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Review Series

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Role of prostanoids in gastrointestinal cancer

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Chronic inflammation is a risk factor for gastrointestinal cancer and other diseases. Most studies have focused on cytokines and chemokines as mediators connecting chronic inflammation to cancer, whereas the involvement of lipid mediators, including prostanoids, has not been extensively investigated. Prostanoids are among the earliest signaling molecules released in response to inflammation. Multiple lines of evidence suggest that prostanoids are involved in gastrointestinal cancer. In this Review, we discuss how prostanoids impact gastrointestinal cancer development. In particular, we highlight recent advances in our understanding of how prostaglandin E_2 induces the immunosuppressive microenvironment in gastrointestinal cancers.

Introduction

Clinical and epidemiologic evidence indicates that chronic inflammation is a major risk factor for several gastrointestinal malignancies, including esophageal, gastric, colorectal, hepatic, and pancreatic cancer. For example, patients with persistent hepatitis B infection, *Helicobacter pylori* infection, or autoimmune disorders such as inflammatory bowel diseases (IBD) face an increased lifetime risk for liver cancer, gastric cancer (GC), or colorectal cancer (CRC), respectively. In addition, solid tumors themselves exhibit certain characteristics found in inflamed tissues, referred to as tumor-induced inflammation. The common pathological features of chronic inflammatory diseases and solid cancers include elevation of proinflammatory mediators such as cytokines, chemokines, and lipids, massive infiltration of deregulated immune cells, and recruitment of endothelial cells and fibroblasts (1–3). The observation that nonsteroidal antiinflammatory drugs (NSAIDs) reduce the incidence, metastasis, and mortality of various solid tumors (4–10), including gastrointestinal cancer, supports the concept that chronic inflammation promotes tumor initiation, growth, and progression. NSAIDs are the most commonly used drugs that help reduce inflammation and relieve fever and pain. It is well accepted that NSAIDs primarily target the cyclooxygenase enzymes COX-1 and COX-2 in reducing inflammation and relieving pain and/or fever.

COX-1 is constitutively expressed in most tissues and is thought to provide basal levels of prostanoids, a subgroup of eicosanoids including prostaglandins (PGs), thromboxanes, and prostacyclins that are important for tissue homeostasis and platelet function. In contrast, COX-2 is an immediate-early response gene that is usually absent in healthy tissues and organs, but is highly inducible at sites of inflammation and is overexpressed in certain cancers, such as those that arise in the gastrointestinal tract. For example, COX-2 expression is elevated in approximately 50% of colorectal adenomas and 85% of adenocarcinomas (11–13). Similarly, COX-2 overexpression is also observed in esophageal and gastric cancer (14,

15). Elevation of COX-2 expression is also associated with a shorter survival time among patients with CRC and esophageal cancer (16, 17). However, conflicting results have been reported in the association between COX-2 expression and survival in patients with GC (18). COX enzymes convert arachidonic acid into an endoperoxide intermediate that can be further metabolized to prostanoids, including PGs such as PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2 (TxA_2) via specific PG synthases (Figure 1). Moreover, prostanoids exert their cellular functions by binding cell surface G protein–coupled receptors. These cell surface receptors are designated EP (EP1, EP2, EP3, and EP4) for the PGE_2 receptors, DP1 and DP2 for the PGD_2 receptor, FP for the $PGF_{2\alpha}$ receptor, IP for the PGI_2 receptor, and TP for the TxA_2 receptor (Figure 1).

The roles of prostanoids in acute inflammation have been recognized very early, and their levels are immediately elevated before leukocyte infiltration in acute inflammation. Among prostanoids, both PGE_2 and PGI_2 have been shown to induce acute inflammation in the majority of animal models (19). In contrast, PGD_2 has been shown to suppress acute inflammation via binding to its DP receptors and via enzymatically independent generation of 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) in animal models (20). 15d-PGJ_2 mainly binds to PPARγ and directly inhibits the NF-κB signaling pathway (21, 22). Similarly, PGE_2 and PGI_2 also enhance chronic inflammation (23) and play a key role in arthritis and IBD (24, 25). The role of PGD_2 in chronic inflammation is context-dependent. On the one hand, PGD_2-derived 15d-PGJ_2 inhibits adjuvant-induced arthritis in vivo (26). On the other hand, PGD_2 facilitates allergic inflammation (25). The roles of $PGF_{2\alpha}$ and TxA_2 in inflammation remain unclear.

