

Minireview

Prostaglandin EP receptors: Targets for treatment and prevention of colorectal cancer?

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Abstract

The importance of the prostaglandin (PG) synthesis pathway, particularly the rate-limiting enzymatic step catalyzed by cyclooxygenase, to colorectal carcinogenesis and development of novel anticancer therapy is well established. The predominant PG species in benign and malignant colorectal tumors is PGE₂. PGE₂ acts via four EP receptors termed EP1 to EP4. Recently, EP receptors have been identified as potential targets for treatment and/or prevention of colorectal cancer. This review summarizes existing knowledge of the expression and function of the EP receptor subtypes in human and rodent intestine during tumorigenic progression and describes the current literature on targeting EP receptor signaling during intestinal tumorigenesis. [Mol Cancer Ther 2004;3(8):1031–9]

The Prostaglandin Synthesis Pathway during Colorectal Carcinogenesis

The importance of the prostaglandin (PG) synthesis pathway (Fig. 1) as a potential target for treatment and/or prevention of colorectal cancer is well established (1, 2). The rate-limiting step of the PG pathway is catalyzed by the cyclooxygenase (COX) enzyme, whereby arachidonic acid is converted to an unstable PG endoperoxide intermediate PGH₂ (Fig. 1). PGH₂ is converted to a series of different PGs dependent on the profile of specific PG synthases present in a particular cell or tissue (Fig. 1; ref. 3). PGs have a relatively short half-life and are believed to act over short distances in an autocrine or paracrine manner via specific cell surface and/or nuclear receptors (3). There are five classes of cell surface PG receptors corresponding to the

main PG species termed EP, DP, FP, IP, and TP (Fig. 1; ref. 3). In addition, nuclear peroxisome proliferator-activated receptors δ and γ are receptors for PGI₂ and cyclopentenone PGs such as 15-d-PGJ₂ (a breakdown product of PGD₂), respectively (4).

Until recently, the inducible isoform of COX, COX-2, was the focus of attention for cancer researchers, as a role for this isoform has been described during the early stages of intestinal tumorigenesis (benign adenoma development) as well as at later stages of colorectal carcinogenesis (invasion and metastasis of cancer cells; refs. 1, 2). However, the emergence of a role for the constitutive isoform of COX, COX-1, during intestinal tumorigenesis (5, 6), the fact that COX-2 expression by human colorectal neoplasms is not invariable (7), and the increased understanding that selective COX-2 inhibitors are not free of unwanted side effects (particularly on renal and cardiovascular systems; ref. 8) have led to a reevaluation of other potential targets in the PG synthesis pathway downstream of COX for treatment and/or prevention of gastrointestinal (GI) carcinogenesis, including PGE synthases (6, 9). Another attractive target for inhibition of the activity of the PG synthesis pathway is inhibition of downstream receptor signaling. Currently, there is little known about the expression and function of DP, FP, IP, and TP receptors in the intestine. Although these PG receptor families are deserving of attention, this particular review is restricted to a summary of current knowledge on the expression and function of EP receptor subtypes, as this class of receptors has been studied most intensely, given that PGE₂ is established as the predominant PG present in colorectal tumors.

PGE₂ Is the Predominant PG during Colorectal Carcinogenesis

Although PGE₂ is widely considered to be the most important PG species with regard to colorectal carcinogenesis, there are surprisingly little comparative data on tissue levels of different PGs in human and rodent intestinal tumors. This is perhaps related to the difficulty in measuring true steady-state mucosal PG levels following the tissue disruption necessary for tissue procurement. This is important when considering the relevance of the effects of different concentrations of PGE₂ in *in vitro* experiments. PGE₂ has been reported to be the predominant PG product of *ex vivo* COX biosynthesis in normal human colonic mucosal homogenates (10). Moreover, levels of PGE₂ have been noted to be higher than those of other measured PGs such as PGD₂ and PGI₂ (measured as its breakdown product 6-keto-PGF_{1 α}) in human colorectal adenomas and adenocarcinomas (11, 12).

