57BL/6 × FVB/N background. We intercrossed these various offspring to obtain additional mice with desired genotypes. Fifteen of the 104 mice used were C57BL/6 × FVB/N F<sub>1</sub> mice, and 89 were from matings of F<sub>1</sub> mice or mice in later generations (all 50% C57BL/6).

**Intestinal histopathology and definitions of tumor sizes**

Adenomas were counted histologically at 6 or 14 weeks, without knowing genotypes. We used 24 Swiss roll sections spaced 150 μm apart for PTGDS transgenic mice, Ppar knockout mice, and their controls. We used 10 Swiss roll sections (250 μm apart) for Ptgdr knockout mice and their controls. Tumors sizes were gauged by the number of sections spanned. Small tumors were defined as those seen in only 1 section. Large tumors were those with profiles in multiple sections. Mitotic figures were identified as described.

**Statistical analyses of tumor data**

Tumor data were analyzed by nonparametric methods (Kruskal–Wallis and Mann–Whitney), because numbers of tumors per mouse did not follow a Gaussian distribution. We analyzed total, small, large, and colon tumors. We also calculated ratios of the geometric mean number of tumors in genetically modified mice to the geometric mean number in controls. Ratios were estimated from differences in logarithm-transformed tumor numbers. For the colon, we added 0.5 to all numbers of tumors before taking logarithms, to handle zero values. Data from 6- and 14-week-old mice were analyzed separately. These statistical methods were also used to reanalyze tumor data from Apc<sup>Min/+</sup> mice with transgenic HPGDS (and controls) from earlier work.

**Immunohistochemistry**

Antibodies used were: mouse monoclonal anti-human PTGDS; rabbit polyclonal anti-human HPGDS; mouse monoclonal anti-rat proliferating cell nuclear antigen (PCNA); and rat monoclonal anti-mouse CD31. Staining for HPGDS, PCNA, and CD31 was done on slides from Apc<sup>Min/+</sup> mice with transgenic HPGDS from earlier work.

**In situ hybridization**

Digoxigenin-labeled probes were prepared by in vitro transcription from a linearized plasmid vector containing the mouse PTGDR cDNA (DIG RNA labeling kit; Roche; Indianapolis, IN). T7 RNA polymerase was used to make anti-sense probes. SP6 RNA polymerase was used to prepare control sense probes.
mRNA analyses by reverse transcription and real-time PCR (RT-PCR)

Primers, probes, and procedures for preparing RNA and determining copy numbers of RNA transcripts are in Supporting Information. Assays for v-my c avian myelocytomatisis viral oncogene homolog (MYC), GAPDH, and vascular endothelial growth factor A (VEGFA) were performed with kits (Applied Biosystems; Grand Island, NY; Mm00487803_m1, Mm99999915_g1, Mm00437304_m1, respectively).

Results

Tumor scoring

We histologically examined >35,000 tumors in Swiss roll sections (Fig. 1A–E), including 9837 tumors from 147 mice in Ptgdr knockout experiments, 21,763 tumors from 104 mice in experiments on PTGDS transgenic and Pparg knockout mice, and 3431 tumors reexamined from 39 HPGDS transgenic mice and controls from earlier work [5].

The earliest tumors were uniglandular, intravillar lesions with a simple cystic configuration, or intravillar neoplasms, also known as intravillous microadenomas [17, 18], dysplastic crypts [19], and cystic crypts (Fig. S1A and S1D) [20, 21]. More advanced early tumors may have other dysplastic features, such as extension of dysplastic cells (Fig. S1E), multiple lumina (Fig. S1C and G), loss of epithelial cell polarity (Fig. S1G), and stratification, or crowding (Fig. S1F).

Intravillar tumors progressed by enlarging, forming adjoining cysts (Figs. S1C and S2), or erupting through the villus surface into the bowel lumen (Figs. S1B and S3). Although early tumors arise from crypts [17, 22], we found only a few examples of out-pouching of cysts from crypts (Fig. S4). Serial sections from two tumors (75 sections each) showed that early tumors may have no crypt connection (Fig. S5) [21]. Examples of early colon tumors are shown in Fig. S6.

Ptgdr knockouts and intestinal tumors

At 6 weeks (Fig. 2A), homozygous Ptgdr knockouts raised total numbers of tumors (medians 64 vs. 49.5; P = 0.0086; Table S1A) and numbers of small tumors (medians 58 vs. 42.5; P = 0.0089; Table S1B). Large tumors and colon tumors were not affected by Ptgdr knockouts at 6 weeks.

