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COX-Derived Prostanoid Pathways in Gastrointestinal Cancer Development and Progression: Novel Targets for Prevention and Intervention

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Abbreviations: AOM, azoxymethane; APC, adenomatous polyposis coli; CRC, colorectal cancer; OAC, oesophageal adenocarcinoma; AA, arachidonic acid; COX, cyclooxygenase; PGDS, prostaglandin D synthase; DP, prostaglandin D2 receptor, 15d-PGJ2; 15-deoxy-delta(12,14)prostaglandin J2, PGES, prostaglandin E synthase; PGE₂, prostaglandin E₂; EP, prostaglandin E₂ receptor; PGIS, prostacyclin synthase; PGI₂, prostacyclin; IP, prostacyclin receptor; PPAR, peroxisome proliferator activated receptor; TXS, thromboxane synthase; TXA₂, thromboxane A₂; TP, thromboxane receptor.

Abstract

Arachidonic acid metabolism through cyclooxygenase (COX) pathways leads to the generation of biologically active eicosanoids. Eicosanoid expression levels vary during development and progression of gastrointestinal (GI) malignancies.

COX-2 is the major COX-isoform responsible for G.I. cancer development/progression. COX-2 expression increases during progression from a normal to cancerous state. Evidence from observational studies has demonstrated that chronic NSAID use reduces the risk of cancer development, while both incidence and risk of death due to G.I. cancers was significantly reduced by daily aspirin intake. A number of randomised controlled trials (APC trial, Prevention of Sporadic Adenomatous Polyps trial, APPROVe trial) have also shown a significant protective effect in patients receiving selective COX-2 inhibitors. However, chronic use of selective COX-2 inhibitors at high doses was associated with increased cardiovascular risk, while NSAIDs have also been associated with increased risk. More recently, downstream effectors of COX-signalling have been investigated in cancer development/progression. PGE₂, which binds to both EP and PPAR receptors, is the major prostanoid implicated in the carcinogenesis of G.I. cancers. The role of TXA₂ in G.I. cancers has also been examined, although further studies are required to uncover its role in carcinogenesis. Other prostanoids investigated include PGD₂ and its metabolite 15d-PGJ₂, PGF_{1α} and PGI₂. Targeting these prostanoids in G.I. cancers has the promise of avoiding cardiovascular toxicity associated with chronic selective COX-2 inhibition, while maintaining anti-tumor reactivity.

A progressive sequence from normal to pre-malignant to a malignant state has been identified in G.I. cancers. In this review, we will discuss the role of the COX-derived prostanoids in G.I. cancer development and progression. Targeting these downstream prostanoids for chemoprevention and/or treatment of G.I. cancers will also be discussed. Finally, we will highlight the latest pre-clinical technologies as well as avenues for future investigation in this highly topical research field.

1. Introduction

Cancer causes seven million deaths annually, accounting for 12.5% of deaths worldwide. It is the second leading cause of death in the developed world and is among the three leading causes of death for adults in developed countries. It is estimated that, by 2020 there will be 16 million new cases every year, representing a 50% increase in cancer incidence [1].

The arachidonic acid pathway is responsible for the generation of a wide variety of bioactive metabolites. These metabolites, otherwise known as eicosanoids, have been shown to be involved in many different pathologies, including inflammation and cancer [2]. Arachidonic acid can be metabolised into the biologically active eicosanoids via the action of three separate groups of enzymes: cyclooxygenases (COX), lipoxygenases (LOX), and epoxigenases (cytochrome *P450*). The COX enzymes catalyse the first step in the synthesis of prostanoids from arachidonic acid [3]. COX was shown to exist as two distinct isoforms in the early 1990's. These included the constitutively expressed COX-1 and the inducible form of COX-2, associated with inflammation [4]. A third COX isoform has also been identified, known as COX-3 [5]. However, subsequent studies have shown that it has no COX activity and is therefore unlikely to have prostaglandin-producing activity in human tissues [6]. COX-derived prostanoids, prostaglandins and thromboxanes, are biologically active lipid mediators involved in a wide range of physiological processes such as modulation of vascular tone, the inflammatory response and gastric cytoprotection. Prostanoids have also been implicated in various disease states such as arthritis, heart disease and pulmonary hypertension [7].

The development of cancer in both humans and experimental animals is consistently linked to an imbalance in COX signaling [3, 8]. There has been a significant interest in COX-2 and its role in the development and progression of cancer over the past decade, with a number of phase II studies examining celecoxib as a potential chemopreventative agent

[9-12]. COX-2 expression has been associated with a poor prognosis in a variety of cancer states [13-15]. A number of clinical trials have been carried out to examine the role of COX-2 inhibition in cancer chemoprevention [16-22], with further human trials ongoing. However, conflicting studies aimed at examining the role of non-specific COX or selective COX-2 inhibition will lead to difficulty interpreting these results. For example, an inhibition in mouse lung tumorigenesis has been observed in studies using non-selective COX inhibitors [23, 24]. However, selective COX-2 inhibition with celecoxib resulted in reduced pulmonary inflammation, but no differences in tumor multiplicity and an increase in tumor size in an initiator promoter lung tumor mouse model [25]. In addition to these observations, chronic administration of selective COX-2 inhibitors at high concentrations has been associated with an increased risk of cardiovascular events, such as thrombosis, stroke, and myocardial infarction [20, 21, 26]. The mechanism whereby these drugs contribute to cardiac complications is thought to be through a disruption in the fine balance between PGI₂ and TXA₂, which modulate platelet activation and aggregation, thereby regulating blood clotting. The role of these respective prostanoids in the regulation of coagulation was demonstrated using a rat model of pulmonary hypertension [27], and subsequently confirmed in a *COX*-gene disrupted mouse model [28]. This model demonstrated that *COX-2* gene deletion exacerbated the pulmonary hypertensive response to hypoxia, an effect mediated at least in part through enhanced sensitivity to TXA₂, and a resulting increase in intravascular thrombosis. These findings are further supported by seminal studies of the cardiovascular effects of selective COX-2 inhibitors and the pharmacological roles of downstream receptors [29, 30].

The effects of COX expression in cancer is thought to be related to the expression profile of down-stream COX-derived prostanoids. Evidence for a role for the prostanoids in carcinogenesis emerges from the numerous epidemiological studies demonstrating that chronic intake of non-steroidal anti-inflammatory drugs (NSAIDs), particularly aspirin, prevents development of the disease [31]. However, the relationship of the prostanoid profile to cancer growth is not yet completely understood [32]. Previous studies have proposed that the prostaglandin biosynthesis profile of malignant cells may differ from that of normal tissue [33, 34]. Increased COX-2 expression has been associated with increased

levels of downstream enzymes required for prostanoid synthesis, suggesting that the tumor-promoting effects of COX-2 overexpression may be attributable to specific downstream products of arachidonic acid metabolism [35]. The past number of years has seen considerable interest in targeting downstream effectors of the cyclooxygenase signaling pathway in cancer. Selective targeting of these downstream effectors could have the potential of avoiding the cardiovascular effects associated with selective COX-2 inhibition, while maintaining anti- cancer properties.

There has recently been a significant rise in the number of reports examining the fractional roles of COX-derived prostanoids in the development and progression of gastrointestinal cancers. In this review, we will discuss the current state of knowledge regarding the contributions of the COX-derived prostanoids and the signalling pathways activated by these prostanoids in GI cancer development and progression. We will assess a role for the prostanoids as potential targets for chemoprevention and/or treatment of these tumor types, which may be used alone, or in combination with other agents/conventional therapies. Finally, we will highlight novel pre-clinical screening technologies, which may be used as platforms for development of targeted therapies against gastrointestinal malignancies.

2. Cyclooxygenase Metabolism: Generation and Classification of Cyclooxygenase-Derived Prostanoids.

Arachidonic acid (A.A.) is virtually undetectable under basal conditions. However, it can be mobilized from the plasma membrane in response to a variety of growth factors, cytokines, and hormones, and converted to a range of bioactive lipids, or eicosanoids, *via* three distinct pathways: cyclooxygenase (COX), lipoxygenase (LOX) or P-450 epoxigenase. Cyclooxygenase (COX) is a rate limiting enzyme in the synthesis of the prostanoids. It catalyses the conversion of A.A. to PGG₂, and subsequently to PGH₂, which is then further converted to the prostanoids by a range of cell specific synthases and isomerases. These prostanoids include PGD₂, PGE₂, PGF_{2α}, PGI₂ (prostacyclin) and TXA₂ (thromboxane A₂).