Although NSAIDs exhibit antitumor effects, the molecular mechanisms underlying their effects, especially aspirin, are not fully understood. Although other mechanisms have been proposed to explain the antitumor effects of these drugs, and "off-target" effects do exist, COX-1 and COX-2 remain primary targets. For example, celecoxib, belonging to a family of COX-2–selective inhibitors (COXIBs), was initially approved by the FDA for use as adjuvant therapy for patients with familial adenomatous polyposis (FAP), but is no longer recommended for that indication. However, long-term use of celecoxib and other COXIBs as well as nonse-
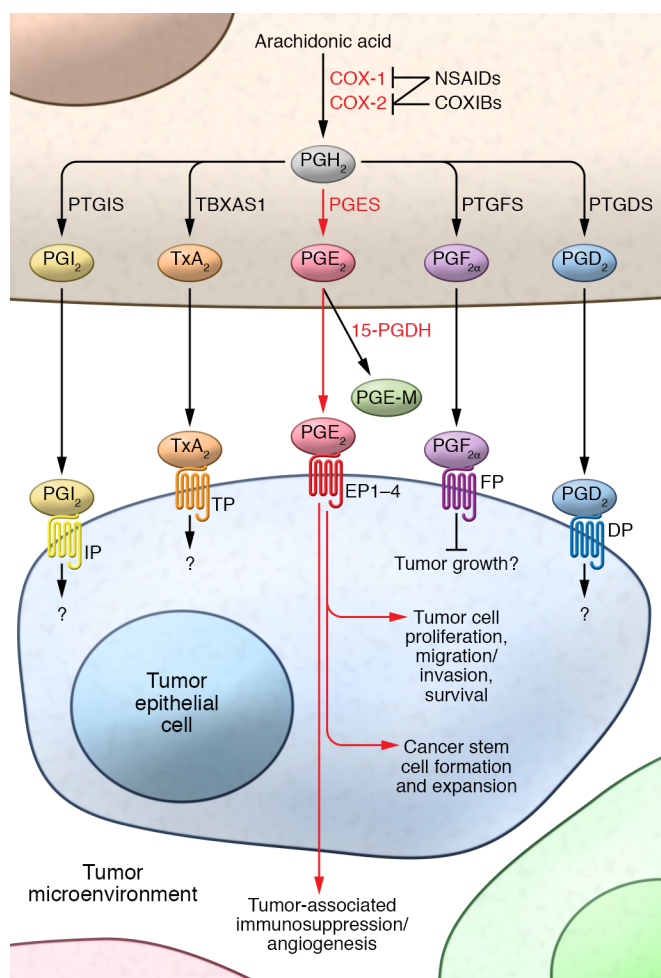
Figure 1. An overview of prostanoid synthesis pathways. Free arachidonic acid can be metabolized to PGH_2 by COX-1 and COX-2. NSAIDs inhibit activity of COX-1 and COX-2, whereas COXIBs inhibit activity of COX-2. PGH_2 is sequentially metabolized to PGI_2 by PTGIS, TxA_2 by TBXAS1, PGE_2 by PGES, $PGF_{2\alpha}$ by PTGFS, and PGD_2 by PTGDS. PGE_2 binding at EP1–EP4 receptors is known to promote cancer development via multiple mechanisms (described in detail in the text and Figures 2 and 3). The roles of other prostanoids and their receptors in gastrointestinal cancer remain unclear (also detailed in the text).

lective NSAIDs (except for aspirin) at high doses is associated with unacceptable cardiovascular side effects in patients, especially in those with a history of atherosclerotic heart disease (27).

Unlike COXIBs and other nonselective NSAIDs, long-term daily use of aspirin is beneficial for prevention of both cancer and cardiovascular diseases. Daily use of aspirin has been shown to suppress polyp growth in FAP patients in two double-blind randomized controlled trials (28, 29) and to reduce risk of adenoma recurrence in patients with a history of colorectal adenomas in four randomized controlled trials (30–33). More intriguingly, recent observational and clinical studies revealed that daily use of aspirin was associated with a reduced risk of metastatic spread (5) and inhibited the spread of primary tumor cells to other organs after the diagnosis of localized disease, in particular CRC (7). Based on recent clinical and epidemiologic evidence, the United States Preventive Services Task Force endorsed aspirin as the first agent

for CRC prevention in individuals between the ages of 50 and 69 with specific cardiovascular risk profiles (34). In addition, aspirin has been shown to enhance the efficacy of chemoradiation for patients with stage II–III rectal cancer (35), indicating that aspirin can be used as a neoadjuvant agent for certain cancer treatments. Aspirin has also been shown to reduce risk of esophageal cancer and GC (10, 36, 37). Moreover, epidemiologic studies have shown that regular use of aspirin specifically reduced risk of the subgroup of patients whose colon tumors expressed higher levels of COX-2 (38), and its use after the diagnosis of CRC at stages I, II, and III prolonged overall survival, especially among individuals whose tumors overexpressed COX-2 (39). These results suggest that antitumor effects of aspirin on CRC might depend on the presence of COX-2. In addition to COX-2 expression, *PIK3CA* mutation and HLA class I antigen expression levels also affect the efficacy of aspirin in improving the overall survival rate of CRC patients (40, 41). However, it is not clear how *PIK3CA* mutations and HLA class I antigen expression are involved in antitumor effects of aspirin.

Since NSAIDs are known to cause gastrointestinal and/or cardiovascular side effects, one of the ways to avoid these side effects would be to target only the COX-2–derived prostanoids that mediate the tumor-promoting effects of COX-2. In this Review, we highlight our current understanding of the role of specific prostanoids in gastrointestinal cancer. Understanding how these bioactive lipids regulate tumor formation, growth, progression, and metastasis may provide a rationale for developing novel and more effective strategies in cancer prevention and treatment that avoid side effects associated with NSAID use.

