

## The PGE<sub>2</sub>-EP Receptor Axis in Colorectal Cancer and Angiogenesis

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### ABSTRACT

Colorectal Cancer is the third most common cause of cancer-related mortality worldwide with rapidly increasing incidence. Substantial evidence suggests that elevated PGE<sub>2</sub> expression, frequently observed in colorectal tumours, enables development of a number of cancer hallmarks, including angiogenesis, which is initiated relatively early in disease and enables tumour progression. However, current therapies targeting COX-2 have an adverse safety profile owing to global prostanoid suppression. Thus, the search for therapies which more specifically modulate components of the PGE<sub>2</sub> lifecycle has gained momentum. The prostanoid receptors, specifically the EP-series of receptors via which PGE<sub>2</sub> exerts its effects, may be a particularly promising target as they sit at the initiating node of relevant signaling pathways. The enzymes regulating PGE<sub>2</sub> synthesis and degradation may also represent potential targets. This article critically examines the literature regarding the PGE<sub>2</sub>-EP receptor axis and the prospect of targeting its components for anti-angiogenic therapy.

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**Key words:** Colorectal Cancer; inflammation; PGE<sub>2</sub>; prostanoids; EP1; EP2; EP3; EP4; Angiogenesis; COX-2

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### INTRODUCTION

#### Colorectal cancer

Colorectal cancer (CRC) is currently the third most common cancer globally and is responsible for around 8% of cancer deaths. In 2008, over 1.2 million people worldwide were diagnosed with CRC with approximately a 50% mortality rate in the same year<sup>[1]</sup>. Although males and females have similar incidence rates, there is significant disparity in the geographic distribution of CRC incidence. The incidence of CRC in developed areas such as Australia and North America can be 8 to 10-fold higher than in certain parts of Africa and south-central Asia. CRC is the fourth most common cause of cancer-related death worldwide and stage of detection is the single most important prognostic indicator<sup>[2]</sup>. Patients diagnosed with stage I disease have five-year survivals of approximately 74% while for patients with stage IV disease this is less than 10%<sup>[3]</sup>. The vast majority of malignant colon and rectal cancers arise from adenomas following 5-10 year latency in individuals over 50. A number of environmental and genetic risk factors have an established role in CRC development. High visceral adiposity and dietary consumption of red meat and alcohol account for a significant CRC risk in western countries<sup>[2]</sup>. Inflammatory bowel diseases (IBD) comprising Chron's Disease and Ulcerative Colitis confer a significantly elevated CRC risk<sup>[4,5]</sup>. Furthermore, the autosomal dominant hereditary disorders Familial Adenomatous Polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC) together account for 5-10% of CRC cases. Briefly, FAP arises from mutations in *APC*, a gatekeeper that controls wnt signaling and thus regulates proliferation and differentiation of stem cells in colon crypts<sup>[6]</sup>. HNPCC arises from mutations in the mismatch repair genes *MLH1* and *MSH2* that facilitates acquisition of a mutator phenotype<sup>[7]</sup>.

#### Angiogenesis

The multistep nature of CRC development and progression has been well characterized, with normal colonic epithelium giving rise to adenomas, which progress to carcinoma *in situ* and finally to invasive disease. Initially, mutations in *APC* drive neoplasia and subsequent

mutations in k-ras and the tumour suppressor P53 lead to a loss of differentiation and increase cell proliferation and survival<sup>[8]</sup>. As in most solid tumours, nascent colorectal tumours require adequate supply of oxygen and nutrients for sustained growth. The diffusion of growth factors and oxygen from existing capillaries becomes insufficient once tumours grow beyond 1-2 mm<sup>[9]</sup> and it is necessary for hypoxic transformed cells to release paracrine mediators that enable recruitment of endothelial cells (ECs) from an existing vascular bed into the tumour mass for new vessel formation. This highly-regulated multistep process is referred to as angiogenesis. Normal differentiated adult vasculature is maintained in a quiescent state through the opposing actions of pro-angiogenic growth factors and their anti-angiogenic counterparts. Endogenous Angiogenesis-inducing factors include vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) while angiogenesis-inhibiting factors include angiostatin and vasostatin. For tumour angiogenesis to commence it is necessary for pro-angiogenic mediators to overcome the activity of anti-angiogenic factors, an event referred to as the 'angiogenic switch'. Briefly, the steps involved in angiogenesis consist of release of pro-angiogenic factors from tumour cells, binding to cognate receptors on ECs, EC proliferation, extracellular matrix (ECM) degradation, directional migration, tube formation and recruitment of mural cells (smooth muscle cells and pericytes) for stabilization. For an excellent in-depth review detailing the steps in angiogenesis and the regulatory processes see the article by Otrick and colleagues<sup>[10]</sup>.