At least two isoforms of COX have been identified to date; COX-1 and COX-2. COX-1 is constitutively expressed in most tissue types. Prostanoids produced by this isoform generally mediate 'housekeeping' functions such as cytoprotection of the gastric mucosa, regulation of renal blood flow, and platelet aggregation. However, COX-1 has also been shown to be inducible, particularly at sites of inflammation [36-38]. Many studies have demonstrated that expression of COX-1 may be induced in endothelial cells in response to a range of stimuli [39-41]. COX-1 is the only isoform expressed within platelets [42, 43] and is an important mediator of platelet aggregation. COX-2 on the other hand is a highly inducible enzyme under physiological conditions, although it is constitutively expressed in some tissues such as the brain, spinal cord, [44], and kidneys [45]. While COX-2 expression is highly restricted under basal conditions, its expression is dramatically up-regulated at sites of inflammation in response to cytokines such as interferon γ , TNF α and IL-1 β . [46, 47], hormones [48], growth factors [49-51] and hypoxia [52, 53]. COX-2 expression has also been observed in neoplastic and endothelial cells within many different tumors [54]. A third isoform of COX was discovered by Chandrasekharan *et al*, and termed COX-3 [5]. This is a splice variant of COX-1, in which intron-1 has been retained in the mRNA. This isoform is unlikely to have prostaglandin-producing activity in human tissues, however. Rat COX-3 has been fully expressed and demonstrated to have no COX-activity [6].

The key regulatory step in the COX-signalling pathway is the enzymatic conversion of arachidonate to PGG₂, which is then reduced to an unstable endoperoxide intermediate, PGH₂. PGH₂ is then catalytically converted to the various prostanoids via reduction, rearrangement, or isomerisation by the terminal synthase enzymes (prostaglandin-E-synthase, prostaglandin-D-synthase, prostaglandin-F-synthase, prostacyclin synthase and thromboxane synthase). The resulting prostanoid (prostaglandins and thromboxanes) products are unstable compounds and are therefore rapidly metabolised *in-vivo* [55]. COX-derived prostanoids are readily generated by a number of cell types. Platelets, mast cells and monocytes/macrophages synthesize TXA₂, PGD₂, PGE₂ and PGF_{2 δ} , while endothelium is the major source of PGI₂ [56]. Arachidonic acid-derived prostanoids are biologically active lipid mediators involved in a wide range of physiological processes such as

modulation of vascular tone, the inflammatory response and gastric cytoprotection. However, they have also been implicated in various disease states such as arthritis, heart disease and pulmonary hypertension [57]. The prostanoids exert their cellular functions by binding to cell surface receptors belonging to a family of seven transmembrane domain G-protein-coupled receptors [58]. The prostanoid receptor nomenclature is assigned based on the ligand bound by as opposed to genetic or functional relationships. TP_{α}/TP_{β} binds TXA_2 , DP binds PGD_2 , EP1-4 binds PGE_2 , FP binds $PGF_{2\delta}$, while IP binds PGI_2 . In some cases, prostanoids and their metabolites bind their nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) [59]. Three distinct isotypes of PPARs have been identified to date and may be generally designated as $PPAR\alpha$, $PPAR\beta/\delta$ and $PPAR\gamma$ [60, 61] (Fig. 1).

3. Cyclooxygenases and Gastrointestinal Cancer

3.1 Gastrointestinal cancer incidence and development

Esophageal cancer is the ninth most common malignancy worldwide and the fifth most common cancer in the developed world, with its incidence increasing. 5-year survival is currently less than 15%, regardless of whether patients have undergone surgery or not. Given its poor prognosis and increasing incidence, the development of preventative and/or novel treatment strategies are vital. Esophageal cancer consists of two separate histological subtypes; squamous cell carcinoma (SCC) and adenocarcinoma (AC), whose incidence has increased markedly over the past number of years. The major risk factor for the development of esophageal adenocarcinoma is reflux esophagitis, where chronic inflammation can lead to intestinal metaplasia known as Barrett's esophagus [62]. Patients with Barrett's esophagus have a 35-125-fold increased risk of developing adenocarcinoma of the esophagus [63], the incidence of which has increased markedly over the past 20 years [64]. In contrast, there is no evidence to show that chronic gastrointestinal reflux disease leads to squamous cell carcinoma of the esophagus [65]. The risk factors involved in the development of this cancer subtype are multifactorial, but cigarette smoking and alcohol consumption are two of the leading risk factors [66]. While squamous cell carcinomas occur mainly in the proximal third of the esophagus, esophageal adenocarcinomas occur in the distal two thirds of the esophagus and gastroesophageal junction [67].

Gastric cancer is the fourth most common cancer worldwide and has the third highest mortality rate, accounting for 11% of cancer-related deaths [68]. Although its incidence has decreased in the US and in Western Europe, it remains a major cause of cancer-related death in many countries [69]. While the overall incidence of gastric cancer has decreased significantly over the past 70 years or so, cancers of the gastric cardia have increased over the past 2 decades [70]. This increase has been attributed to the rising incidence of Barrett's esophagus [71]. The development of gastric cancer occurs in a multistep process, going from normal gastric mucosa to chronic active gastritis, gastric atrophy, intestinal metaplasia, and finally, dysplasia and cancer [72]. Epidemiologic evidence suggests that *Helicobacter pylori* (*H. pylori*) play an important role in this process. This bacterium has

been shown to induce COX-2 expression, with a concomitant generation of PGE₂ in both malignant and pre-malignant lesions [73, 74]. The molecular mechanism of *H pylori* involvement in gastric carcinogenesis remains to be elucidated however.

Colorectal cancer is the third most common cancer globally and has the fourth highest mortality rate, accounting for 7.6% of cancer-related deaths worldwide [68]. Colorectal cancer may be described as a heterogeneous disease, with at least three major forms identified: hereditary, sporadic, and colitis-associated CRC. Patients with familial adenomatous polyposis (FAP), caused by a germline mutation in the tumor suppressor gene *adenomatous polyposis coli* (*Apc*), have an almost 100% risk of developing CRC by 40 years of age if left untreated [75, 76]. Hereditary colorectal cancer due to inherited mutations in genes responsible for DNA mismatch repair is responsible for approximately 2-7% of all CRC cases. Other risk factors for CRC development include chronic inflammation and aging. An abnormal mucosal immune response of the colorectum is thought to lead to a chronic inflammatory state known as inflammatory bowel disease (IBD). IBD represents a complex group of disorders, the two major forms of which include ulcerative colitis and Crohns disease. In combination with the hereditary forms of FAP and hereditary CRC, IBD is among the top three high risk conditions for CRC development [77].

3.2 Cyclooxygenases and gastrointestinal malignancies

The incidence of COX-2 tissue expression gradually increases with the development of esophageal lesions, ranging from 75% expression in metaplasia, to 83% in low grade dysplasia, to 100% in high-grade dysplasia and esophageal adenocarcinoma [78]. Selective COX-2 inhibitors induced both growth arrest and apoptosis in esophageal cancer cells [79-82]. Selective COX-2 inhibition has also demonstrated suppressive effects on the epithelium of Barrett's esophagus *in-vivo*. This effect was proposed to be mediated *via* increased apoptosis and reduced cell proliferation [80, 83]. In addition, selective COX-2 inhibition has also been shown to prevent esophageal cancer development *in-vivo* [84, 85]. In light of these previous observations, a Chemoprevention for Barrett's Esophagus Trial (CBET) was started in 2003 for the study of celecoxib in patients with Barrett's esophagus