Prostanoids and gastrointestinal cancer

The biological functions of COX-1/2 enzymes depend on which COX-derived prostanoids are produced in cancers. Among prostanoids, PGE_2 is the most abundant in human gastrointestinal cancers, including CRC and GC (42, 43). More importantly, only PGE_2 and PGI_2 levels are elevated in CRC specimens as compared with matched normal tissues (44). The steady-state accumulation of PGE_2 in tumor tissues depends on the relative rates of COX-2/PGE_2 synthase–dependent biosynthesis and 15-hydroxyprostaglandin dehydrogenase–dependent (15-PGDH–dependent) degradation (Figure 1). 15-PGDH first converts PGE_2 into an inactive 15-keto PGE_2 that is then further metabolized to a stable end metabolite (PGE-M) in a series of steps. 15-PGDH is highly expressed in normal tissues but is ubiquitously lost in many human cancers, including CRC, GC, and esophageal cancer (45–48). Since measurement of the urinary PGE_2 metabolite PGE-M is an effective way to quantify systemic PGE_2 production in vivo, much work has been done to evaluate whether urinary PGE-M levels could serve as a promising biomarker for predicting cancer risk and prognosis. Emerging epidemiologic evidence and a phase II biomarker study showed that urinary PGE-M levels were associated with an increased risk of developing CRC and GC (49–53). These results suggest that urinary PGE-M could be used as a biomarker for predicting gastrointestinal cancer risk and prognosis. More importantly, epidemiologic evidence revealed a strong inverse association between aspirin use and levels of urinary PGE-M in healthy humans (54) and breast cancer patients (55, 56). Moreover, a recent study showed that low-dose aspirin (100 mg/d
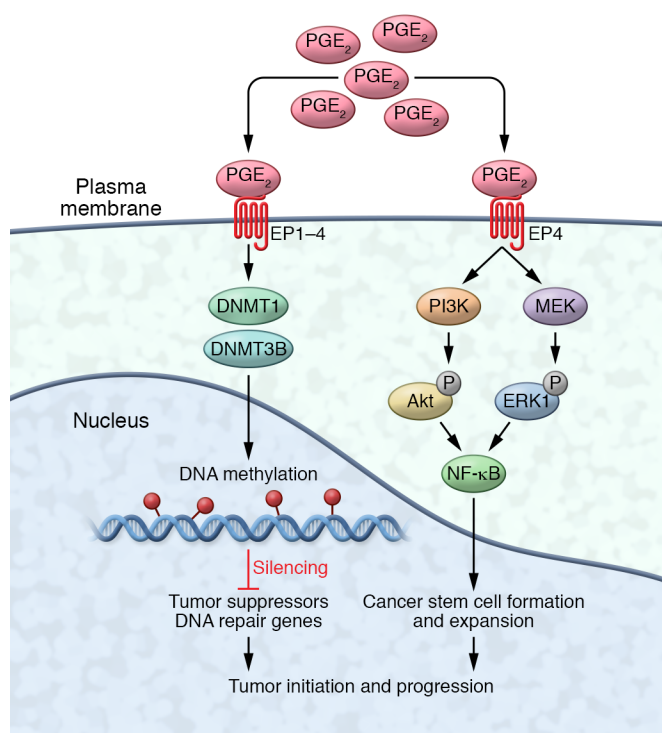
Figure 2. PGE$_2$ regulation of tumor initiation. PGE$_2$ promotes tumor initiation and growth via DNA methylation and cancer stem cells. PGE$_2$ binds to its receptors (EP1–EP4) to suppress certain tumor suppressor and DNA repair genes by DNA methylation via induction of expression of the DNA methyltransferases DNMT1 and DNMT3B. PGE$_2$ also promotes cancer stem cell formation and expansion by activating NF-κB via EP4-dependent PI3K/MAPK pathways.

for 7 days) reduced PGE$_2$ levels in human colorectal mucosa by 46% (57). In addition, one epidemiologic study showed that regular use of aspirin more effectively reduced CRC risk in patients with higher 15-PGDH expression compared with low 15-PGDH expression in colonic mucosa (58). These findings further support the hypothesis that PGE$_2$ mediates some of the tumor-promoting effects of COX-2 as well as the notion that the COX-2/PGE$_2$ pathway is a legitimate target for cancer prevention and treatment.

Direct evidence that PGE$_2$ promotes tumor growth came from animal studies. In mouse models of FAP and/or sporadic CRC, PGE$_2$ treatment dramatically increased both small and large intestinal adenoma burden in $Apc^{Min/+}$ mice and significantly enhanced azoxymethane-induced (AOM-induced) colon tumor incidence and multiplicity (59, 60). Furthermore, elevating endogenous PGE$_2$ by genetically deleting 15-$Pgdh$ promotes colon tumor growth in $Apc^{Min/+}$ and AOM mouse models (61). PGE$_2$ also reverses the antitumor effects of an NSAID in $Apc^{Min/+}$ mice (62), suggesting that PGE$_2$ is one of the important NSAID targets for cancer prevention and treatment. In accordance with the above results, inhibition of endogenous PGE$_2$ by genetic deletion of microsomal PGE$_2$ synthase 1 ($mPges$-1) suppresses intestinal tumor formation and growth in Apc-mutant and AOM models (63, 64). In mouse models of GC, simultaneous overexpression of COX-2 and mPGES-1 in gastric epithelial cells was sufficient to induce gastric tumor formation (65). Moreover, deletion of EP1 or EP4, but not EP3, atten-

uates AOM-induced aberrant crypt foci (66, 67). In $Apc^{Δ716}$ mice, loss of EP2, but not EP1 and EP3, reduces intestinal tumor burden (68). Interestingly, one report showed that loss of EP3 promoted colon tumor development in AOM-treated mice (69). In a mouse model of colitis-associated CRC, loss of EP2 reduced the number of colon tumors, whereas deletion of EP1 or EP3 increased colon tumor numbers (70). Collectively, these results demonstrate that PGE$_2$ promotes intestinal tumorigenesis via EP2 and EP4, but not EP3. The role of EP1 in CRC remains unclear.