There is evidence to suggest that angiogenesis begins relatively early in CRC development at the adenoma stage. Staton *et al* were the first group to examine microvessel density (MVD), a surrogate marker of angiogenesis, in each step of the adenoma-carcinoma sequence in patient samples<sup>[11]</sup>. Their results indicate a steady increase in expression of VEGF, the archetypal pro-angiogenic peptide, during progression. Angiogenesis was found to be induced early in dysplastic transformation at the adenoma stage. Other studies show MVD to be inversely correlated to overall patient survival, suggesting angiogenesis may accelerate tumour growth and metastasis<sup>[12]</sup>.

### CRC and COX-2

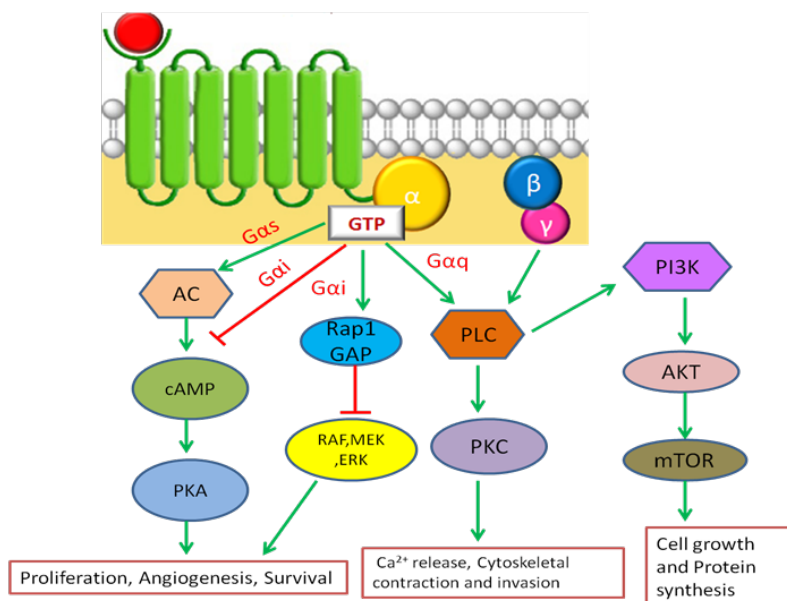
Despite our extensive understanding of the molecular mechanisms underlying angiogenesis, the assortment of regulatory factors and receptors that are involved have not been fully characterized. This in part reflects the complexity of the tumour microenvironment, with cells including macrophages, lymphocytes, fibroblasts and ECs themselves playing an important role. Eicosanoids, oxygenated derivatives of omega-6 20-carbon saturated fatty acids, predominantly arachidonic acid (AA), are released from a number of the aforementioned cell types, including tumour cells themselves. Much work in recent years has focused on unraveling the role of eicosanoids in tumour physiology in a bid to identify pertinent therapeutic targets. Eicosanoids include thromboxanes, leukotrienes and prostaglandins that are produced downstream of AA by three major pathways. Initially, AA is released from cell membrane glycerophospholipids through esterification of the sn-2 domain by phospholipase A2<sup>[13]</sup>. AA can be metabolized via the 5-lipoxygenase (5-LOX) pathway to generate 5-hydroperoxyeicosatetraenoic acid (5-HPETEs), precursors of leukotrienes which have roles in physiologic inflammation, including smooth muscle contraction and leukocyte chemotaxis, but also have been demonstrated to contribute to tumour metastasis<sup>[14]</sup> and angiogenesis<sup>[15-17]</sup>. A second pathway for AA metabolism occurs via cytochrome P450 epoxygenases resulting in the generation of epoxyeicosatrienoic acids (EETs) which are

involved in regulating a number of endothelial processes including calcium flux<sup>[18]</sup>, vascular tone<sup>[19]</sup> and tissue regeneration<sup>[20]</sup>.