and low or high-grade dysplasia [86]. The authors found that 200 mg of celecoxib twice daily did not appear to prevent progression of Barrett's dysplasia to cancer [22]. However, it was suggested that this dose may have been too low to have an effect on cancer development. The addition of a selective COX-2 inhibitor, rofecoxib, to acid inhibition therapy did not affect the cellular proliferation index in Barrett's esophagus cells following 6 months of treatment. However, increased cellular apoptosis was observed, in combination with a reduction in both COX-2 and VEGF expression [87]. In a rat model of reflux-induced esophageal adenocarcinoma, treatment with sulindac or a selective COX-2 inhibitor was significantly associated with a reduction in tumor incidence [84]. A more recent study in a small number of patients with Barrett's esophagus indicated that 12 days of treatment with the selective COX-2 inhibitor rofecoxib, reduced COX-2 expression, PGE₂ release, and cell proliferation [88]. COX-2 up-regulation was shown to be a late occurrence in esophageal adenocarcinoma, which was independently associated with reduced survival following surgery. In contrast, COX-2 expression in squamous cell carcinoma was not found to be associated with a poor outcome, suggesting that the significance of COX-2 expression may vary between tumor subtypes [89]. While COX-2 expression gradually increased during the progression to adenocarcinoma, COX-1 expression was down-regulated in neoplastic tissue, relative to normal controls [90, 91]. A second study found that COX-1 was expressed in normal esophageal mucosa, but only occasionally induced in well-differentiated squamous cell carcinoma. In contrast, COX-2 expression was only observed in dysplasia and carcinoma, suggesting a role in tumorigenesis [92]. These observations suggest that while there is a clear role for COX-2 in oesophageal carcinogenesis, the role of COX-1 is questionable.

The relationship between COX-2 and gastric cancer has been frequently reported and is well known [93]. COX-2 expression increases throughout the process of gastric cancer development, ranging from 10% in superficial gastritis, to 69.5% in cancer [94]. Over-expression has been reported in pre-cancerous lesions of the stomach (metaplastic and adenomatous gastric cells), suggesting a role for this enzyme in carcinogenesis [95]. Expression levels are higher in gastric tissue infected with *Helicobacter pylori*, while the intensity of expression is similar in the presence or absence of malignant tissue [74]. COX-

2 expression in gastric cancer patients is associated with a poor prognosis [96]. In addition, COX-2 expression is significantly associated with expression of apoptosis and proliferation markers such as p53 and Ki-67. However, the precise mechanisms underlying the effects of COX-2 expression on tumor development and progression remain unclear. The role of COX-2 in gastric carcinogenesis was further validated using a transgenic model over-expressing both COX-2 and microsomal PGE synthase [97]. In these animals, the development of inflammation-associated hyperplastic gastric tumors was suppressed following 4 weeks of treatment with NS-398. Expression of both COX-2 and microsomal PGE synthase also converted preneoplastic lesions into dysplastic gastric tumors by 20 weeks in transgenic mice expressing Wnt-1, suggesting a role for both Wnt and PGE₂ pathways in gastric cancer development [98]. Unlike COX-2, there is little known of the role of COX-1 in gastric cancer. While a number of studies have examined the effect of aspirin and NSAIDs on gastric carcinogenesis, the role of COX-1 has not been directly assessed. However, an early clinicopathologic study revealed no association of COX-1 expression with prognosis in gastric adenocarcinoma [99].

The observation that COX-2 was up-regulated by 2 to 50 fold in human colorectal adenomas and adenocarcinomas has stimulated intense research activity to understand the association between COX-2 and cancer [100]. COX-2 was subsequently found to be up-regulated in the *Apc*^{min/+} mouse model, which exhibits mutations in the adenomatous polyposis coli gene and serves as a model for familial adenomatous polyposis [101]. A number of further studies later confirmed that COX-2 is consistently up-regulated in a significant number of malignant and pre-malignant tumors [77]. It was suggested that COX-2 may be a target for the future treatment of CRC. Later studies confirmed these observations and found elevated COX-2 expression in approximately 50% of adenomas and 85% of adenocarcinomas [102, 103]. Direct molecular evidence for a role for COX-2 in CRC carcinogenesis was obtained from *in-vivo* studies, where disruption of the COX-2 gene resulted in reduced tumor formation in both small intestine and colon of *Apc*^{Min/+} mice [104]. *Apc* mutations leads to uncontrolled growth of intestinal epithelial cells and are commonly associated with the earliest stages of CRC carcinogenesis [75]. Both genetic and pharmacological studies support the hypothesis that COX-2 is negatively regulated by *Apc*

and that *Apc* mutation and subsequent dysregulation of COX-2 expression promotes CRC development and progression [105]. In patients with familial adenomatous polyposis (FAP), the use of both NSAIDs and selective COX-2 inhibitors has been shown to reduce both the size and number of intestinal adenomas [106-108]. While the relationship between colorectal cancer (CRC) and COX-2 expression is one of the most fully investigated in research into colorectal polyps, adenoma, and cancer [69], there is also evidence for a role for COX-1 in colorectal carcinogenesis. Spontaneous polyp formation in *APC1309* mice was reduced by both COX-1 and COX-2 inhibition, while dual inhibition of both isoforms doubled this effect [109]. Selective COX-1 inhibition suppressed intestinal carcinogenesis in both AOM rats and *APC1309* mice [110]. This effect was similar to that seen with selective COX-2 inhibition, suggesting that both COX-isoforms can contribute to intestinal tumorigenesis. These observations were confirmed in a further study of selective COX-1 inhibition in colon carcinogenesis [111]. A recent study demonstrated reduced proliferation and increased macroautophagy in colon cancer cells following COX-1 inhibition [112]. More recently, it was demonstrated that tumor formation in colitis-associated colon cancer was not dependant on COX-1 or COX-2 expression, suggesting that the mechanism of tumour promotion may vary for different forms of CRC [113]. Selective COX-1 but not COX-2 inhibition was found to mimic the anti-tumor and anti-angiogenic effects of a chemically modified NSAID [114]. While a role for COX-2 in colorectal carcinogenesis is clear, there is also clear evidence to implicate COX-1 in tumor development and progression, which should not be overlooked

4. Cyclooxygenase-Derived Prostanoids in the Development and Progression of Gastrointestinal Malignancies

In addition to their physiological roles, COX-derived prostanoids are involved in a range of pathological processes, including inflammation and cancer. In the last number of years, numerous studies have examined the role of individual prostanoids in the process of carcinogenesis and the hallmarks of cancer (Table 1). Of these COX-derived prostanoids, PGE₂ is the most widely investigated in G.I. cancers, with overexpression frequently reported in G.I. tract tumors [115]. More recently, there has been significant interest in uncovering the role of PGD₂ (and its dehydration product 15d-PGJ₂) and TXA₂ in G.I. malignancies. These studies have been particularly aimed at identifying novel potential targets for chemoprevention and/or treatment.

4.1 Prostaglandin D₂ pathway

Deletion of the gene for hematopoietic PGD synthase was found to accelerate tumor growth in *Apc*^{Min/+} mice. In addition, transgenic overexpression of hemopoietic PGD synthase results in few intestinal adenomas than corresponding controls [116]. The role of PGD₂ in colon cancer remains unclear, although it has been shown to regulate IBD through its anti-inflammatory effects [117]. However, studies carried out to examine the role of the downstream DP receptor in CRC suggest that the corresponding PGD₂ prostanoid does not inhibit tumor growth. DP receptor deletion was found to have no effect on colon tumor formation in AOM-treated mice [118]. In addition, selective DP antagonism significantly reduced aberrant crypt foci development in post-colitis rats [119]. Two possible explanations have been put forward for this. Firstly, as PGD₂ also exerts its biological functions via the nuclear PPAR γ receptor, the anti-tumor effects of PGD₂ may be mediated through activation of this receptor. This would result in growth arrest of the tumor cells via inhibition of cell proliferation and/or induction of apoptosis. Secondly, PGDS overexpression may shift the conversion of PGH₂ away from PGE₂, thereby suppressing tumor growth [120].