In evaluating the role of other PGs in CRC, contradictory results have been reported in mouse models of CRC. For PGD$_2$, loss of hematopoietic PGD$_2$ synthase (PTGDS) accelerated intestinal tumor growth in $Apc^{Min/+}$ mice (71), and deletion of $Ptgds$ in mast cells enhanced colitis-associated tumorigenesis in an AOM/ dextran sulfate sodium (DSS) model (72). In addition, deletion of DP resulted in an increase of intestinal tumor numbers in $Apc^{Min/+}$ and AOM/DSS-treated mice (70, 73). In contrast, $Apc^{Min/+}$ mice expressing transgenic human hematopoietic PTGDS exhibited fewer intestinal adenomas than controls (71). These results suggest that PGD$_2$ serves as a tumor suppressor in CRC. However, one study showed that disruption of DP did not affect colon tumor formation in AOM-treated mice (74). For PGI$_2$, one report showed that loss of PGI$_2$ synthase (PTGIS) facilitated colon carcinogenesis in AOM-treated mice (75). However, the results that loss of IP did not affect colon tumor formation in AOM/DSS–treated mice (70, 74) do not support the antitumor effect of PGI$_2$ in the colon via the IP receptor. For PGF$_{2α}$ and TxA$_2$, one study showed that disruption of FP or TP did not affect colon tumor burden in AOM-treated mice (74), whereas loss of TP increased the number of colon tumors in AOM/DSS–treated mice (70). More intriguingly, the expression of DP, FP, and IP receptors is reduced in human CRC specimens as compared with adjacent normal colon tissues (76). Clearly, the question of whether PGD$_2$, PGI$_2$, PGF$_{2α}$, TxA$_2$, and their receptors are involved in gastrointestinal cancer needs to be further investigated.

To understand the mechanisms underlying effects of PGE$_2$ on cancer development, researchers have been investigating precisely how PGE$_2$ promotes tumor formation, growth, progression, and metastasis. Numerous reports suggest that PGE$_2$ promotes cancer development via multiple mechanisms, including regulation of tumor epithelial cell biology, promotion of tumor-associated angiogenesis, and suppression of tumor immunity (Figure 1).

PGE$_2$ and tumor epithelial cells

The mechanisms by which PGE$_2$ promotes tumor epithelial cell proliferation, survival, and migration/invasion as well as tumor-associated angiogenesis have been reviewed in detail elsewhere (refs. 22, 77; and Figure 1). Here we highlight emerging evidence indicating that PGE$_2$ may be a targetable link between chronic inflammation and tumor initiation (Figure 2). PGE$_2$ has been shown to promote intestinal tumor initiation and growth by silencing certain tumor suppressor and DNA repair genes via DNA methylation (78). In addition, cancer stem cells (CSCs) are thought to be responsible for tumor initiation, growth, metastatic spread, relapse, and recurrence. The observation that the expression of stem cell factors (i.e., CD44, LGR5, SOC-2, and OCT4) is associated with a worse prognosis in CRC (79) sup-
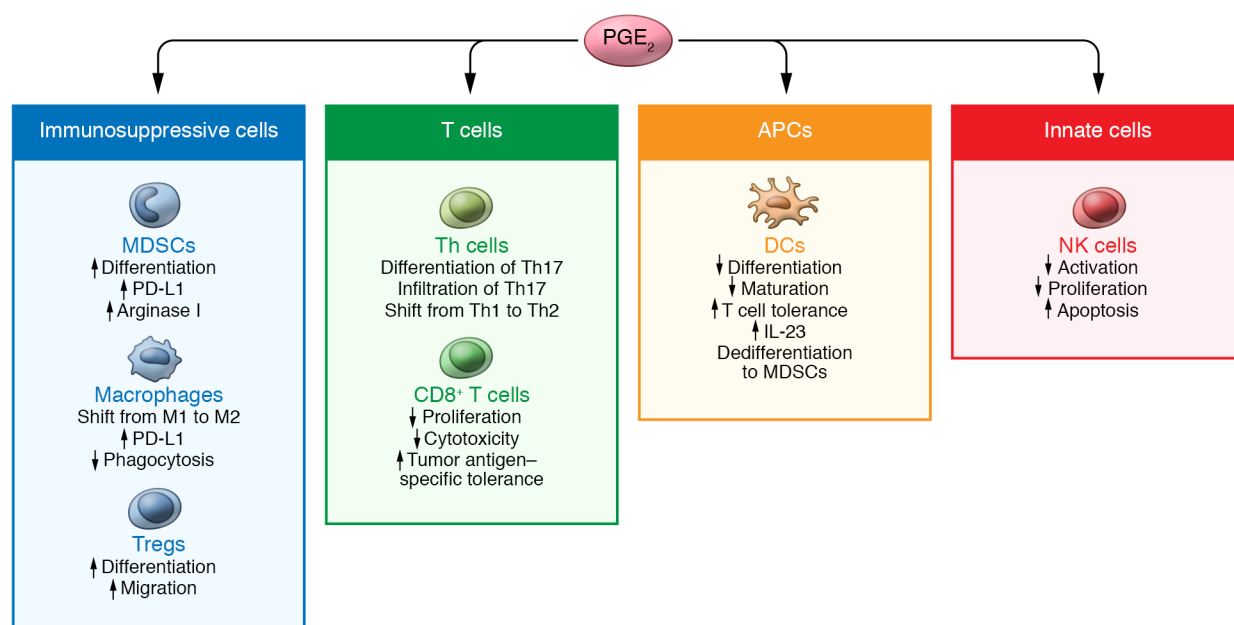
Figure 3. A model of PGE$_2$-regulated tumor-associated immunosuppression. PGE$_2$ regulates immunosuppressive cells and their functions by (a) inducing MDSC differentiation and production of PD-L1 and arginase I; (b) shifting macrophages from M1 to M2, inducing PD-L1 expression, and reducing macrophage phagocytosis; and (c) inducing differentiation and migration of Tregs. PGE$_2$ regulates DCs and their functions through inhibition of differentiation and maturation; induction of T cell tolerance and IL-23 expression; and induction of dedifferentiation of DCs to MDSCs. PGE$_2$ regulates Th cells and their functions by inducing differentiation and recruitment of Th17, and shifting Th cells from Th1 to Th2. PGE$_2$ regulates CD8$^+$ T cells and their functions by induction of proliferation and tumor antigen–specific tolerance and reduction of CD8$^+$ T cell cytotoxicity. PGE$_2$ regulates NKs and their functions via suppression of cell activation and proliferation and induction of cell apoptosis.