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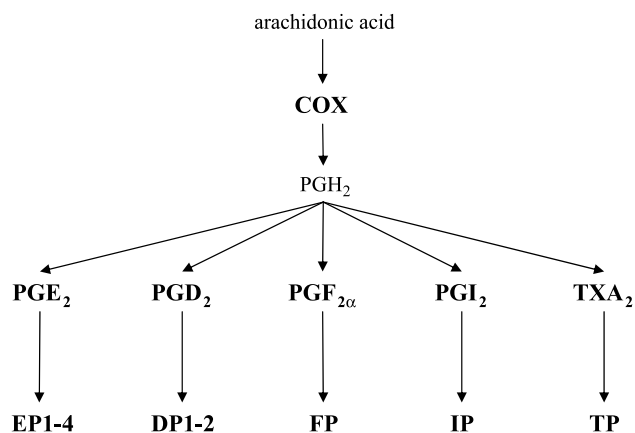


Figure 1. The five main PG species produced from the PG synthesis pathway with their cognate cell surface receptors. Plasma membrane-derived arachidonic acid, which is produced by phospholipase A₂, is converted by either of the two COX isoforms (COX-1 or COX-2) into PGH₂. Subsequently, PGH₂ is converted to a series of PG end products by specific PG synthases (e.g., PGE synthase). There are at least three PGE synthase isoforms (cytosolic PGE synthase and microsomal PGE synthase-1 and PGE synthase-2) that couple functionally to individual upstream COX isoforms (3). For example, inducible microsomal PGE synthase-1 preferentially uses PGH₂ from the inducible isoform of COX (COX-2). PGs act in an autocrine and/or paracrine manner via individual families of cell surface, seven-transmembrane domain, G protein-coupled receptors. For example, PGE₂ acts via a family of four EP receptors termed EP1 to EP4 (3).

A larger body of evidence exists that PGE₂ levels are increased in neoplastic colorectal lesions compared with normal mucosa. Several studies, using different methodologies for measurement of tissue PGE₂ content, have shown that PGE₂ levels are significantly increased in benign and malignant human and rodent colorectal tumors compared with paired histologically normal colorectal mucosa (11–15). Increased PGE₂ levels are apparent in benign adenomas as well as in established adenocarcinomas, although whether there is a quantitative increase in PGE₂ content during adenoma-carcinoma progression requires further investigation (13). The PGE₂ level increases in a size-dependent manner in colorectal adenomas in familial adenomatous polyposis patients (11). The PGE₂ content of venous blood draining from human colorectal cancers also increases as the tumor size increases (16). In both dimethylhydrazine-induced and *Apc^{Min/+}* mouse models of intestinal tumorigenesis, PGE₂ levels have been noted to be higher in macroscopically normal intestinal mucosa compared with mucosa from untreated or wild-type animals (17, 18). These findings have been mirrored in one human study, in which *ex vivo* PGE₂ synthesis by histologically normal mucosa from patients with a colorectal cancer was significantly higher than that of colorectal mucosa from control patients (13).

PGE₂ Production by Human Colorectal Cancer Cells *In vitro*

There is also a paucity of data on relative PG production by cultured malignant colorectal epithelial cells *in vitro*. In general, PGE₂ has been used as a “readout” of COX activity

in colorectal epithelial cells without detailed analysis of synthesis of other PGs. The small amount of comparative data that exist is in keeping with the data from tissue analysis of PGE₂ levels, in that PGE₂ also seems to be a major PG exported from cultured colorectal cancer cells [e.g., HCA-7 and Caco-2 cells (19–21)], although there are cell line-specific differences in the relative production of individual PGs (19, 21). PG production and release from human colorectal epithelial cells cannot be inferred from levels of cellular COX expression (20, 21). For example, HT-29 human colorectal cancer cells constitutively express COX-2 but do not synthesize detectable levels of PGE₂ (20). However, transfection of the HT-29 COX-2 gene into HCT116 human colorectal cancer cells promoted PGE₂ synthesis, implying that another factor intrinsic to HT-29 cells controls PGE₂ synthesis and/or export in this particular cell line (20).

It has also become clear recently that the stromal cell population in tumors is also capable of significant PG synthesis. For example, isolated fibroblasts from hereditary nonpolyposis colorectal cancer tumors produce large quantities of PGI₂, which has antiapoptotic activity on cocultured HCA-7 human colorectal cancer cells (22), and *ex vivo* tumor-associated macrophages from human colorectal cancers produce significant levels of PGE₂ (23).

EP Receptors

PGE₂ is the ligand for four EP receptor subtypes termed EP1 to EP4, which are the products of separate genes (Table 1; ref. 24). In addition, multiple splice variants of EP3 are recognized (3, 24). The known physiologic roles of the EP receptors in the GI tract are summarized in Table 1 (25–30). Understanding of the pharmacology and physiologic roles of each of the EP receptors has been enhanced greatly by derivation of individual EP receptor “knockout” mouse models, data from which have recently been reviewed extensively elsewhere (31, 32). Detailed discussion of the physiology of PGE₂ and EP receptor signaling is beyond the scope of this review but has recently been summarized in a series of articles (33–36). In general, knowledge of the roles of each of the EP receptor subtypes has lagged behind understanding of the effects of PGE₂ on GI physiology.