Inhibition of PPAR γ ligand activation by 15-deoxy-delta-PGJ₂ has been examined in esophageal cancer progression. PPAR γ is involved in growth inhibition of several tumor

types, possibly through induction of cell cycle arrest and/or apoptosis. Inhibition of PPAR γ by ligands such as 15-deoxy-delta-PGJ₂ inhibited proliferation and induced apoptosis in adenocarcinoma and squamous cell carcinoma cell lines [121, 122], suggesting that inhibition this nuclear hormone receptor has potential as a future treatment of the disease. PPAR γ has been implicated in *Helicobacter*-related gastric carcinogenesis, suggesting that PPAR γ agonists may be of therapeutic benefit in gastric cancer [123]. 15d-PGJ₂ suppressed gastric cancer cell angiogenesis via down-regulation of the pro-angiogenic factor, ang-1 [124]. PPAR γ ligands (including 15d-PGJ₂) inhibited gastric cancer cell growth in a dose-dependant manner, while prostacyclin, a PPAR δ agonist, had no effect [125]. PPAR γ agonist treatment was associated with an up-regulation in pro-apoptotic gene expression (*bad*, *p53*) and a down-regulation in anti-apoptotic gene expression (*bcl-2*, *bcl-xl*). Furthermore, a PPAR γ ligand significantly reduced gastric tumor volume *in-vivo*, suggesting that agonists of PPAR γ , such as 15d-PGJ₂ may play a role in gastric cancer prevention/treatment. This hypothesis was further supported by the observation that 15d-PGJ₂ inhibited gastric cancer cell growth and induced apoptosis and cell arrest in a concentration and time-dependent manner [126].

While there is limited evidence for PGJ₂ formation at concentrations sufficient for PPAR interaction *in-vivo*, both PGJ₂ and 15d-PGJ₂ induced proliferation of colorectal cancer cells [127]. However, a subsequent study found that apoptosis was induced following treatment with 15d-PGJ₂ [128]. This observation was supported by a number of further studies [129-134]. These subsequent studies also identified that 15d-PGJ₂ treatment induced cell cycle arrest [130]. Investigation of the mechanisms underlying the pro-apoptotic effects of 15d-PGJ₂ treatment uncovered an association with reduced levels of anti-apoptotic genes *NF-kappaB* and *bcl-2* [128]. Further gene-expression profiling identified a down-regulation in *c-myc* expression and up-regulation in *c-jun* and *gadd153* [132]. An up-regulation in death receptor 5 following 15d-PGJ₂ treatment was also reported, an effect which was independent of PPAR γ and p53, but dependent on C/EBP homologous transcription factor gene expression [134]. Down-regulation of *hTERT* by 15d-PGJ₂ was also shown to mediate its pro-apoptotic effect [131]. Combined treatment with 15d-PGJ₂ and a histone deacetylase (HDAC) inhibitor had a synergistic effect on caspase-

dependent apoptosis, leading to ROS generation and endoplasmic reticulum stress to decrease expression of anti-apoptotic bcl-X(L) and XIAP and increase expression of CAAT/enhancer binding protein homologous protein and death receptor 5 [129]. The authors suggested that co-treatments with clinically relevant HDAC inhibitors and PPAR γ ligands are promising for the treatment of a wide range of malignancies. 15d-PGJ2 treatment also induced apoptosis in an *in-vivo* model, resulting in the generation of reactive oxygen species (ROS) *via* JNK activation and Akt inactivation [133]. A role for 15d-PGJ2 in angiogenic mechanisms has also been identified, with the anti-tumor properties of this prostanoid shown to be mediated via inhibition of induction of AP-1-dependent genes such as *VEGF* or *COX-2* [135].

4.2 Prostaglandin E₂ pathway

Of all the prostanoids, PGE₂ is the most extensively studied in gastrointestinal carcinogenesis, and there is strong evidence to suggest that PGE₂ mediates both the pro-inflammatory and tumor-promoting effects of COX-2 on tumor development and progression.

4.2.1 Prostaglandin-E-synthase

Microsomal prostaglandin-E-synthase (PGES-1) inhibition significantly inhibited serum-induced proliferation and increased apoptosis in esophageal cell lines expressing COX-2. In addition, mPGES-1 inhibition had no effect on endothelial prostacyclin (PGI₂) generation, which is required to avoid the cardiovascular risk associated with selective COX-2 inhibition. mPGES-1 expression was up-regulated in both an *in-vivo* model of Barrett's esophagus and human esophageal adenocarcinoma [136, 137]. In light of these observations, inhibition of this enzyme may have therapeutic benefit in the prevention and/or treatment of OAC [138]. Both mPGES-1 and mPGES-2 were independent prognostic factors in gastric cancer overall [139], while mPGES-1 expression was associated with the formation of dysplastic gastric tumors in transgenic mice expressing Wnt-1 [98]. Genetic deletion of mPGES-1 and subsequent inhibition of endogenous PGE₂ also suppressed intestinal tumor formation in *Apc*^{Min/+} and AOM (azoxymethane) mouse models [140].

4.2.2 Prostaglandin E_2

COX-2 protein expression corresponded with PGE_2 generation in cell lines from both histological subtypes of esophageal cancer. Selective COX-2 inhibition was shown to reduce tumor multiplicity in carcinogen-induced murine model of esophageal carcinoma *via* a reduction in PGE_2 levels [141]. Both cell proliferation and PGE_2 levels were significantly reduced following selective COX-2 inhibition. However, the effects on cell proliferation were not reversed by addition of exogenous PGE_2 to COX-2 inhibitor treated cells, suggesting that these effects are PGE_2 independent [142]. As with COX-2, PGE_2 levels are also elevated in OESS. The PGES enzyme been shown to be up-regulated in response to hypoxia is OESS [143]. Hypoxia also stimulated PGE_2 generation, an effect which was HIF-1 α dependant.

COX-2 over-expression in gastric cancer was significantly correlated with PGE_2 levels and microvessel density, but not with either TXB_2 or 6-keto-PGF $_{1\alpha}$ [144]. It has been suggested that both the synthetic machinery and receptors for PGE_2 may play a central role in PGE_2 tumorigenesis. These were found to be predominantly expressed by T-lymphocytes in the gastric mucosa, at the boundary of normal mucosa with gastric tumor cells [145]. It is well known that PGE_2 has strong immunosuppressive effects, suggesting that generation of this prostanoid may allow tumors to evade immune surveillance, thereby promoting tumor growth [146]. Recently, COX-2 dependant generation of PGE_2 has been shown to play an important immunomodulatory role during *Helicobacter* infection [147]. Systemic PGE_2 administration prevented development of premalignant pathology in mouse models of gastric preneoplasia. Pre-existing lesions were also completely reversed via suppression of interferon- γ production, although increased *Helicobacter* colonization was observed in all models. These effects were caused by the immunosuppressive effects of PGE_2 on CD4 $^+$ T-helper cells, with T-cell inhibition shown to be caused by silencing of *IL-2* gene transcription. A further study aimed to examine a mechanism whereby tumor-infiltrating Treg cells with increased expression of Foxp3 may mediate immune suppression [148]. Levels of these immune cells were associated with TNM stage in patients with gastric cancer. Expression of Foxp3 in Treg cells also correlated with both COX-2 and PGE_2 levels. Finally, these cells inhibited proliferation of autologous

CD4⁺CD25⁻ T-cells, an effect which was reversed by both COX-inhibition and PGE₂ receptor antagonism. These studies further our understanding of the immunomodulatory mechanisms of COX-2/PGE₂ in gastric cancer and suggest that overcoming these effects may provide some benefit in gastric cancer treatment. In addition to its immunomodulatory role, PGE₂ has also been shown to up-regulate VEGF expression *via* transactivation of the EGFR/MAP kinase signalling pathways in gastric cancer cells. This suggests a pro-angiogenic role of PGE₂ in gastric cancer and provides a potential signalling mechanism underpinning the pro-tumor effects of COX-2 [149]. PGE₂ is the most strongly expressed prostanoid in human CRC [115]. PGE₂ protected small intestinal adenomas from NSAID-induced regression in *Apc*^{Min/+} mice [150]. Furthermore, treatment with PGE₂ significantly increased both intestinal adenoma burden in *Apc*^{Min/+} mice and significantly increase AOM-induced colon tumor incidence and multiplicity [151, 152].