ports this hypothesis. It is also believed that chemotherapy/radiation resistance is due to the presence of CSCs that are not being properly targeted (80). Moreover, the observation that chemotherapeutic and/or radiation therapy enhances COX-2 expression and PGE$_2$ production in cancers prompted investigators to postulate that PGE$_2$ regulates CSC biology. NSAIDs have been shown to eliminate oncogenic intestinal stem cells via inducing apoptosis in $Apc^{Min/+}$ mice (81) and to inhibit sphere formation in human colorectal carcinoma cells in vitro (82). Strikingly, PGE$_2$ promotes colonic CSC formation and expansion as well as liver metastasis by activating NF-κB via EP4-dependent PI3K/MAPK pathways in vivo (83, 84). Similarly, simultaneous overexpression of COX-2 and mPGES-1 in the gastric epithelial cells is sufficient to induce CD44$^+$ slow-cycling tumor cell expansion in vivo (85), indicating that PGE$_2$ induces gastric CSC expansion. In addition, PGE$_2$ released following chemotherapy-induced apoptotic tumor cells promotes neighboring CSC repopulation in a xenograft model of bladder cancer (86). Collectively, these results suggest that reduction of PGE$_2$ levels and/or inhibition of PGE$_2$ signaling pathways may not only suppress tumor cell proliferation, survival, and migration/invasion, but may also eliminate CSCs. Targeting CSCs may thus present a novel therapeutic approach for cancer patients.

PGE$_2$ and tumor-associated immunosuppression

The role of PGE$_2$ in regulating immunity and host defense against viral, fungal, and bacterial pathogens has been reviewed in detail elsewhere (87). Here we focus on the role of PGE$_2$ in tumor-associated immunosuppression. The tumor microenvironment not only supports tumor growth, progression, and spread by angiogenesis, but also allows tumor cells to evade host immunosurveillance. This tumor-associated immunosuppression is characterized by enhancement of immunosuppressive cells, a defect of antigen-presenting cell function, a shift from Th1 to Th2 and Th17 immune responses, and impairment of cytotoxic activity of CD8$^+$ T and natural killer (NK) cells. Reversing immunosuppression remains one of the major challenges in cancer immunotherapy. It is becoming increasingly evident that PGE$_2$ has a broader impact on tumor-associated immunosuppression than previously thought (Figure 3). However, the mechanisms by which PGE$_2$ induces tumor-associated immunosuppression remain largely unclear. Understanding the mechanisms underlying PGE$_2$ induction of tumor-associated immunosuppression may provide a rationale for developing more effective therapeutic strategies to subvert tumor-induced immunosuppression for patients with gastrointestinal cancer.

PGE$_2$ and immunosuppressive cells

Myeloid-derived suppressor cells. In healthy individuals, immature myeloid cells differentiate into mature myeloid cells including macrophages, DCs, and granulocytes. However, this normal physiological process is interrupted in cancer patients. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature myeloid cells that have been demonstrated to contribute to cancer immunosuppression by suppression of effector T cell activation, proliferation, trafficking, and viability; by inhibition of NKs; and by activation/expansion

of regulatory T cells (Tregs) (88). The levels of MDSCs in the blood and/or tumor tissue correlated with clinical cancer stage, metastatic tumor burden, or poor survival in patients with colon, esophageal, gastric, or pancreatic cancer (89–93). Animal studies have demonstrated that MDSCs mediate one of the protumor effects of chronic inflammation. For example, depletion of MDSCs attenuated colitis-associated tumorigenesis in a mouse model of IBD-associated carcinogenesis (94). Along the same lines, transfer of MDSCs promoted chronic colonic inflammation and colitis-associated tumor development via suppression of colonic CD8+ T cell cytotoxicity against tumor cells in a mouse model of colitis-associated carcinogenesis (95). Moreover, liver-infiltrating MDSCs formed a premetastatic niche that ultimately promoted liver metastases without involvement of T and NK cells in a mouse model of metastatic spread of CRC (96). MDSCs isolated from premetastatic livers of immunodeficient NSG mice bearing cecal tumors inhibited colorectal carcinoma cell apoptosis induced by serum deprivation in cell culture without cell-cell interaction of malignant cells and MDSCs, suggesting that MDSCs must secrete factors that promote tumor cell survival (96). In addition, MDSCs have also been shown to directly enhance CSC formation and protect proliferating tumor cells from senescence without involvement of T and NK cells in vivo (97, 98).