EP receptors are all cell surface, seven-transmembrane domain, rhodopsin-type G protein-coupled receptors (3, 24). There is also some evidence that EP receptors (particularly EP1) can localize to the nuclear membrane in cultured endothelial cells (37). EP receptors are highly conserved between mammalian species (24). However, there are significant differences in the structure and pharmacology of the EP receptor subtypes within species (Table 1). EP1 signaling is coupled to phospholipase C/inositol trisphosphate signaling, leading to mobilization of intracellular calcium, whereas EP2 and EP4 receptor signaling generates increased intracellular cyclic AMP (cAMP) levels via coupling to G_s proteins. EP3 has generally been considered to couple to a G_i protein leading to reduction in intracellular cAMP levels. However,

Table 1. EP receptor signaling pathways and cellular localization in the normal large intestine

Receptor	Second Messenger Signal	Tissue Localization	Physiologic Role in the GI Tract
EP1*	Phospholipase C/inositol trisphosphate	Epithelium (H), epithelium, [†] glial cells, and longitudinal muscle (R)	GI tract motility (R)
EP2	Increased cAMP	Epithelium [†] (R)	Chloride secretion (H)
EP3 [‡]	Decreased cAMP	Epithelium (H), epithelium, [†] glial cells, and longitudinal muscle (R)	Duodenal bicarbonate secretion (M) and GI tract motility (R)
EP4	Increased cAMP	Epithelium and lamina propria cells (H), and epithelium [†] (M and R)	Gastric mucus production (R) and chloride secretion (H)

NOTE: H, human (25–27); M, mouse (28); and R, rat (29, 30).

*There are two human EP1 splice variants.

[†]mRNAs for all four EP receptors are strongly expressed by goblet cells in rat colonic epithelium (29).

[‡]There are eight human EP3 splice variants; at least one of which mediates an increase in intracellular cAMP levels via G_s. Activation of at least two EP3 isoforms also leads to increased inositol trisphosphate levels via G_q.

activation of at least one EP3 splice variant leads to increased cAMP levels, suggesting functional coupling to a G_s protein (38). In keeping with data on other G protein-coupled receptors, there may be plasticity of EP receptor signaling such that PGE₂ binding and G protein coupling to individual EP receptors may alter depending on the local PGE₂ concentration and other cell-specific and tissue-specific factors (39).

Recently, EP2 and EP4 receptor activation has been linked to increased β -catenin/T-cell factor transcriptional activity in human embryonic kidney cells via phosphorylation and hence inhibition of glycogen synthase kinase-3 (GSK-3; refs. 40, 41). Increased β -catenin/T-cell factor transcription secondary to loss of *adenomatous polyposis coli* (*APC*) tumor suppressor gene function is a pivotal event that commonly initiates colorectal carcinogenesis (42). However, the relevance of EP2/EP4 receptor-induced β -catenin up-regulation in colorectal epithelial cells, which already contain only mutant *APC*, remains to be determined. EP2 inhibits GSK-3 predominantly via a protein kinase A-dependent mechanism, whereas EP4 preferentially uses a phosphatidylinositol 3-kinase-dependent pathway, involving AKT/protein kinase B, which also drives activation of extracellular signal-related kinase (ERK) signaling (40, 43). It is currently unclear whether EP4 receptor activation is directly linked to the phosphatidylinositol 3-kinase pathway or whether this activity is mediated by activation of a receptor tyrosine kinase such as the epidermal growth factor receptor (EGFR). Recently, PGE₂ has been implicated in EGFR activation (either by direct intracellular receptor tyrosine kinase phosphorylation or via extracellular release of a membrane-bound EGFR ligand such as heparin-binding epidermal growth factor) in human colorectal cancer cells *in vitro*, although no EP receptor subtype was implicated in these studies (44, 45). PGE₂ has also been reported to induce expression of the EGFR ligand amphiregulin in LS174T human colorectal cancer cells via a protein kinase A-dependent mechanism (46), thus providing another mechanistic link between the PG pathway and EGFR signaling *in vitro*. EP1 receptor signaling has also recently been implicated in up-regulation of vascular endothelial cell growth factor

(VEGF) production by HCT116 human colorectal cancer cells via ERK signaling (47). Current knowledge of the relationship between EP receptor signaling events and other signal transduction pathways is summarized in Fig. 2.