4.2.3 Prostaglandin E₂ receptors

The biological effects of PGE₂ are known to be mediated by EP receptors, of which there are four different subtypes (EP1-EP4). PGE₂ exerts its effects by binding to specific EP receptors (1-4). A recent report has shown that both COX-2 and EP2 were increased in the sequence from Barrett's esophagus to esophageal adenocarcinoma. EP4 receptor expression was also increased in esophageal adenocarcinoma. The authors suggested that in addition to COX-2, both EP2 and EP4 receptors could be novel targets in the prevention and/or treatment of Barrett's-associated adenocarcinoma [153]. Antagonism of both EP1 and EP4 receptors significantly reduced tumor cell growth in an adenocarcinoma cell line, suggesting a role for selective EP antagonists in the treatment of this tumor subtype [154]. EP2 overexpression was correlated with depth of tumor invasion and was associated with a significantly lower overall survival in esophageal SCC, particularly among patients at earlier stages of disease [155]. In addition, PGE₂ and an EP2 agonist, butaprost, have been shown to induce cell proliferation and ERK phosphorylation in esophageal squamous cell carcinoma without affecting EGFR phosphorylation. It has been suggested that the effects of PGE₂ and EP2 on proliferation in esophageal squamous cell carcinoma is mediated through activation of the protein kinase C/ERK signalling cascade, and downstream immediate early growth-response genes, such as *AP-1*, *fos*, *jun*, and *c-myc* [8]. In addition

to esophageal reflux, obesity is a second major risk factor for the development of esophageal adenocarcinoma (EAC). The increasing incidence of EAC has been shown to parallel that of obesity [156]. In support of these observations, leptin (secreted predominantly by adipose tissues) stimulated esophageal tumor cell proliferation and inhibited apoptosis. These effects were found to be dependant on COX-2 expression and were mimicked by the addition of PGE₂. The pro-tumor effects of leptin and PGE₂ were diminished by an EP-4 antagonist. Furthermore, the authors found that PGE₂-mediated transactivation of the epidermal growth factor receptor and JNK activation was vital to the leptin-mediated effects [157]. In addition to leptin, PGE₂ has been shown to mediate the actions of a range of growth factors of esophageal adenocarcinoma, including acid, acidified bile, and glycine-extended gastrin [158-160].

Both COX-2 inhibition and EP2 receptor antagonism have been shown to inhibit angiogenesis and tumor invasion in gastric cells [161]. This effect was shown to be mediated through suppression of the urokinase-type plasminogen activator (uPA) system, which plays an important role in these tumor survival pathways. These observations implicate PGE₂ as the main downstream prostanoid mediating the effects of COX-2, potentially through effects on tumor survival pathways such as angiogenesis.

Transgenic mice deficient in EP receptors 1 and 4 are resistant to azoxymethane-induced aberrant crypt foci formation in the colon [118, 162]. *EPI* gene disruption significantly reduced colon cancer incidence and multiplicity as well as tumor volume in and AOM-induced model of colon cancer [163]. Disruption of the gene encoding the EP2 receptor reduced the multiplicity and size of intestinal polyps, extent of angiogenesis, and VEGF expression in mice genetically susceptible to intestinal polyp development [164, 165]. EP3 knockout mice were also found to develop less tumor-associated vessels, due to the reduction in VEGF expression [166]. The proliferative effect of low PGE₂ doses in CRC cells was shown to be mediated via G-proteins, most likely via the EP3 receptor. Interestingly, at higher doses, this effect was superimposed by a second cAMP-dependant anti-proliferative effect, suggesting that the proliferative action of PGE₂ on CRC cells is dose-dependant. EP4 has also been shown to mediate the pro-neoplastic effects of PGE₂ in

CRC cells [167]. The authors suggested that EGFR transactivation by EP4 may be a potential mechanism mediating these effects. There is mounting evidence to suggest that the mechanism through which EP activation promotes carcinogenesis includes the involvement of epidermal growth factor receptor (EGFR), protein kinase C (PKC) and the transcription factor β -catenin. It should be noted however, that PGE₂ does not necessarily activate all three components in a single cell to mediate its pro-tumor effects. In addition to being highly specific for cell and tissue-type, these signalling cascades are readily influenced and regulated by other pathways [8].

4.2.4. 15-Hydroxy-prostaglandin dehydrogenase

PGE₂ levels in cancer are regulated by degradation of an important enzyme in prostaglandin catabolism is 15-hydroxy-prostaglandin dehydrogenase (15-PGDH). 15-PGDH is a ubiquitously expressed enzyme, which catalyses oxidation of the 15(S)-hydroxyl group of prostaglandins to form 15-keto metabolites with greatly reduced biological activity [168]. 15-PGDH expression is down-regulated in gastric cancer, potentially leading to tumor progression. In addition, the pro-inflammatory cytokine IL-1 β suppressed 15-PGDH expression, at least in part through inhibition of gene promoter activity [169]. IL-1 β activity is therefore thought to play a dual role in gastric cancer. While it up-regulates COX-2 expression, it also down-regulates 15-PGDH expression, subsequently leading to increased PGE₂ expression levels. Further examination of the expression patterns of this enzyme revealed that reduction of 15-PGDH is an independent predictor of poor survival and is associated with increased cell proliferation in gastric adenocarcinoma [170]. 15-PGDH is widely expressed in normal colon mucosa, but its expression is lost in most human colorectal cancers, relative to matched normal tissue [171].

4.3 Prostaglandin F_{2 α} pathway

Biosynthesis of PGF_{2 α} can occur via three distinct pathways acting on PGH₂, PGD₂ or PGE₂. Studies carried out on PGF_{2 α} and its receptor FP suggests that that they do not play a role in the pathogenesis of IBD [172]. Also, PGF_{2 α} failed to induce cell proliferation in CRC cell lines [173], while FP receptor disruption had no effect on tumor growth in AOM-

treated mice [118]. However, a more recent report showed that $\text{PGF}_{2\alpha}$ induced cell motility with equivalent potency to PGE_2 in both colorectal adenoma and carcinoma-derived cell lines and also increased the invasiveness of colorectal cancer cells. The role of $\text{PGF}_{2\alpha}$ in carcinogenesis of colon cancer is therefore unclear. Its role in both oesophageal and gastric cancer also remains to be elucidated [174].

4.4 Prostaglandin I_2 pathway

Very little is known regarding the role of PGI_2 signalling in G.I. cancer. Prostacyclin synthase (PGIS) overexpression in a murine xenograft model of CRC resulted in a significant reduction in tumor growth and tumor-associated angiogenesis [175]. The effects of overexpression were reversed by specific PGIS inhibitors, suggesting that PGIS and its prostanoid, PGI_2 , are anti-cancer in this model. PGI_2 has been shown to activate the nuclear receptor $\text{PPAR}\delta$ in CRC cells [176]. Stromal generation of PGI_2 has been shown to promote survival in colonic epithelial cells, an effect which was proposed to be mediated via $\text{PPAR}\delta$ activation [177]. Inhibition of COX-2 derived PGI_2 activated $\text{PPAR}\delta$, resulted in up-regulation of pro-apoptotic genes such as *14-3-3epsilon*. Increased 14-3-3epsilon protein expression was subsequently shown to enhance sequestration of the pro-apoptotic Bad protein, leading to an induction of apoptosis [178]. Activation of this receptor also significantly increased intestinal tumor growth in *Apc^{Min/+}* mice [179]. Levels of the PGI_2 metabolite, 6-keto- $\text{PGF}_{1\alpha}$, were higher in adjacent areas of colon, relative to cancerous tissue, suggesting that expression of this prostanoid is lost in CRC and therefore not essential to carcinogenesis. The PGI_2 receptor, IP, is not involved in colon tumor formation in AOM-treated mice [118]. IP has also been shown to have no involvement in chronic inflammation in an animal model of IBD [172]. IP receptor expression was significantly reduced in colorectal tumor tissue, relative to adjacent normal colon tissue. However, expression was not related to clinical parameters such as tumor stage and tumor cell differentiation, suggesting no clear-cut relationship to disease progression [180]. The role of PGIS signalling in CRC development and progression therefore remains unclear. Further studies are warranted to uncover the role of this signalling pathway in CRC and the precise mechanisms through which it exerts its effects.