Multiple studies have shown that inhibition of COX-2 suppressed tumorigenesis by inhibiting tumor-associated MDSC infiltration in mouse models of CRC and glioma (99, 100) as well as in mice with implanted mesothelioma and mammary carcinoma (101, 102). Moreover, PGE$_2$ promoted tumor progression via inducing the development of MDSCs from bone marrow myeloid progenitor cells, whereas inhibition of PGE$_2$ signaling by deletion of EP2 or its antagonists blocked this differentiation in mice with implanted 4T1 murine mammary carcinoma tumors (102). An EP4 antagonist, E7046, has been shown to reduce tumor-infiltrating MDSCs and to enhance the antitumor effect of anti–CTLA-4 antibodies in syngeneic mouse models of cancer (103), indicating that EP4 mediates the effect of PGE$_2$ on MDSCs. An in vitro study showed that PGE$_2$ blocked differentiation of monocytes into DCs and promoted MDSC development (104). Moreover, PGE$_2$ enhanced immunosuppressive function by inducing MDSC-specific hypermethylation via DNMT3A (105) and by inducing PD-L1 expression (106) and arginase I expression (107) in vitro. However, the role of PGE$_2$ in enhancement of gastrointestinal tumor-infiltrating MDSCs is still largely unknown, and the mechanisms by which PGE$_2$ regulates MDSC differentiation, expansion, and immunosuppressive functions are also not fully understood.

Regulatory T cells. Tregs are essential for suppressing immune responses and maintaining self-tolerance by regulating the activity of other immune cells. The frequency of Tregs (CD4+CD25+Foxp3+) is elevated in the peripheral blood and in primary tumors of CRC and GC patients (108, 109). Tumor-infiltrating Tregs are also associated with GC progression and a poor survival rate (110, 111). There is a positive correlation between PGE$_2$ levels and the numbers of Foxp3+ Tregs in the peripheral blood, tumor tissues, and draining lymph nodes of CRC patients (112). In addition, Foxp3 expression in tumor-infiltrating Tregs correlates with COX-2 expression and PGE$_2$ levels in GC (109).

In mouse models of cancer, inhibition of COX-2 by celecoxib resulted in reduction of tumor burden and proportion of Tregs in intestinal lamina propria lymphocytes in *Apc$^{Min/+}$* mice (113). Deletion of *mPges-1* attenuated AOM-induced tumor formation with reduction of Tregs in the colon-draining mesenteric lymph nodes (64). In addition, treatment with an EP4 antagonist resulted in a decreased number of Tregs in the peripheral lymph nodes after UV irradiation (114). An EP1 antagonist inhibited tumor growth with reduction of tumor-infiltrating Tregs in a colon tumor implantation model (115). Consistent with these findings, PGE$_2$ promoted tumor growth with induction of Treg expansion and activity in a mouse model of lung cancer (116). In vitro studies further demonstrate that PGE$_2$ induces Treg differentiation and migration. For example, PGE$_2$ can directly enhance the differentiation of naive CD4+ T cells into Tregs (117). PGE$_2$ secreted from breast cancer cells directly induces Treg migration via EP2 and EP4 (118). In addition, PGE$_2$ indirectly attracts Tregs via induction of CCL22 in mature DCs (119). Collectively, these studies indicate that PGE$_2$ enhances tumor formation and growth via tumor-infiltrating Tregs (Figure 3).

Macrophages. Macrophages are highly plastic and can be activated in two polarization states: classically M1 (Th1 response) or alternatively M2 (Th2 response), depending on the context of their microenvironment. Tumor-associated macrophages (TAMs) resemble an M2-like phenotype and are a major subpopulation of tumor-infiltrating immune cells (120). Multiple lines of evidence indicate that TAMs promote cancer progression and metastasis by supporting tumor-associated angiogenesis, enhancing tumor cell migration/invasion and intravasation, and suppressing immunosurveillance (121). For example, TAMs contribute to immunosuppression by suppressing CD8+ T cell cytotoxic activity via stimulation of expression of immune checkpoint receptor ligands such as PD-L1 and B7-H4 and/or via recruitment of Tregs (122, 123). TAMs are recognized as a poor prognostic sign in CRC (124). Moreover, a meta-analysis of 55 studies indicated that high density of TAMs correlated with overall poor survival of GC (125).