The Effect of PGE₂ on Colorectal Epithelial Cells *In vitro*

There are rather contradictory reports of the effect of PGE₂ and individual EP receptor agonists on intestinal epithelial cell proliferation and apoptosis, which are likely to reflect cell line-specific differences in EP receptor expression and use of different proliferation and apoptosis assays. These studies have generally employed micromolar

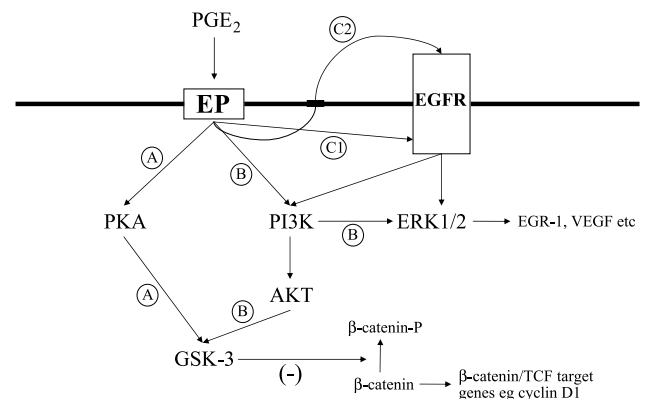


Figure 2. The relationship between EP receptor (*EP*) activation by PGE₂ and other signal transduction pathways. Signaling through EP2 leads to GSK-3 phosphorylation via a protein kinase A-dependent mechanism (A). EP4 receptor activation also leads to GSK-3 phosphorylation, but this occurs via a mechanism involving phosphatidylinositol 3-kinase and AKT (B). GSK-3 inactivation by EP2 and EP4 signaling in human embryonic kidney cells has been shown to lead to increased transcriptional activity of β -catenin, presumably via an increase in β -catenin protein levels, consequent on reduced β -catenin phosphorylation by GSK-3. EP4 receptor signaling also leads to ERK signaling. EP1 receptor signaling can also activate ERK signaling in human colorectal cancer cells. Whether activation of phosphatidylinositol 3-kinase and ERK signaling occurs directly from EP receptors or indirectly through a mechanism that could involve intracellular (C1) or extracellular (C2) EGFR activation is currently unknown. Abbreviations: (-), inhibition; *EGR*, early growth response factor.

concentrations of PGE₂. Qiao et al. (48) have described dose-dependent and time-dependent proliferative activity of PGE₂ and its stable analogue 16,16-dimethyl-PGE₂ (dmPGE₂) on HT-29 and SW1116 human colorectal cancer cells. Interestingly, there was a "bell-shaped" dose-response relationship for SW1116 cells suggesting differential activation of EP receptors at different PGE₂ concentrations (this phenomenon has also been reported following treatment of HT-29 human colorectal cancer cells with PGE₁; ref. 49). However, PGE₂ had no effect on apoptosis in this study. By contrast, another study failed to show any effect of similar concentrations of PGE₂ on proliferation of HT-29 cells and could not detect changes in either intracellular cAMP or calcium concentrations (50). Similar findings were reported by Parker et al. (21) who found that PGE₂ did not alter human colorectal cancer cell (including HT-29 as well as SW480, SW848, and Caco-2 cell lines) proliferation until antiproliferative effects became apparent at concentrations above 20 μmol/L. The HT-29 human colorectal cancer cell line consists of a heterogeneous population of colorectal epithelial cells including cells with goblet cell and absorptive cell phenotypes (51). Differences in EP receptor expression between subpopulations of cells in HT-29 cell cultures could explain discrepant data from investigators using different HT-29 cell cultures. For example, dmPGE₂ promotes proliferation of a mucus-secreting HT-29 human colorectal cancer goblet cell clone (52). Growth stimulatory activity of PGE₂ has also been described in LS174T and HCA-7 human colorectal cancer cells (53, 54), but antiproliferative activity against HCT116 human colorectal cancer cells has been noted in the same study (54). PGE₂ also inhibits apoptosis of HCA-7 human colorectal cancer cells, which is associated with increased BCL-2 protein expression (54). The human small intestinal epithelial cell line T84 is also protected by PGE₂ from apoptosis induced by staurosporine or anti-FAS antibody (55). However, PGE₂ alone has been reported to have no effect on nontransformed rat IEC-6 intestinal epithelial cells (56, 57).

PGE₂ has also been shown to have other effects on colorectal epithelial cells, which may be associated with growth modulation. Mucin release is increased by PGE₂ administration to LS174T and HT-29-18N2 human colorectal cancer cells (52, 58), and T84 cell barrier function is

enhanced by PGE₂ (25). Sheng et al. (53) have described an increase in motility and invasive behavior of LS174T human colorectal cancer cells by nanomolar concentrations of PGE₂.