4.5 Thromboxane A₂ pathway

While the role of PGE₂ in esophageal cancer has been the subject of considerable interest, thromboxane A₂ (TXA₂) signalling has been investigated in a range of cancer types [181-183]. This has not yet been examined in esophageal cancer. However, preliminary studies carried out in our lab have found a significant increase in TXB₂ levels (the downstream metabolite of TXA₂) in esophageal adenocarcinoma (OAC) patients, relative to disease-free (age and sex-matched) controls (data not shown). Selective TXS inhibition has also been shown to significantly inhibit tumor cell growth in both OA and SCC cell lines (data not shown). These findings suggest that this enzyme and its corresponding downstream prostanoid may play a role in esophageal cancer and warrant further investigation.

While the role of TXA₂ signalling in gastric cancer has not been investigated, a number of studies have examined this prostanoid in CRC. Thromboxane synthase (TXS) is over-expressed in human colorectal carcinoma tissue and protein samples, relative to matched normal mucosa [184]. These observations were validated by findings in our lab of an increase in TXS expression in CRC tissue, relative to matched normal colon controls (Fig. 2). TXA₂ has been shown to play an important role in endothelial migration and angiogenesis [185]. Depletion of TXS protein expression significantly reduced colorectal tumor cell proliferation, an effect that was rescued by direct addition of a stable TXA₂ analogue [184]. The authors suggested that TXA₂ may be involved in tumor growth of CRC. Thromboxane synthase (TXS) overexpression has been shown to promote tumor growth and tumor-associated angiogenesis in a xenograft murine model of the disease [175]. The effects of overexpression were reversed by specific TXS inhibitors. Furthermore, a second *in-vivo* study also reported that a TXS inhibitor inhibits colorectal liver metastasis [186]. Thromboxane metabolite (TXB₂) levels appeared to be increased in colon cancer tissue, relative to adjacent cancer-free colon [187]. In patients with CRC, urinary excretion of the enzymatic metabolite of TXB₂ was significantly increased, relative to age-matched controls. Moreover, daily administration of aspirin for 5 consecutive days to CRC patients led to a cumulative inhibition of platelet activity both *in-vivo* and *ex-vivo*. The authors concluded that increased platelet activation occurs in CRC patients and

suggested that targeting platelet COX-1 with low-dose aspirin may promote an anti-tumor response [188]. Levels of the thromboxane receptor (TP) were significantly higher in RNA samples extracted from colorectal tumor tissue samples, relative to adjacent normal mucosa controls. However, these increases in expression were not found to be related to disease progression [180]. In addition, TP disruption had no effect on colon tumor formation in AOM-treated mice [118]. These conflicting observations suggest that the role of thromboxane signalling in G.I. carcinogenesis is still unclear. Further studies are therefore required to fully elucidate the role of this prostanoid and its corresponding enzyme in G.I. cancer development and progression.

5. Targeting the Cyclooxygenase-Signalling Pathway for Chemoprevention and/or Treatment.

5.1 Non-steroidal anti-inflammatory drugs and gastrointestinal cancers

Evidence from epidemiologic studies has suggested that long-term use of non-steroidal anti-inflammatory drugs (NSAID) may reduce the risk of developing several types of cancer, including GI malignancies [142]. There is strong epidemiological evidence to suggest that regular/occasional use of aspirin or other NSAIDs is inversely related to the risk of esophageal cancer [189-191]. A recent review of 8 published epidemiologic studies found that the reduction in the relative risk of esophageal cancer approaches 73% with daily NSAID intake [192]. A recent study of cancer-related deaths following randomized daily aspirin trials revealed a survival benefit for daily aspirin intake after only 5-years follow-up [193]. This effect was still apparent after 20-years and was greatest for the adenocarcinoma subtype. The AspECT trial (Aspirin Ezomperazole Chemoprevention Trial) is currently underway to determine the effect of inhibition of gastric acid secretion in combination with aspirin on the conversion from Barrett's esophagus to adenocarcinoma or high grade dysplasia [194]. Accrual for this trial is continuing, with the first interim analysis anticipated in 2011. Studies in both colon cancer and stomach cancer have demonstrated a 40-60% reduced risk with continuous NSAID use [195]. In addition, regular use of aspirin prevents the subgroup of colon cancers in which COX-2 is most highly induced [196]. A recent study by Rothwell *et al.*, demonstrated that the long term incidence and mortality due to colorectal cancer was significantly reduced when at least 75mg aspirin was taken

daily for several years [197]. The authors noted greatest benefit for cancers of the proximal colon, which are not effectively prevented by conventional screening methods. A more recent study of long-term risk of death from cancer following randomized trials of daily aspirin versus control found a significantly reduced risk in the treatment groups. Benefit was not associated with dose, but increased with age [193]. Treatment with NSAIDs has also been shown to attenuate tumorigenesis in rodent models of GI cancers, while indomethacin has demonstrated anti-tumor effects in animal models of esophageal cancer [45, 195].

5.2 Selective cyclooxygenase-2 targeting

Epidemiological, experimental and early clinical evidence suggests that COX-2 is a potential molecular target for treatment and/or prevention of esophageal cancer [198]. However, a recent clinical trial of selective COX-2 inhibition in combination with conventional therapy in advanced esophageal cancer patients was closed prematurely due to external safety concerns regarding celecoxib [199]. An increase in COX-2 expression has been reported in both esophageal squamous [200-202] and adenocarcinoma [88, 203], and has generally been associated with a poor prognosis [89]. Daily use of a selective COX-2 inhibitor was associated with a significant reduction in the number and multiplicity of chemically induced esophageal tumors in a rat model [85]. A second study found that the selective COX-2 inhibitor MF-tricyclic significantly reduced the relative risk of esophageal cancer in an animal model of Barrett's adenocarcinoma [84]. Both selective and non-selective COX-2 inhibitors prevented the development of reflux-induced adenocarcinoma in a murine model of Barrett's esophagus [84]. However, a chemoprevention trial of selective COX-2 inhibition failed to prevent the progression from Barrett's dysplasia to cancer, suggesting that further studies are required to fully determine the effects of COX-2 inhibition in esophageal tumorigenesis [22]. In addition, a number of trials have examined combinations of COX-2 inhibitors and pre-operative chemo-radiotherapy, with no clear benefit observed for the addition of celecoxib to conventional treatment [199, 204].

In gastric cancer, selective COX-2 inhibition reduced the growth of tumor xenografts [205]. More recently *H pylori* eradication therapy followed by selective COX-2 inhibition

with celecoxib improved gastric precancerous lesions by inducing apoptosis and inhibition cell proliferation and angiogenesis [206]. Furthermore, long-term treatment with the selective COX-2 inhibitor, etodolac, reduced metachronous cancer development in metaplastic gastritis patients, suggesting that long-term inhibition may prevent gastric cancer development [207].

The first evidence of a link between COX-2 and carcinogenesis came from a study on colorectal cancer [100], with direct molecular evidence obtained from animal studies [104, 208]. Clinically, the selective COX-2 inhibitor celecoxib was shown to enhance the effects of radiotherapy [209]. Furthermore, combined treatment of a selective COX-2 inhibitor with an oral flouropyrizine drug reduced liver metastasis to a greater extent than either drug alone [210]. Treatment with the selective COX-2 inhibitor rofecoxib did not improve overall survival or protect from disease recurrence in the adjuvant setting of CRC. It was suggested that premature closure of this trial and the short duration of treatment may have offset any therapeutic benefit for patients who were randomly assigned. Recently, it has been shown that adenoma prevention with celecoxib requires the presence of the prostaglandin degradation enzyme 15-PGDH [211]. While rofecoxib did not improve overall survival in the adjuvant setting in colorectal cancer, further phase III trials of celecoxib in the adjuvant CRC setting are about to commence [212]. While selective COX-2 inhibition has shown considerable promise for CRC prevention and/or treatment, its association with increased cardiovascular toxicity in chemoprevention trials has dampened enthusiasm for chronic use of this class of inhibitors [20, 21].