Treatment with celecoxib resulted in reduction of polyp burden and conversion of TAMs from M2 to M1 in *Apc$^{Min/+}$* mice (126). In a colon tumor implantation model, overexpression of 15-PGDH in tumor tissue is sufficient to redirect the differentiation of intratumoral CD11b cells from immunosuppressive M2-oriented TAMs to M1 macrophages (127). It has been reported that macrophages express EP2 and EP4, but not EP1 or EP3 (128), and that EP3 and EP4 have higher affinity for PGE$_2$ than EP1 and EP2 (129). Therefore, deletion of EP4 in myeloid cells resulted in a reduction of tumor burden in *Apc$^{Min/+}$* mice (130). An EP4 antagonist, E7046, has also been shown to shift TAMs from M2 to M1 macrophages and to enhance the antitumor effect of anti–CTLA-4 antibodies in syngeneic murine models of cancer (103). In vitro studies showed that PGE$_2$ promoted M2 macrophage polarization via a CREB/CRTC pathway in bone marrow–derived macrophages (131) and eliminates CD8+ T cells by inducing PD-L1 expression in TAMs (106). PGE$_2$ also inhibited macrophage phagocytosis in vitro (132). In addition to macrophage function, *H. pylori* and PGE$_2$ cooperated to upregulate CCL2, which recruited macrophages to gastric tumors (133). Collectively, these results demonstrate that PGE$_2$ promotes tumor growth via induction of M2 macrophages (Figure 3).

PGE$_2$ and antigen-presenting cells

Dendritic cells. Professional antigen-presenting cells include dendritic cells (DCs), macrophages, and B cells. Among antigen-presenting cells, DCs are central to the host immune response to tumor antigens. Since little is known about the role of B cells in gastrointestinal cancer and other solid tumors, we will focus on DCs here. Circulating DC levels and activity are reduced in CRC patients as compared with healthy controls, and this correlates with the stage of disease (134, 135). Moreover, highly mature tumor-infiltrating DCs correlate negatively with tumor stage in patients with CRC (136) and are associated with better survival in GC patients (137). DCs include both conventional DCs (cDCs) and plasmacytoid DCs (pDCs). The studies evaluating levels of circulating cDCs and pDCs in CRC patients have resulted in conflicting data. One report showed that the levels of pDCs, but not cDCs, in blood were reduced in CRC patients (138). In contrast, another study showed that levels of both circulating cDCs and pDCs were reduced in CRC patients (139). Further studies with large numbers of patients are necessary to clarify this discrepancy.

In tumor implantation models of colon cancers, PGE$_2$ promoted tumor growth by suppressing differentiation of DCs from bone marrow progenitors (140). Indeed, PGE$_2$ suppresses DC differentiation and maturation in vitro and in vivo (141, 142). Moreover, PGE$_2$ inhibits the antigen presentation ability of bone marrow–derived DCs by reduction of MHC II expression and upregulation of IL-10 via EP2 and EP4 (143). PGE$_2$ has also been shown to switch the function of DCs from induction of immunity to T cell tolerance via upregulation of CD25 and indoleamine 2,3-dioxygenase (IDO), a rate-limiting enzyme in the kynurenine pathway (144). Furthermore, PGE$_2$ shifted the IL-12/IL-23 balance in DCs signaling via EP2 and EP4 receptors in favor of IL-23, which in turn increases the number of Th17 cells in vitro (145). More interestingly, PGE$_2$ has recently been shown to redirect the differentiation of human DCs into monocytic MDSCs (146). Further work is necessary to determine whether PGE$_2$ promotes tumorigenesis by inhibition of DC differentiation, maturation, and function in spontaneous mouse models of gastrointestinal cancer (Figure 3).

PGE$_2$ and T cells

CD4$^+$ T helper cells. T helper (Th) cells include Th1, Th2, and Th17 cells. Th1 and Th2 cells are characterized by secretion of Th1 cytokines (IFN-γ, TNF-α, and IL-2) and Th2 cytokines (IL-4, IL-10, and IL-6), respectively, whereas Th17 cells are characterized by secretion of IL-17. High Th1/Th2 ratios in tumor tissues are associated with better overall survival in CRC patients (147). In addition, tumor-infiltrating Th1 cells are associated with a positive prognosis, whereas intratumoral Th17 cells are associated with a poor prognosis in CRC (148, 149). Similarly, high Th1/Th2 ratios in peripheral blood are associated with a positive postoperative prognosis, whereas high circulating Th17 cells correlate with tumor progression and poor survival in GC patients (150, 151).

Although an in vivo study indicated that Th17 cells promoted intestinal tumor burden (152), little is known about the impact of PGE$_2$ on the imbalance of Th1/Th2 response and Th17 cells in the tumor microenvironment. In vitro studies showed that PGE$_2$ shifted CD4$^+$ T cells to Th2 cells by downregulation of Th1 cytokines and upregulation of Th2 cytokines (153, 154). However, another study revealed that low concentrations of PGE$_2$ induced Th1 differentiation and high concentrations inhibited Th1 differentiation (155). In addition, PGE$_2$ exacerbated inflammation and disease severity by increasing infiltration of Th17 cells into the colonic tissue in a murine model of IBD (145). Moreover, an EP4 antagonist was found to reduce accumulation of both Th1 and Th17 cells in regional lymph nodes and suppressed disease progression in an animal model of chronic inflammation (155). Indeed, in vitro studies revealed that PGE$_2$ facilitated IL-23–induced differentiation of Th17 from naive T cells (156). PGE$_2$ also directly promotes differentiation of memory CD4$^+$ T cells to Th17 cells by induction of IL-17 expression and reduction of IFN-γ expression (157). Clearly, further research is needed to determine whether PGE$_2$ promotes gastrointestinal tumorigenesis via Th cells.