At 14 weeks (Fig. 2B), heterozygous Ptgdr knockouts increased the median number of large tumors (33 vs. 24; P = 0.023; Table S2C). Also at 14 weeks, homozygous Ptgdr knockouts raised median numbers of total tumors (100 vs. 71; P = 0.0060; Table S2A) and large tumors (38 vs. 24; P = 0.0040; Table S2C). Ptgdr knockouts did not affect small or colon tumors at 14 weeks (Table S2B and D).

To obtain data on occurrence of the earliest tumors, we also scored tumors in ten 3-week-old mice: six ApcMin/+ mice (3–8 tumors each); three ApcMin/+ mice with heterozygous Ptgdr knockouts (5–11 tumors each); and one ApcMin/+ mouse with homozygous Ptgdr knockouts (11 tumors). However, data from these 10 mice were not included in statistical analyses, because of the age difference.

In situ hybridization for PTGDR mRNA showed consistent, but weak, staining of inflammatory cells in the mucosal stroma (lymphocytes or monocytes, or both; Fig. 1F–G). There was no detectable staining in epithelial cells of crypts or villi. Staining with PTGDR antibodies was not conclusive (not shown).

Expression of transgenic PTGDS

Human PTGDS transgenes were highly expressed in the intestines, as measured RT-PCR. Specifically, we found 1.61 × 105 and 8.13 × 105 copies of human PTGDS transcripts per nanogram of total RNA in two transgenic mice (mean geometric, 3.6 × 105 copies). These values were comparable to levels for HPGDS transgenes in earlier work (7.5 × 105 copies—a 375-fold increase in expression of transgenic HPGDS over endogenous mouse Hpgds) [5]. Immunohistochemistry showed heavy staining of transgenic PTGDS in all intestinal cells (Fig. 1H–I). Endogenous mouse PTGDS mRNA was not detectable in the colon.

Transgenic PTGDS and large tumors

With 104 ApcMin/+ mice, we scored intestinal tumors in relation to transgenic PTGDS, with and without heterozygous Pparg knockouts. Among mice without Pparg knockouts, only large tumors were reduced in number by transgenic PTGDS (medians were 52 vs. 83 for controls; P = 0.011; Fig. 3A–D; Table S3). Tumor suppression was also reflected by the ratio of the geometric mean number of large tumors in PTGDS transgenic mice to the geometric mean number in controls (ratio = 0.56 for large tumors; 95% confidence interval 0.34–0.92; Table S3C). Large tumors were >150–300 μm in diameter, based on the spacing between sections.

We measured colon mRNA levels for VEGFA and MYC, relative to endogenous GAPDH transcript levels. PTGDS transgenes lowered median levels of MYC and VEGFA transcripts by 50% in ApcMin/+ mice (Fig. S7).
Figure 1. (A–E) Swiss roll section (14 weeks). (A) The box outlines B. Scale bar, 5 mm. (B) The top, middle, and bottom boxes outline E, C, and D, respectively. Scale bar, 1 mm. (C) An early adenoma abutting against a larger adenoma. Scale bar, 200 μm. (D) An early adenoma expanding the villus base. Scale bar, 200 μm. (E) A large adenoma. Scale bar, 200 μm. (F–G) In situ hybridization for PTGDR (12 weeks). (F) Detection of PTGDR mRNA with antisense probes. PTGDR mRNA appears as blue deposits in stromal cells, in a pattern consistent with lymphocytes or monocytes, or both. (G) Sense probes showed no staining (negative control). Counterstained with neutral red. Scale bar, 100 μm. (H–O) Immunohistochemistry (14 weeks). (H–I) High production of human PTGDS and HPGDS in transgenic mice, shown by immunoperoxidase staining (with rabbit polyclonal anti-human PTGDS [H, I] or HPGDS [J, K] antibodies). Staining (brown) occurred in all cell types of the small bowel and colon (epithelium and stroma). Scale bars, 50 μm. (H) Small bowel villi from a wild-type mouse. Antibody labeling is mostly in the cytosol of some epithelial cells, with occasional stromal cell staining. (I) A small bowel villus from a PTGDS transgenic mouse. Antibody binding is heavy throughout the villus, with a cytoplasmic staining pattern. (J) Small bowel villi from a wild-type mouse. HPGDS staining is mainly within the stroma of villi, not epithelial cells. Earlier studies showed these cells to be macrophages and monocytes. (K) Small bowel villi from an HPGDS transgenic mouse, showing heavy antibody staining in all cells (as in I). (L, M) Staining for PCNA in intravillar tumors from ApcMin/+ mice, with and without HPGDS transgenes. There was no consistent difference in staining between mice with and without transgenic HPGDS. Scale bars, 100 μm. (L) An intravillar adenoma from an ApcMin/+ mouse. (M) An intravillar tumor from an HPGDS transgenic ApcMin/+ mouse. (N, O) Staining for microvessels with anti-CD31 antibodies in tumors from ApcMin/+ mice with and without HPGDS transgenes. There was no consistent difference in staining between mice with and without transgenic HPGDS. Scale bars, 100 μm. (N) A tumor from an ApcMin/+ mouse. (O) A tumor from an HPGDS transgenic ApcMin/+ mouse.
Heterozygotic Pparg knockouts and transgenic PTGDS