Many studies have attempted to investigate the role of endogenously produced PGE₂ in colorectal epithelial cells using COX inhibitors [including nonsteroidal anti-inflammatory drugs (NSAID)], but analysis of the role of PGE₂ in these experiments is difficult because of potential concomitant changes in levels of non-E-type PGs and the existence of possible COX-independent mechanisms of action of NSAIDs (59).

Only a subset of the *in vitro* studies on colorectal epithelial cells have contained experiments that implicate particular EP receptors in the bioactivity of PGE₂. Some studies have implicated EP2 and/or EP4 signaling based on increased cAMP levels (25, 55). Other studies have used EP receptor agonists to implicate EP4 receptor signaling in promotion of a protumorigenic phenotype and mucin release in LS174T human colorectal cancer cells (53, 58). EP1 receptor signaling seems to be necessary for stimulation of VEGF production (and by inference, proangiogenic behavior) by PGE₂ in HCT116 human colorectal cancer cells (47).

The Effect of PGE₂ on Colorectal Epithelial Cell Proliferation and Intestinal Tumorigenesis *In vivo*

Administration of dmPGE₂ to normal BALB/c mice for 15 days has been reported to increase mouse colonocyte proliferation (measured by flow cytometric proliferating cell nuclear antigen expression by isolated colonocytes; ref. 48). However, in other experimental settings, PGE₂ analogues seem to be mitotically inactive (60, 61). There have been several studies in which NSAIDs such as indomethacin have been used to infer the activity of endogenous PGE₂ on intestinal epithelial turnover *in vivo* (62). However, it is difficult to draw conclusions regarding the role of PGE₂ from these studies for the reasons outlined above.

In keeping with the general theme that the effect of PGE₂ on GI epithelial cells is context dependent, data that have emerged from the use of rodent intestinal tumorigenesis

Table 2. The effect of PGE₂ and PGE₂ analogues on rodent intestinal tumorigenesis

Model	PGE ₂ Analogue	EP Receptor Activity	Weekly Dose* (μg)	Duration (wk)	% Untreated Tumor Number	Change in Tumor Size
<i>Apc</i> ^{Min/+} mouse (63)	dmPGE ₂ + 17-phenyl-trinor-PGE ₂	EP2-EP4	180	1 [†]	83	↓
<i>Apc</i> ^{Min/+} mouse (65)	dmPGE ₂	EP1, EP3 EP2-EP4	180 0.03	7	~50	↓
Rat azoxymethane-induced colon carcinogenesis (64)	PGE ₂ [‡]	EP1-EP4	100	25	280	↑

*All dosings were by i.p. injection apart from the study by Hansen-Petrik et al. (63), in which 10 μg of each analogue were given by daily gavage.

[†]6 days.

[‡]PGE₂ β-cyclodextrin clathrate.

models have not been consistent (Table 2). PGE₂ analogues have been shown to reverse abrogation of tumor growth by the NSAID sulindac in the *Apc^{Min/+}* mouse model of familial adenomatous polyposis (63), and reduction of systemic PGE₂ availability using a neutralizing anti-PGE₂ antibody decreases tumor multiplicity in this model (63). Others have reported that i.p. administration of PGE₂ promotes azoxymethane-induced colonic tumors (Table 2; ref. 64). However, data exist that challenge the idea that PGE₂ is the main COX-derived PG species responsible for protumorigenic activity in the *Apc^{Min/+}* mouse model (Table 2). Two independent groups have reported that i.p. administration of the PGE₂ analogue dmPGE₂ alone (65) or in combination with 17-phenyl-trinor-PGE₂ (63) is associated with a reduction in the number and size of *Apc^{Min/+}* mouse adenomas (Table 2). Possible explanations for discrepancies in these data include the use of PGE₂ analogues with differing EP receptor specificity (Table 2). It should also be noted that the majority of adenomas occur in the small intestine (not the colon) of the *Apc^{Min/+}* mouse, and possible tissue-specific differences in EP function during intestinal tumorigenesis between the mouse small intestine and colon (see below) should be taken into consideration. The use of the i.p. route of administration of PGE₂ means that systemic activity of PGE₂, which could affect intestinal tumorigenesis (by alteration of mesenteric blood flow, for example), cannot be ruled out in the above studies.