CD8$^+$ cytotoxic T cells. The density of tumor-infiltrating CD8$^+$ T cells is associated with better survival of CRC and GC patients (148, 158). Although the role of PGE$_2$ in regulation of tumor-associated CD8$^+$ T cells in vivo remains unclear, one in vivo study showed that PGE$_2$ suppressed cytotoxic T lymphocyte (CTL) survival and function during chronic lymphocytic choriomeningitis virus infection (159). Moreover, a number of in vitro studies have demonstrated that PGE$_2$ inhibits CD8$^+$ T cell proliferation and impairs the CD8$^+$ CTL function. PGE$_2$ can directly inhibit CD8$^+$ T cell proliferation by promoting replicative senescence (160). PGE$_2$ also suppresses the cytotoxic activity of CD8$^+$ T cells by upregulation of CD94 and the NKG2A C-type lectin receptor complex (161) or by attenuating T cell receptor–induced IFN-γ release (162). Moreover, PGE$_2$ produced by metastatic renal carcinoma cells shifted CD8$^+$ CTLs toward tumor antigen–specific tolerance during interaction of CTLs and tumor cells (163). Clearly, these in vitro results need to be confirmed in animal models of gastrointestinal cancer.

PGE$_2$ and innate leukocytes

Innate leukocytes include NK cells, mast cells (MCs), and phagocytic cells. The role of MCs in gastrointestinal cancer remains elusive, because contradictory results have been reported in human CRC specimens and mouse models of CRC. For example, tumor-infiltrating MCs have been shown to correlate with either positive or negative prognoses in CRC (164–166). Similarly, elimination of MCs resulted in reduction of tumors in *Apc^{Δ468}* mice and mice treated with carcinogenic 1,2-dimethylhydrazine (167, 168), indicating that MCs promote polyp formation. In contrast, absence of MCs led to induction of tumors in *Apc$^{Min/+}$* mice (169), suggesting that MCs inhibit tumor formation. Therefore, more work needs to be completed in this area before any definitive conclusions can be made.

NK cells. NK cells are able to recognize and kill transformed or virus-infected cells but spare normal cells in the absence of antigen-specific priming. Interestingly, one in vitro result showed that NK cells selectively recognized and killed colonic cancer stem cells (CSCs) (170). Suppressed NK cell activity has been found in human CRC and is an important prognostic factor for the development of distant metastases (171, 172). Similarly, tumor-infiltrating NK cell levels are associated with an improved survival in GC (173). More intriguingly, intratumoral NK cell levels are negatively correlated with levels of COX-2 expression in GC (173).

Although there are no available in vivo data showing that PGE$_2$ suppresses NK cell cytotoxicity in gastrointestinal cancer, in vivo studies demonstrated that treatment of rats with PGE$_2$ inhibited

NK cell activity and enhanced lung metastases (174) and reversed enhancement of NK cell activity by an NSAID (175). Modulation of EP4 receptor signaling mediated the effects of PGE_2 on promotion of breast cancer metastasis and suppression of NK cell function in a syngeneic murine model of metastatic breast cancer (176). Substantial in vitro evidence has further demonstrated that PGE_2 suppresses NK cell function via multiple mechanisms. PGE_2 suppressed NK cytotoxicity by inhibiting NK receptors via a cAMP/PKA pathway (177). Moreover, PGE_2 not only directly inhibited NK cell production of IFN-γ, which is essential for NK cell functions, but also attenuated IL-12–induced or IL-18–induced IFN-γ expression in NK cells via EP2 receptor (178, 179). In addition to NK function, PGE_2 secreted from GC cells also inhibited NK cell proliferation and induced apoptosis (173). Taken together, these findings suggest that PGE_2 not only suppresses NK functions, but also inhibits NK cell proliferation and survival (Figure 3). More work is needed to evaluate whether PGE_2 promotes gastrointestinal tumorigenesis via suppression of NK cells.

Summary

Our focus on prostanoids indicates that PGE_2 has been shown to promote gastrointestinal tumor formation, progression, and metastasis by multiple mechanisms. In addition to the direct effect of PGE_2 on tumor cell proliferation, survival, and migration/invasion, PGE_2 has been shown to promote CRC initiation, growth, and metastasis by silencing certain tumor suppressor and DNA repair genes via DNA methylation and by induction of CSC formation and expansion. Strikingly, PGE_2 is also one of the tumor-associated immunosuppressive mediators that help to stimulate immunosuppression by enhancement of immunosuppressive cells, a defect in antigen-presenting cell function, a shift from Th1 to Th2 and Th17 immune responses, and/or impairment of functions of CD8+ cytotoxic T cells and NK cells, resulting in escape of tumor cells from effective immunosurveillance. Therefore, more selective pharmacologic inhibitors of PGE_2 signaling not only target tumor cells, including CSC, but also subvert tumor-induced immunosuppression. It is clear that effective therapies should include elimination of tumor cells, especially CSCs, inhibition of tumor-associated angiogenesis, and subversion of tumor-induced immunosuppression. Clinical studies are warranted to evaluate the efficacy and toxicity of these inhibitors, such as EP2 and EP4 antagonists, in gastrointestinal cancer.

Acknowledgments

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