Without PTGDS transgenes, the numbers of tumors in heterozygotic Pparg knockout mice were comparable to numbers in mice without Pparg knockouts (Fig. 3; Table S3; see “Control”). Thus, heterozygous Pparg knockouts alone did not increase tumors in ApcMin/+ mice.

On the other hand, ApcMin/+ mice with both transgenic PTGDS and heterozygotic Pparg knockouts had intermediate numbers of large tumors. Specifically, going by medians, there were 52 large tumors in mice with PTGDS transgenes alone, 88 in mice with heterozygotic Pparg knockouts alone, and 70 in mice with both mutations (Table S3C). Similarly, the ratio of the mean number of large tumors in PTGDS transgenic mice to the mean number in controls was 0.56 for mice without heterozygotic Pparg knockouts (95% confidence interval, 0.34–0.92), compared to 0.78 for mice with heterozygotic Pparg knockouts (95% confidence interval, 0.48–1.26).

PTGDS versus HPGDS

As mentioned above, RT-PCR showed similar expression of transgenic PTGDS, compared to transgenic HPGDS (as measured in our previous work) [5]. Also, immunohistochemistry showed high levels of PTGDS and HPGDS (Fig. 1H–K). Both experiments scored tumors in the same way (24 Swiss roll sections; 150 μm between sections). Therefore, we reanalyzed slides from HPGDS transgenic mice from our first report [5] to directly compare PTGDS to HPGDS (Fig. S8; Table S4). Ratios of the mean total number of tumors in transgenic mice to the mean total in controls were 0.70 for PTGDS, compared to 0.28 for HPGDS (Tables S3A and S4A). Thus, HPGDS may be two times stronger than PTGDS in suppressing tumors.

We assessed tumor cell proliferation in relation to HPGDS transgenes, by use of immunohistochemistry with anti-PCNA antibodies. Again, we used slides from our earlier work on ApcMin/+ mice with HPGDS transgenes [5]. We focused on intravillar tumors, because they are fairly uniform in size. There was no difference in PCNA staining in intravillar tumors in HPGDS transgenic versus nontransgenic ApcMin/+ mice (Fig. 1L–M). We also counted mitotic figures in all intravillar tumors of 24 HPGDS transgenic mice and 15 ApcMin/+ controls. There were 0.18 mitoses per tumor in HPGDS transgenic ApcMin/+ mice (33 mitotic figures in 182 intravillar tumors), compared to 0.11 mitoses per tumor in non-transgenic ApcMin/+ mice (64 mitotic figures in 587 intravillar tumors). Thus, transgenic HPGDS did not reduce tumor cell proliferation.

Immunohistochemistry with anti-CD31 antibodies showed no consistent difference in microvessel staining between HPGDS transgenic and nontransgenic tumors (Fig. 1N–O). Thus, microvessel growth does not appear to explain occurrence of fewer tumors with PGD2.

Tumors in eight mutants with altered PGD2 synthesis or binding

We have now analyzed tumors in eight different ApcMin/+ mouse mutants that have altered PGD2 production or binding, due to knockouts or transgenes. Some experiments used different procedures for cutting sections. For example, we used up to 24 Swiss roll sections for scoring tumors in our first report [5] and in the PTGDS and PPARG experiments shown here. Alternatively, we used 10 sections per Swiss roll in the

Figure 2. Tumors in ApcMin/+ mice, with and without Ptgdr knockouts (total, small, large, and colon). (A) Tumors at 6 weeks. (B) Tumors at 14 weeks. +/+ control ApcMin/+ mice. +/− and −/−, ApcMin/+ mice with heterozygous and homozygous Ptgdr knockouts, respectively. Filled symbols: females. Open symbols: males. Horizontal bars: medians. * P < 0.025. See Tables S1 and S2 for details.
PTGDR experiments, because reanalysis of earlier data showed that the same conclusions can be reached with 8–10 sections.