The cyclooxygenase (COX) system accounts for the majority of AA metabolism and is responsible for generating prostaglandins and thromboxanes (collectively termed 'prostanoids'). Two major COX isoforms exist in mammalian cells, COX-1 and COX-2. COX-1 is constitutively expressed across most tissue types and is involved in housekeeping functions including gastric cytoprotection, platelet aggregation and renal electrolyte homeostasis<sup>[21,22]</sup>. In contrast, COX-2 constitutive expression is restricted to tissues including the lung, brain and kidney where it performs homeostatic functions<sup>[23]</sup>. In the majority of healthy tissues, however, COX-2 is not expressed but is rapidly upregulated by inflammatory stimuli, including TLR4 signalling<sup>[24]</sup> and high levels of reactive oxygen species (ROS)<sup>[25]</sup>. Both COX-1 and COX-2 function as homodimers to mediate conversion of AA into PGG<sub>2</sub>, a highly unstable intermediate which is rapidly converted into PGH<sub>2</sub> by the peroxidase activity of the COXs<sup>[26,27]</sup>. As a major source of pro-inflammatory mediators, COX enzymes have long been considered as an important therapeutic target. Non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin, inhibit both COX isoforms and prolonged NSAID use has been associated with loss of gastric mucosal homeostasis, ulceration and renal toxicity<sup>[28]</sup>. These effects are thought to be a result of COX-1 inhibition, leading to reduced expression of homeostatic prostaglandins. The development of COX-2 specific inhibitors, such as celecoxib, aimed to minimize these complications through inhibition of the inflammatory prostaglandins downstream of COX-2. While successful in a large proportion of patients, selective COX-2 inhibitors have been linked to significantly elevated risk of cardiovascular toxicity, especially myocardial infarction and stroke, across a number of population studies<sup>[29,30]</sup>. It is believed that downregulation of anti-thrombotic PGI<sub>2</sub> expression with sustained levels of COX-1 derived pro-thrombotic TXA<sub>2</sub> may underlie these events<sup>[31]</sup>. Moreover, celecoxib has been shown to interact with non-specific targets including carbonic anhydrase and PDK1<sup>[32,33]</sup>. These molecules have important physiologic roles and their disruption could lead to unexpected toxicity. The issues described here relating to NSAIDs and COX-2 inhibitors have led to a search for specific therapeutic targets downstream of COX which may be involved in tumour development or progression. Prostanoids, as described below, are involved in various steps of tumorigenesis including proliferation, evasion of apoptosis, metastasis and angiogenesis and therefore represent promising targets.

## PROSTANOIDS AND THEIR RECEPTORS IN ANGIOGENESIS

COX-2 is the rate-limiting enzyme mediating prostaglandin synthesis in inflammation and disease. The endoperoxide PGH<sub>2</sub> is converted into the various prostaglandins via terminal prostaglandin synthase (tPGS) enzymes, namely prostaglandin-D synthase (PGDS), PGES, PGFS and PGIS involved in production of prostaglandins D, E, F and I respectively<sup>[34]</sup>. Following synthesis and release of prostaglandins, these metabolites are able to bind to their cognate receptors in autocrine or paracrine fashion with high specificity. All prostaglandin receptors are rhodopsin-type heptahelical G-protein coupled receptors. Depending on their expression, they are capable of inducing a multitude of intracellular cascades that upregulate pro-inflammatory genes<sup>[35]</sup> but which are also important in driving tumorigenesis by facilitating acquisition of several hallmarks including proliferation<sup>[36]</sup>, invasion<sup>[37]</sup> and metastasis<sup>[38]</sup>. Figure 1



**Figure 1:** G-protein coupled receptors are plasma membrane-bound structures comprised of an N-terminal ligand binding domain, seven membrane-spanning domains and a C-terminal domain which interacts with heterotrimeric G proteins. Following binding of PGs (red circle) and conformational changes in the receptor, the dissociation of GTP-bound Gα subunits and the βγ complex leads to activation of a number of signaling pathways that govern tumorigenic processes including cell growth, proliferation, survival, angiogenesis and invasion. Each member of the Gα family exerts different effects on these pathways. AC: adenylylase; cAMP: cyclic adenosine monophosphate; PKA: Protein Kinase A; Rap1: Gtpase activating protein; PLC: phospholipase C; PKC: protein Kinase C; PI3K: phosphatidylinositide-3 kinases; mTOR: mammalian target of rapamycin.

below highlights several important signaling pathways implicated in cancer via PG receptor activation.