Expression of Individual EP Receptor Isoforms in Normal Large Intestine and during Intestinal Tumorigenesis

The majority of studies of intestinal EP receptors have investigated expression at the mRNA level [by reverse transcription-PCR (RT-PCR) or *in situ* hybridization] due to the lack of well-characterized antibodies to EP receptors. Antibody characterization has been hampered by the discovery of different molecular weight forms of EP receptors (e.g., EP4) in different tissues, which may be explained by differential glycosylation (66). Data on localization of EP receptors EP1 to EP4 in rodent and human intestine are summarized in Table 1 (25–30). In summary, each of the EP receptor subtypes has been localized to epithelial cells, particularly mucus-producing goblet cells (29). In addition, expression of EP1 and EP3 receptors is prominent in longitudinal muscle and nerve plexuses. It is important to note that colorectal epithelial cells have been noted to have more prominent EP receptor expression than small intestine (28, 29), which is likely to be relevant to interpretation of data from different rodent tumorigenesis models.

RT-PCR analysis of murine colorectal mucosal mRNA has revealed an increase in EP1, EP2, and EP4 receptor mRNAs in azoxymethane-induced colorectal cancer tissue compared with adjacent macroscopically normal mucosa (64, 67). EP2 and EP4 mRNA levels are also increased in *Apc^{A716}* mouse small intestinal and colonic polyps (68). This study also reported that EP3 mRNA levels were

lower in polyps than macroscopically normal mucosa (68). *In situ* hybridization for EP2 receptor mRNA showed that transcripts for this receptor were predominantly localized to stromal cells within intestinal adenomas of *Apc^{A716}* mice compared with the epithelial cell compartment of these tumors in which only a faint signal was detected (68).

Surprisingly, no studies of EP receptor expression in human colorectal neoplasms have been published to date. However, Roche et al. have studied EP receptor expression in human colorectal mucosa involved by ulcerative colitis, which predisposes affected individuals to colorectal cancer development (25, 26). In active ulcerative colitis, EP4 receptor expression is increased by lamina propria T lymphocytes, and increased levels of EP2 and EP3 receptors are apparent in epithelial cells (25, 26). Although it is well established that PGE₂ levels are significantly increased in active ulcerative colitis (69), the roles of PGE₂ and individual EP receptors in the pathogenesis of ulcerative colitis remain unknown (36).

The Role of Individual EP Receptor Isoforms during Intestinal Tumorigenesis

Several studies have now been published (70–78), which have explored the role of individual EP receptor subtypes during intestinal tumorigenesis using genetic deletion and pharmacologic manipulation of EP receptors (Table 3). EP1, EP2, and EP4 receptor signaling have all been implicated in intestinal tumorigenesis in different rodent models (Table 3). By contrast, EP3 receptor “knockout” does not seem to impact on intestinal tumorigenesis (Table 3). Genetic deletion or inhibition of a single EP receptor has consistently been shown to reduce intestinal adenoma or aberrant crypt focus (ACF) development by 40% to 60%. This compares with an 80% to 90% reduction in neoplasia in the same models associated with genetic deletion of either COX isoform (particularly COX-2). Therefore, these data suggest that more than one EP receptor subtype is involved in intestinal tumorigenesis in these models and/or that other PGs (acting via alternative PG receptors) may contribute to the promotion of tumorigenesis by COX. A recently published study of the concurrent use of EP1 and EP4 antagonists in the *Apc¹³⁰⁹* mouse model of familial adenomatous polyposis is consistent with this concept, in that the effects of the two inhibitors were additive and produced a polyp number 44% of untreated control animals (79). In a similar pattern to previous reports (67, 70), treatment with the EP1 antagonist was associated with a prominent reduction in polyp number, whereas a reduction in tumor size was linked to EP4 antagonism (79). This suggests that different EP receptors may have roles at different stages (initiation versus progression) of intestinal tumorigenesis.

It should again be remembered that adenomas in *Apc^{Min/+}*, *Apc^{A716}*, and *Apc¹³⁰⁹* mouse models occur predominantly in the small intestine and that the relevance of chemically induced ACFs to sporadic colorectal carcinogenesis remains unclear (80). Therefore, the relevance of

Table 3. The number of colonic ACF or intestinal polyps in mouse models of intestinal tumorigenesis following either genetic deletion or pharmacologic inhibition of EP receptor subtypes or COX isoforms