To compare data across experiments, we converted the total number of tumors for each mouse to a “multiple of the median” value. Specifically, we divided the total number of tumors for each mouse by the median number of tumors for that mouse’s controls. By this analysis, the most tumor-promoting mutations were Hpgds knockouts and homozygous Ptgdr knockouts—raising tumor numbers 40% above control values (Fig. 4; all mice were analyzed at 14 weeks). In contrast, HPGDS transgenes were the most tumor-suppressing mutations—lowering numbers of tumors by 70–80% (Fig. 4).

**Female versus male ApcMin/+ mice**

To assess female–male differences in tumor numbers at 14 weeks, we used current data and two earlier reports [5, 23] (for a total of 61 female and 75 male ApcMin/+ mice; Fig. S9). Males and females had similar numbers of intestinal tumors (ratio of tumors in males vs. females, 0.82; \( P = 0.069 \)). However, males had more colon tumors (ratio of colon tumors in males vs. females, 1.6; \( P = 0.0002 \)). Results are consistent with McAlpine et al. [24].

**Discussion**

**PTGDR and intestinal tumors**

Homozygous deletion of the gene for PGD\(_2\) receptor PTGDR led to 30–40% more intestinal tumors in ApcMin/+ mice. The result supports an interpretation that PTGDR mediates tumor inhibition by PGD\(_2\) in these mice. We now have data on eight different ApcMin/+ mouse mutants, each with a different alteration in PGD\(_2\) production or binding. Homozygous Ptgdr and homozygous or heterozygous Hpgds knockout mutations are the most pro-tumorigenic. On the other hand, HPGDS transgenes are the most tumor-suppressive mutations—lowering numbers of tumors by 70–80% (Fig. 4).
There was no detectable staining of PTGDR mRNA in the epithelium of intestinal crypts or villi. However, PTGDR mRNA was consistently detected in inflammatory cells in the mucosal stroma (Fig. 1F–G). Tissue-specific gene knockouts will be needed to more conclusively identify the cells that respond to PGD2.

Mutoh et al. [25] treated homozygous Ptgdr knockout mice with azoxymethane starting at 7 weeks and examined colons at 12 weeks. They did not find more aberrant crypt foci in the colons of knockout mice, compared to controls. Our results are consistent with Mutoh et al., because we did not observe more colon tumors at 6 or 14 weeks with Ptgdr knockouts (Fig. 2A–B). However, a role for PTGDR in colon tumor growth is supported by human data. Gustafsson et al. [26] found fivefold lower expression of PTGDR in colorectal cancers, compared to normal tissues (62 tumors and 43 normal tissues, from 62 patients). Galamb et al. [27] showed a trend toward decreased PTGDR expression going from normal tissues, to adenomas, to early cancers, and to advanced cancers.

Comparison of PTGDS and HPGDS effects

Transgenic PTGDS in Apc^{Min/+} mice reduced numbers of large adenomas (>150–300 μm; Fig. 3C; Table S3C). In this way, PTGDS had a tumor blocking effect. However, transgenic PTGDS was less effective than transgenic HPGDS in suppressing tumors (Fig. 4). Reasons are unknown. A difference between PTGDS and HPGDS is secretion of PTGDS into body fluids, whereas HPGDS stays in the cytosol [28]. We recognize that our comparison between PTGDS and HPGDS is based on only one transgenic mouse line for each mutant. But these lines had comparable numbers of PTGDS or HPGDS mRNA transcripts in the intestines (3.6 × 10^5 and 7.5 × 10^5 copies, respectively).

Transgenic PTGDS was associated with lower colon expression of MYC (Fig. S7). MYC is a major part of WNT signaling following Apc loss [29]. Moreover, disruption of Myc restores the normal appearance of intestinal crypts in mice with intestine-specific Apc knockouts [30]. Thus, lower intestinal levels of MYC may be part of the tumor preventive mechanism of PGD2.

Reduced levels of VEGFA mRNA were also seen in PTGDS transgenic mice (Fig. S7). The finding is consistent with VEGFA effects in Apc mice [31]. However, we did not see a decrease in microvessel density in large tumors in Apc^{Min/+} mice with transgenic HPGDS (Fig. 1N–O). Thus, tumor suppression by PGD2 did not appear to involve antiangiogenesis in our experiments [32], at a level detectable by anti-CD31 immunohistochemistry.