5.3 Cyclooxygenase-derived prostanoids

The toxic effects of NSAIDs and selective COX-2 inhibitors have significantly hampered their use as chemo-prophylactics to prevent cancer. Prolonged use of traditional NSAIDs have been associated with both gastrointestinal bleeding and nephropathy, effects which were attributed to their additional inhibitory effect on COX-1. Selective COX-2 inhibitors were subsequently developed in an effort to counteract these toxicities. However, chronic use of selective COX-2 inhibitors at high concentrations has been associated with an increased risk of adverse cardiovascular events [20, 21, 26]. A larger systematic analysis

further confirms that in addition to selective COX-2 inhibitors, the use of NSAIDs is also associated with a heightened cardiovascular risk [213, 214]. A potential mechanism underlying the adverse cardiovascular effects associated with selective COX-2 inhibition has been suggested to involve shuttling the hemostatic balance between the anti-aggregate PGI₂ and pro-aggregate TXA₂ in the circulation. However, chronic use of selective COX-2 inhibitors at high concentrations is required to promote a pro-coagulant effect, as endothelial cells have the ability to generate new COX-2, thereby replacing depleted downstream anti-thrombotic PGI₂ [215]. Targeting downstream pathways of COX-metabolism has the potential of avoiding the unfavorable safety profile associated with chronic COX-2 inhibition, while maintaining anti-tumor reactivity. This concept has gained considerable momentum in recent years, and is reviewed in the following sections.

There are no reports of PGD₂ targeting for G.I. cancer prevention and/or treatment. However, the last number of years has seen significant interest in the role of its hydration product, 15d-PGJ₂ as a potential treatment. This has been investigated in esophageal [121], gastric [216], and colorectal cancers [127-135]. While the role of 15d-PGJ₂ has been investigated *in-vivo* in colorectal cancer [133], most studies to date have been limited to cell lines. Further investigation using *in-vivo* models will confirm preliminary observations.

While a number of reports support a role for PGES in G.I. cancers [140, 217], few studies have been carried out to examine PGES inhibition as a potential therapeutic target. 15d-PGJ₂ has been shown to down-regulate mPGES-2 expression in colon cancer cells, implicating this synthase as a potential target for intervention [218]. A product of olive oil, dihydroxyphenylethanol, inhibited both mPGES and VEGF expression and also reduced tumor angiogenesis *in-vivo* in CRC [219]. The natural product, curcumin (derived from the spice tumeric), which exhibits anti-inflammatory and anti-carcinogenic properties, has also been shown to inhibit both PGES and PGE₂ [220]. The chemopreventative action of curcumin is proposed to be *via* modulation of arachidonic acid metabolism. Dietary curcumin significantly inhibited colon carcinogenesis *in-vivo*, and also reduced levels of PGE₂, PGF_{2α}, PGD₂, 6-keto-PGF_{1α} and TXB₂, as well as a number of lipoxygenase products [221]. Combined treatment of colorectal cells with curcumin in addition to

celecoxib resulted in a synergistic inhibitory effect on cell growth and induction of apoptosis, while curcumin augmented celecoxib inhibition of PGE₂ synthesis [222]. As PGE₂ has been shown to mediate a significant proportion of the carcinogenic effects of COX overexpression, blockade of EP receptors may also be a novel target for chemoprevention/treatment of GI malignancies. Pharmacological inhibition of EP receptors has had some success in both *in-vitro* and *in-vivo* studies. Selective EP4 antagonism attenuated the formation of aberrant crypt foci in C57BL/6Cr mice as well as intestinal polyp numbers in *Apc*^{Min/+} mice. A selective EP1 antagonist was found to reduce the incidence and multiplicity of chemically (4-nitroquinoline 1-oxide)-induced tongue cancer in a rat model of the disease [223].

In addition to PGE₂, the pro-thrombotic prostanoid, TXA₂ may also be a novel therapeutic target in the prevention/treatment of G.I. malignancies. TXA₂ may promote tumor progression *via* its effects on a range of tumor cell survival pathways such as invasion and angiogenesis [185, 224, 225]. TXA₂ has been shown to be a potent stimulator of angiogenesis both directly, and through induction of VEGF and PDGF secretion from platelets following aggregation [224, 225]. TXA₂ expression also potently regulates thrombosis; one of the most common complications in cancer and a frequent cause of cancer-related mortality [226]. Platelet abnormality and thromboembolic disorders affect 15-20% of cancer patients, with platelet activation and aggregation shown to facilitate tumor angiogenesis and metastasis [227, 228]. Recent evidence has demonstrated a link between cancer development, tumor angiogenesis and metastasis to thrombosis formation [229, 230]. Selective targeting of clotting intermediates in cancer may therefore be a novel approach to future treatment. Biomarkers which showed promise as predictive factors of thrombosis included markers of platelet activation. BM-567, the mixed TXS inhibitor/receptor antagonist was found to reduce tumor-cell induced platelet aggregation (TCIPA) in osteogenic sarcoma cells [231]. Observations of increased TXS [184] and TXB₂ [188] levels in gastrointestinal cancers, relative to normal controls suggests that targeting of this potent clotting intermediate may impact on gastrointestinal carcinogenesis (*via* its effects on coagulation and cancer survival pathways) to restore anti-tumor reactivity. Increased platelet TXA₂ levels in patient with gastrointestinal cancers may

impact on the chronic use of selective COX-2 inhibitors in this setting. Results from several clinical and experimental studies suggest that inhibition of COX-2 dependant PGI₂ formation in the vasculature in the face of unrestrained platelet TXA₂ generation may confer a heightened risk of thrombosis in patients with increased cardiovascular risk [232].

6. Pre-Clinical Screening Models for Anti-Cancer Drug Development.

Most anti-cancer drug discovery efforts to date have involved the use of cell lines and *in-vivo* xenograft models to test the efficacy of novel agents. Cell lines are well utilised in cancer research. However, results from cell lines rarely correlate with the sensitivity of tumors to the drugs in question. While they can be used in the early stages of drug development, the practice of lead optimisation in primary cell cultures and xenografts seems to yield more positive results [233]. Current models of *in-vivo* evaluation generally consist of xenograft implantation of human cancer cell lines into immunodeficient mice. However, these tumor cells represent an extreme deviation from advanced cancers *in-vivo*, and are not associated with the tumor stroma, which is a crucial component in tumor metastasis [234]. Generally, xenograft models have limited ability to predict clinical efficacy of anti-cancer agents.

Tumor explants and three-dimensional tissue models allow examination of cell:cell and cell:matrix interactions. More recently orthotopic xenograft of human tumors into nude mice has provided the ability to reproduce both the histology and metastatic pattern of most human cancers at advanced stage [235]. While not useful in examining the contribution of the immune system in this process, this model is more promising than commonly used subcutaneous (SC) xenografts in preclinical drug screening and development. This model allows the evaluation of therapies in individual human tumors derived from different genetic backgrounds, as opposed to the use of inbred animals with a homogeneous genetic background [235]. However, the ability of this model to predict clinical therapeutic response remains to be established.

It is widely accepted that *in-vivo* models are critical for defining the mechanism of drug activity and for testing therapeutic regimes. However, only a few models are ideal for this

purpose. Zebrafish have become one of the most powerful and versatile models used for biomedical research to identify novel cancer markers/molecular signatures of disease [236]. They are advantageous for drug screening as they account for the complex metabolism that affects drug efficacy or causes toxicity. Since chemicals can be directly delivered into the fish water and proteins can be injected, assessment of cytotoxic, apoptotic or anti-angiogenic effects of potential drug candidates singly, or in combination, is easy and straightforward [237, 238]. The transparency of zebrafish also allows direct imaging of mechanisms of cancer progression including cell invasion, intra-/extravasation, and angiogenesis [239, 240]. The development of xenograft zebrafish models has allowed the propagation and visualisation of human cancer cells engrafted into optically transparent zebrafish [241]. To date, zebrafish are the only animal organism to date with the potential for large-scale, yet cost effective pharmacological screens to identify potential therapies to alter tumor dissemination and/or angiogenesis [238]. Our group is interested in assessing the anti-angiogenic properties of inhibitors of the TXS signaling pathway *in-vivo*. While the effect of TXS pathway inhibitors in tumorigenesis has been investigated by our group and others [184, 242-244], the anti-angiogenic properties of these inhibitors are not fully understood. Using a transgenic line of zebrafish Tg (fli1:EGFP), which specifically expresses green fluorescent protein in vessels, we examined the effect of TXS-pathway inhibition on the morphology of intersegmental vessels (Fig 3A). Selective TXS inhibition with ozagrel (10 μ M) demonstrated clear effects on intersegmental vessel morphology following only 48 h incubation (Fig. 3B), suggesting an anti-angiogenic mechanism for this drug.