Receptor	Azoxymethane-Induced ACF Development*	<i>Apc</i> ^{Min/+} Mouse Polyposis [†]	<i>Apc</i> ^{Δ716} Mouse Polyposis [†]
EP1	65% (GD; ref. 70) 65% (P; refs. 70, 71)	56% (P; ref. 70) No Δ polyp size	No difference (GD; ref. 68)
EP2	ND	ND	58% (GD; ref. 68)
EP3	No difference (GD; ref. 67)	ND	No difference (GD; ref. 68)
EP4	56% (GD; ref. 67) 67% (P; ref. 67)	69% (P; ref. 67) Polyp size decreased	No difference (EP4 ^{+/-} only; ref. 68)
COX-1	60% (P; ref. 72)	23% (GD; ref. 5)	59% [‡] (P; ref. 72)
COX-2	63% (P; ref. 73) 3% [§] (P; ref. 74)	16% (GD; ref. 5) 29% (P; ref. 75)	14% (GD; ref. 76) 45% (P; refs. 77, 78)
Anti-PGE ₂ antibody	ND	67% (63) No Δ polyp size	ND

NOTE: ND, not determined.

*Azoxymethane-induced ACF number following either genetic deletion (GD) or pharmacologic inhibition (P) as a percentage of wild-type or control ACF multiplicity.

[†]Polyp (or adenoma) number following either genetic deletion (GD) or pharmacologic inhibition (P) as a percentage of wild-type or control polyp multiplicity. Δ, change.

[‡]The *Apc*^{T309} mouse model of familial adenomatous polyposis was used in this study.

[§]Percentage of azoxymethane-induced tumor (not ACF) multiplicity in untreated rats.

these rodent data to human colorectal cancer development is uncertain. Advances in the field of EP receptor signaling during human colorectal carcinogenesis will only follow careful delineation of EP receptor subtype localization and levels at different stages of cancer development [i.e., adenoma (polyp) versus cancer] in parallel with *in vitro* studies of EP receptor function in epithelial and stromal cells derived from human colorectal tumors.

Two carefully performed studies have also implicated a role for individual EP receptor subtypes in other solid

tumor models in mice (81, 82). EP3 (and to a lesser extent EP2) has been implicated in tumor-associated angiogenesis in sarcoma-180 and sponge implantation models via stromal fibroblast VEGF production (81). This highlights the potential tissue and model specificity of EP receptor PGE₂ signaling that is becoming apparent, as EP3 is the one EP receptor subtype that has consistently *not* been implicated in the studies using intestinal tumorigenesis models (Table 3). By contrast, in polyps of the *Apc*^{Δ716} model, stromal cell PGE₂-EP2 receptor signaling has been

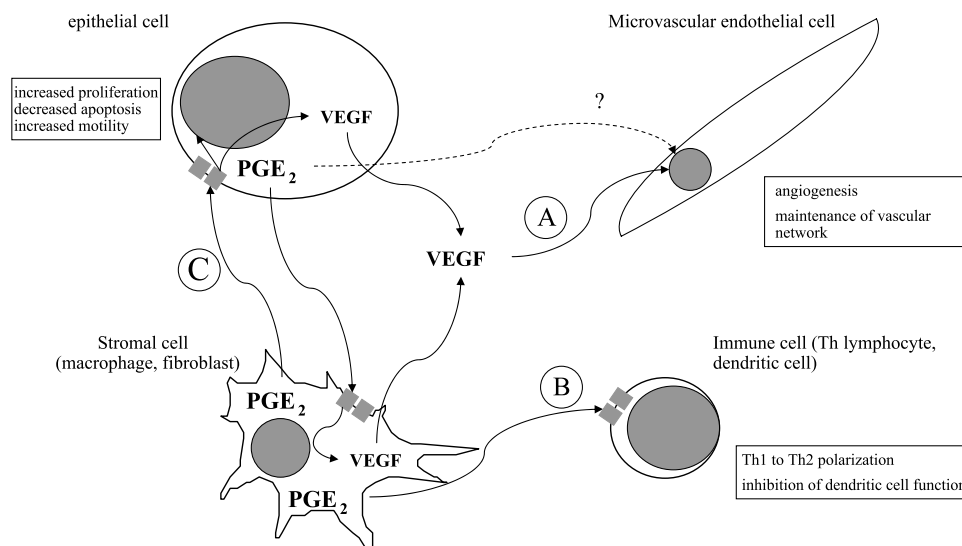


Figure 3. PGE₂-EP receptor signaling during intestinal tumorigenesis. PGE₂ (derived from COX-1-mediated and/or COX-2-mediated PG synthesis pathways) can act in an autocrine and/or paracrine manner in stromal and epithelial cell compartments of tumors. It is unclear what the contribution is of each cellular compartment to PGE₂ bioactivity in colorectal neoplasms. Currently, evidence is perhaps strongest for a role for stromal cell (fibroblast and/or macrophage) EP receptor (subtypes 2 and 3) signaling in promotion of angiogenesis (A) and impairment of host immune antitumor surveillance (B). PGE₂ also contributes to T-lymphocyte development (87) and a switch from a Th1 to Th2 predominant immune response (88). It is unknown whether endothelial cells express EP receptors and so whether PGE₂ has direct activity on the vasculature. At least part of the angiogenic activity of COX-2 is believed to be mediated by increased expression of the proangiogenic factor VEGF. Direct PGE₂-EP receptor signaling (subtypes 2 and 4) in epithelial cells is also likely to be important in intestinal neoplasms *in vivo* (C).