Transgenic HPGDS did not reduce PCNA immunostaining in early tumors in Apc^{Min/+} mice (Fig. 1L–M). PCNA is a marker of intestinal cell proliferation and belongs to the family of sliding DNA clamps that bind factors at replication forks [33]. Similarly, transgenic HPGDS did not lower numbers of mitotic figures in early tumors. Thus, PGD2 does not appear to suppress tumors by lowering rates of tumor cell division.

A possible explanation is increased tumor cell death with PGD2 and PTGDR, as shown by Lewis lung cancer cells implanted onto the backs of mice [6]. Alternatively, PGD2 may prevent tumors by slowing initiation.

PPARG and intestinal tumors

Heterozygous Pparg knockouts alone did not increase the numbers of tumors in our Apc^{Min/+} mice. The result is consistent with earlier reports [24, 34]. However, McAlpine et al. [24] found ~30% more tumors in male Apc^{Min/+} mice with heterozygous or homozygous intestine-specific Pparg deletions.

In our transgenic mice with PTGDS overproduction and reduced adenoma occurrence, the decrease in numbers of large tumors caused by PTGDS appeared blunted in heterozygous Pparg knockout mice (Fig. 3C and Table S3C). Such blunting could be compatible with tumor suppression by PGD2 metabolites bound to PPARG [35], when PGD2 production is increased. A limitation in our experiments with heterozygous Pparg knockouts and PTGDS transgenics was the use of mice with mixed C57BL/6-FVB/N backgrounds.
(all 50% C57BL/6, but not all F1). However, fairly large numbers of mice were used in the Pparg experiments (104 in total). The 147 mice in the Ptgdr knockout experiments were all 100% C57BL/6.

**PGD₂ and inflammation**

Mechanisms for tumor suppression by PGD₂ in the intestines have not been proven, but useful information is available. For example, in the skin [36] and lung [37], PGD₂ delays migration of dendritic cells to draining lymph nodes, where T cells are primed. PGD₂ also reduces the ability of dendritic cells to stimulate naïve T cells [38, 39]. In the intestinal mucosa, dendritic cells produce IL-23, to stimulate release of IL-22 by immune cells (innate lymphoid cells [40, 41], T₁/₁7 cells [42], and T₁/₂₂ cells [43]). In turn, IL-22 induces proliferation of epithelial cells, production of inflammatory mediators, and release of antimicrobial proteins, to guard against invaders [44]. This cytokine can be neutralized by IL-22-binding protein, a soluble receptor also made by dendritic cells in the colon. Huber et al. [45] showed that IL-22 gene knockouts allowed fewer tumors in Apc\(^{Min/+}\) mice, whereas knockouts of IL-22-binding protein caused more (in the colon). Further work is needed to determine if these functions of dendritic cells explain tumor suppression by PGD₂. Identification of mechanisms involving PGD₂ and PTGDR may suggest molecular targets for tumor prevention studies.

**Conclusions**

By scoring tumors in Apc\(^{Min/+}\) mice histologically at 6 and 14 weeks, we showed that homozygous knockouts of the gene for the PGD₂ receptor, PTGDR, raised median numbers of tumors by 30–40%. The results support an interpretation that PGD₂ is a tumor-suppressing molecule, acting through PTGDR. Heterozygous Pparg knockouts had smaller effects in our experiments. The observation that PGD₂ and PTGDR can affect tumorigenesis may have impact for prevention.

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**Conflict of Interest**

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Examples of early stage intravillar neoplasms (A and D) and later stage tumors (B, C, E–G) in the small bowel of ApcMin/+ mice. Arrows indicate single, intravillar, neoplastic glands. (A) An early stage intravillar neoplasm with a single neoplastic gland lying at the level of normal glands and displacing them. (B) Profile of an intravillar neoplasm that progressed by erupting upward through the villus surface and opening into the lumen. Serial sections of this tumor are shown in Figure S3. (C) Profile of an intravillar neoplasm that progressed by forming multiple attached cysts. Serial sections of this lesion are shown in Figure S2. (D) Higher magnification view of an early intravillar neoplasm (not the same tumor as shown in A). (E) A later stage intravillar neoplasm consisting of a single neoplastic gland between the stalk of the villus and underlying normal glands, with extension of neoplastic cells (outlined by asterisks) beyond the gland. (F) A later stage intravillar neoplasm in which the glandular cells display crowding and pseudo-stratification. (G) A more complex, later stage intravillar neoplasm, characterized by polarized neoplastic glandular columnar cells (arrow), multiple neoplastic glandular lumina in a cribriform pattern (two examples are indicated by arrowheads), and solid areas of nonpolarized cells (asterisks). Hematoxylin and eosin staining. Scale bars, 100 μm.