7. Summary and Future Directions

Increased expression of COX-2 is evident in the majority of gastrointestinal cancers, with COX-2 expression seen to gradually increase in the progressive sequence of many GI cancers. While both NSAIDs and selective COX-2 inhibitors may inhibit GI cancer development and progression, the downstream signalling mechanism underlying these effects remains unclear. In addition, increased cardiovascular risk associated with chronic use of selective COX-2 inhibitors suggests that alternative targets of this signalling pathway are warranted.

In light of these observations, recent research efforts have focussed on examining a role for downstream effectors of cyclooxygenase signalling in GI carcinogenesis. PGE₂ is the most extensively studied of the COX-derived prostanoids in GI carcinogenesis, although PGD₂, TXA₂ and other prostanoid pathways have also been investigated. Further downstream, pre-clinical evidence also supports the use of EP receptor antagonists as chemo-preventative and therapeutic agents for GI cancers, while the natural agent curcumin and PPAR γ agonists such as 15d-PGJ₂ have also shown promise. While the COX-derived prostanoids have shown significant promise as potential therapeutics for GI carcinogenesis, further pre-clinical and clinical studies will be required to support preliminary findings. Novel methodologies such as tumor explants, zebrafish screening, and xenotransplantation will provide significant pre-clinical insight into the validity of compounds which inhibit prostanoid metabolism as anti-cancer agents. These agents may have efficacy alone, or in combination with conventional therapeutic approaches.

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Figure 1

Generation of the COX-derived prostanoids *via* arachidonic acid metabolism. Arachidonic acid is metabolized *via* one of three distinct signaling pathways; the cyclooxygenase (COX), the lipoxygenase (LOX), and the P-450 epoxygenase pathways. While LOX and epoxygenase signalling generate metabolites hydroperoxyeicosatetraenoic acids (HPETEs) and epoxyeicosatrienoic acids (EETs) respectively, cyclooxygenases (COX-1 and COX-2) catalyse the formation of PGH₂ from arachidonic acid. PGH₂ is then further converted to the prostanoids through the action of cell-specific synthases/isomerases. Both NSAIDs and selective COX-2 inhibitors disrupt this signalling pathway at the level of the COX-isoforms and have shown considerable promise in G.I. malignancies. More recent studies have focussed on targeting downstream mediators of COX-signalling, with the potential of avoiding adverse side-effects, while maintaining anti-tumor reactivity. The COX-derived prostanoids exert their effects by binding to specific G-protein receptors, with unbound prostanoids generally further metabolised. Prostanoids and their metabolites (such as 15d-PGJ₂) may also bind nuclear receptors such as peroxisome-proliferator-activated receptors (PPARs). Prostanoids such as PGE₂ may also be degraded by the activity of prostaglandin degradation enzymes such as 15-hydroxy-prostaglandin dehydrogenase (15-PGDH).

Figure 2

Overexpression of thromboxane synthase in colorectal tumor tissue, relative to matched normal colon. Increased TXS expression was observed in colon tumor tissue sections, relative to matched normal tissue. Human placental tissue was used as both a positive antibody control (with a blocking peptide to TXS) and a positive tissue control.

Figure 3

Zebrafish as a pharmacological screening tool to identify novel anti-cancer therapies. A transgenic line of zebrafish Tg (fli1:EGFP), which specifically expresses green fluorescent protein in vessels may be used to examine the effect of potential anti-cancer compounds on intersegmental vessel morphology. Zebrafish are mated and eggs collected for screening. Approximately 6 h post-fertilization, developing larvae are added to culture plates (5 per well in duplicate). Screening compounds may be added directly to the zebrafish water for

up to 4-days. Imaging and quantification of vessels is carried out by fluorescent microscopy (A). Selective TXS inhibition with ozagrel (10 μ M) demonstrated clear effects on intersegmental vessel morphology following only 48 h incubation (B), suggesting an anti-angiogenic mechanism for this drug.

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Table 1

The effects of cyclooxygenase-derived prostanoids on the hallmarks of cancer [245]. This table references studies which have examined the roles of these prostanoids in the hallmarks of G.I. cancer (+, cancer hallmark increased; -, cancer hallmark reduced; NE, no effect).

Prostanoid	Cancer Type	Cancer Hallmark	Result	Author
PGD ₂	Oesophageal	Proliferation	-	Takashima <i>et al.</i> , 2001 ¹²⁰
		Apoptosis	+	
		Proliferation	-	Takashima <i>et al.</i> , 2005 ¹¹⁹
		Apoptosis	+	
	Gastric	Proliferation	-	Chen <i>et al.</i> , 2003 ¹¹³
		Apoptosis	+	
		Proliferation	-	Leung <i>et al.</i> , 2004 ¹²³
		Angiogenesis	-	Fu <i>et al.</i> , 2006 ¹²²
		Proliferation	-	Ma <i>et al.</i> , 2009 ¹²⁴
		Cell cycle arrest	+	
		Apoptosis	+	
	Colorectal	Proliferation	+	Chinery <i>et al.</i> , 1999 ¹²⁵
		Apoptosis	+	Chen <i>et al.</i> , 2002 ¹²³
		Apoptosis	+	Shimada <i>et al.</i> , 2002 ¹²⁶
		Angiogenesis	-	Grau <i>et al.</i> , 2004 ¹²³
		Apoptosis	+	Lin <i>et al.</i> , 2007 ¹²⁸
		Cell cycle arrest	+	Su <i>et al.</i> , 2008 ¹³²
		Apoptosis	+	Shin <i>et al.</i> , 2009 ¹³¹
		Apoptosis	+	Morai <i>et al.</i> , 2009 ¹²⁹
		Apoptosis	+	Koyama <i>et al.</i> , 2010 ¹²⁷
		Apoptosis	+	
PGE ₂	Oesophageal	Proliferation	+	Piazuelo <i>et al.</i> , 2006 ¹³²
		Proliferation	+	Ogunwobi <i>et al.</i> , 2006 ¹³⁴
		Proliferation	+	Beales <i>et al.</i> , 2010 ¹³⁴
		Apoptosis	-	
	Gastric	Inflammation	+	Takafuji <i>et al.</i> , 2002 ¹⁴³
		Angiogenesis	+	Ding <i>et al.</i> , 2005 ¹⁴⁷
		Invasion	+	Iwamoto <i>et al.</i> , 2008 ¹⁴⁸
		Angiogenesis	+	
		Inflammation	-	Toller <i>et al.</i> , 2010 ¹⁴³
		Inflammation	-	Yuan <i>et al.</i> , 2010 ¹⁴⁴
		Proliferation	+	Thiel <i>et al.</i> , 2009 ¹⁴⁸
		Metastasis	+	
	Colorectal	Proliferation	+	Tatsunaka <i>et al.</i> , 2010 ¹⁴⁹
		Angiogenesis	+	Sonoshita <i>et al.</i> , 2001 ¹⁴⁴
		Angiogenesis	+	Seno <i>et al.</i> , 2002 ¹⁴³
		Angiogenesis	+	Terruzzi <i>et al.</i> , 2010 ¹⁴⁸
PGF _{2α}	Colorectal	Proliferation	NE	Cassano <i>et al.</i> , 2000 ¹⁷²
		Invasion	+	Qualtrough <i>et al.</i> , 2007 ¹⁷³
PGI ₂	Gastric	Proliferation	NE	Leung <i>et al.</i> , 2004 ¹²³
	Colorectal	Proliferation	-	Pradono <i>et al.</i> , 2002 ¹⁷⁸
		Angiogenesis	-	
		Apoptosis	+	Wu <i>et al.</i> , 2009 ¹⁷⁷
TXA ₂	Colorectal	Metastasis	+	Yokoyama <i>et al.</i> , 1995 ¹⁸⁵
		Proliferation	+	Pradono <i>et al.</i> , 2002 ¹⁷⁸
		Angiogenesis	+	
		Proliferation	+	Sakai <i>et al.</i> , 2006 ¹⁸⁴

Fig. 1

Human Placenta (Blocking Peptide)

Human Placenta (positive)

Normal Colon (40X)

Matched Colon Tumour (40X)

Fig. 2

Fig. 3