associated with increased microvessel number and VEGF expression (83). The concept of tissue heterogeneity in EP receptor function is further strengthened by data from other solid tumor transplantation models (including the MC26 mouse colorectal cancer cell model), in which EP2 has not been implicated in angiogenesis (82). Instead, this study provided evidence for a role for PGE₂-EP2 receptor signaling in tumor-associated inhibition of dendritic cell differentiation and function, thereby leading to impairment of host immune antitumor surveillance (82).

Therefore, the weight of the current evidence for EP receptor signaling in different tumorigenesis models would suggest a significant role for the stromal cell (rather than tumor cell) component of neoplasms, including proangiogenic effects and subversion of the host immune response (Fig. 3; refs. 84–88). The potential contribution of autocrine and/or paracrine PGE₂-EP receptor signaling between tumor cells and between stromal and tumor cells has yet to be investigated thoroughly (Fig. 3). However, it is interesting to note that stromal cell COX-2 drives epithelial cell proliferation in *Apc*^{Δ716} mouse polyps (68) and protumorigenic behavior of intestinal epithelial cells *in vitro* (56). This evidence along with consistent EP receptor expression by colorectal epithelial cells *in vitro* suggests that PGE₂-EP receptor signaling also contributes directly to epithelial cell behavior, as well as angiogenesis and the immune response, during intestinal tumorigenesis.

Therapeutic Implications of EP Receptor Signaling for Treatment of GI Cancer

EP receptors represent ideal targets for pharmacologic agents, and PGE₂ analogues and synthetic drugs, which can selectively activate or antagonize signaling from one or more EP receptor subtypes, have now been developed and used experimentally (31). As outlined above, some of these agents have been shown to have antineoplastic activity, with no reported extraintestinal toxicity, in rodents (67, 70, 71, 79). Pertinent to the suitability of these agents for clinical trials in humans is the question of which EP receptors mediate the various physiologic roles of PGE₂ such as gastric mucosal protection and renal homeostasis. Although a large literature exists on the pharmacologic properties of PGE₂ (in the presence or absence of NSAIDs) on small intestinal permeability and protection as well as on gastric mucosal defense, research into the role of individual EP receptors in these processes is in its infancy (33–36). One relevant study has implicated PGE₂-EP3 (but not EP1) receptor signaling in duodenal bicarbonate secretion and maintenance of mucosal integrity in studies of EP receptor “knockout” mice (89). Potential physiologic roles for EP receptors *in vivo* can also be hypothesized from *in vitro* data. For example, the EP4 receptor mediates mucus production and protects against apoptosis in colonic and gastric epithelial cells (54, 58, 90). Therefore, antagonism of the EP4 receptor could promote mucosal injury *in vivo*. The extent of EP receptor redundancy in individual organs will govern whether individual EP receptor agonists and antagonists have toxicity in humans.

Summary

A large body of evidence exists that the PG synthesis pathway, via PGE₂-EP receptor signaling, plays an important role in colorectal carcinogenesis (Fig. 3). Available data suggest that EP1, EP2, and EP4 receptors all play a role in the early stages of intestinal tumorigenesis (ACF and adenoma development). Other solid tumor models, which perhaps have more relevance to established colorectal cancer, have implicated EP3 and EP2 receptors in the host angiogenic response. In keeping with the idea that there may be redundancy of EP receptor signaling function and that EP receptor function varies at distinct stages (e.g., initiation versus progression) of tumorigenesis, comparison of the effects of single EP receptor and COX isoform gene deletion suggests that antagonism at any one EP receptor will not have the preventative efficacy of inhibition of either or both COX enzymes. More research is now needed to define the activity and toxicity of single/combination EP receptor antagonists in preclinical models to prompt phase I and II clinical evaluation of these agents. Drugs targeting individual EP receptors may eventually find a role as adjunctive therapy (with, for example, selective COX-2 and/or PGE synthase inhibition) in defined groups of patients with colorectal neoplasia.

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