Figure S2. Sections of an intravillar tumor that contains multiple adjoining cystic structures. The sections indicate that intravillar neoplasms can progress by forming new cysts that abut existing cysts. The slides of this tumor (beginning with the section represented by the top left image and ending with the section represented by the bottom right image) spanned 48 sections (4 μm each). However, only 20 sections are shown here, to save space. Scale bar, 100 μm.

Figure S3. Sections of an intravillar tumor that erupted through the villus surface. The sections indicate that intravillar neoplasms can progress by expanding toward the top of the villus, erupting through the villus surface, and opening into the bowel lumen. The slides of this tumor (beginning with the section represented by the top left image and ending with the section represented by the bottom right image) spanned 31 sections (4 μm each). However, only 18 sections are shown here, to save space. Scale bar, 100 μm.

Figure S4. Three examples of intravillar tumors that show a connection to a normal crypt. Scale bar, 100 μm.

Figure S5. Sections of an intravillar neoplasm in the small bowel of an ApcMin/+ mouse, showing a uniglandular, intravillar lesion with a simple cystic structure. Although tumors arise from crypt cells, we did not observe a connection between the cystic structure and the crypt for this tumor. Thus, early tumors may become fully enclosed or “sealed off.” All mounted sections containing profiles for this tumor are shown here. Scale bar, 100 μm.

Figure S6. Examples of colon tumors seen at 6 weeks. Tumors at this age are typically small and lie below the mucosal surface. They would be overlooked without histological examination. The inset in A shows a higher magnification view of the tumor. Scale bar, 100 μm (applies to all panels, except the inset).

Figure S7. Lower expression of VEGFA and MYC in the colon of ApcMin/+ mice with PTGDS transgenes (TG) and without (WT). mRNA was prepared from colon tissue, and expression levels for VEGFA and MYC were quantitated relative to endogenous mouse GAPDH. Plotted points are averages of triplicate measurements in different mice. VEGFA expression in PTGDS transgenic mice was approximately 50% of expression in controls (P = 0.022, Mann–Whitney; t-test). MYC expression was
also 50% lower in PTGDS transgenic mice ($P = 0.041$, Mann–Whitney; $P = 0.050$, $t$-test). Filled symbols: females. Open symbols: males. Horizontal bars show medians. *$P < 0.05$.

**Figure S8.** Numbers of adenomas in $Apc^{Min/+}$ mice with HPGDS transgenes (TG) and without (WT). Transgenic HPGDS was associated with statistically significant reductions in the numbers of tumors in all size categories. See Table S4 for median values, ranges, numbers of mice, $P$-values, and ratios of numbers of tumors in HPGDS transgenic mice to numbers in controls. Filled symbols: females. Open symbols: males. Horizontal bars indicate medians. *$P < 0.05$.

**Figure S9.** Tumor development in female and male $Apc^{Min/+}$ mice at 14 weeks. We combined data from the current experiments with data from two earlier reports [5, 23]. For each mouse, numbers of tumors (in the entire intestine and in the colon) were normalized to the median number among females in the same experiment.

For colon tumors, we added 0.5 to the number of tumors before taking the median. Horizontal bars indicate medians. The dotted horizontal lines indicate 1.0 (which is the median value for females). Males (77 mice) and females (61 mice) tended to have similar numbers of total tumors throughout the intestine (median ratio for males to females = 0.82; $P = 0.069$; A), but males had roughly 60% more colon tumors, compared to females (median ratio for males to females = 1.6; $P = 0.0002$; B).

**Table S1.** Adenomas at 6 weeks in $Apc^{Min/+}$ mice with Ptgd knockouts.

**Table S2.** Adenomas at 14 weeks in $Apc^{Min/+}$ mice with Ptgd knockouts.

**Table S3.** Adenomas in $Apc^{Min/+}$ mice with PTGDS transgenes, with and without heterozygous $Pparg$ knockouts.

**Table S4.** Adenomas in $Apc^{Min/+}$ mice with HPGDS transgenes.