Progress in the identification of prostanoid receptors was initially slow owing to the existence of receptor isoforms and varying spatiotemporal expression. Despite data from the 1960's showing changes in activity and expression of various intracellular secondary messengers following PG receptor ligation, the first prostanoid receptor, that for TXA<sub>2</sub>, was cloned in 1989<sup>[39,40]</sup>. Work to characterize the biochemical properties PG receptors rapidly gained momentum. There are currently 8 prostanoid receptors which have been found to be conserved from mouse to humans; DP receptor for PGD<sub>2</sub>, EPs 1-4 for PGE<sub>2</sub>, FP for PGF<sub>2</sub>, IP for PGI<sub>2</sub> and TP for TXA<sub>2</sub>. The PGE<sub>2</sub>-EP receptor axis is discussed in more detail below

## PGE<sub>2</sub> AND THE TUMOUR MICROENVIRONMENT IN ANGIOGENESIS

### PGE<sub>2</sub> Synthesis

Despite evidence for PGD<sub>2</sub>, PGI<sub>2</sub>, PGF<sub>2</sub> and TXA<sub>2</sub> in angiogenesis and tumorigenesis, PGE<sub>2</sub> is the COX-2 metabolite which has been studied most extensively with regards to CRC and is therefore the main focus of this review. The synthesis of PGE<sub>2</sub> from PGH<sub>2</sub> is mediated by three synthase isoforms, namely cytosolic PGES (cPGES), membrane-bound prostaglandin E synthase 1 (mPGES-1) and mPGES-2. cPGES and mPGES-2 have both characterized as

constitutive enzymes, while mPGES-1 levels correlate with COX-2 induction<sup>[41,42]</sup>. cPGES also appears to preferentially isomerise COX-1 derived PGH<sub>2</sub>, while mPGES-1 uses COX-2 derived PGH<sub>2</sub>. mPGES-2 displays no preference as to which endoperoxide substrate to use. The potential of targeting mPGES-1 to selectively inhibit PGE<sub>2</sub> synthesis is discussed in more detail later in this review.

### Vascular EP Receptors in CRC angiogenesis

Given the abundance of PGE<sub>2</sub> in several tumour types, much work has focused on understanding its receptors and downstream signaling pathways in a bid for novel drug targets. The EP receptors consist of four subtypes viz. EP1-4. These GPCRs are coupled to diverse intracellular signaling pathways and regulate a number of tumorigenic processes, of which angiogenesis is just one. Table 1 below summarizes the biochemical properties of each EP receptor and specific effects of their activation on tumour cells, ECs and mural cells.

EP1 is coupled to Gαq and has roles to play in calcium signaling. Both EP2 and EP4 are coupled to Gas which can elevate levels of cyclic AMP via adenylylase. Paradoxically, it has been shown that EP4 can also be coupled to Gai, although this is likely to be cell-type dependent. EP3 is coupled exclusively to Gai and exerts an inhibitory effect on adenylylase<sup>[43]</sup>.

EP4 is the best characterized receptor with regard to the consequences of its activation on tumour cells and ECs, although its

**Table 1** The signaling pathways downstream of each EP receptor and the functional consequences of EP receptor activation on colorectal cancer cells, endothelial cells, smooth muscle cells and pericytes. Abbreviations: EMT –epithelial to mesenchymal transition, EGFR- epidermal growth factor receptor, HIF1- hypoxia inducible factor 1 .

EP receptor	Signalling pathways	CRC cells	Endothelial cells	Smooth Muscle cells
EP1	Gαq→PLCβ→PKC →IP <sub>3</sub> →Ca <sup>2+</sup> release	Proliferation <sup>[50]</sup> , Immunosuppression <sup>[51]</sup> , EMT <sup>[52]</sup>	HIF1 activation <sup>[45]</sup>	contraction <sup>[53]</sup>
EP2	Gas→AC→cAMP→PKA	Initiation, Proliferation, EMT <sup>[54]</sup> , genomic instability <sup>[55]</sup>	Migration, survival <sup>[56]</sup> , tubulogenesis <sup>[57]</sup>	Proliferation <sup>[58]</sup> , hyaluronan synthesis <sup>[46]</sup> , Inhibition of calcium release <sup>[59]</sup>
EP3	Gai AC→cAMP→PKA G12/13→Rho	Inhibition of proliferation <sup>[60]</sup> , Metastasis <sup>[44]</sup>	HIF1 activation <sup>[45]</sup> , PGE2 synthesis, tubulogenesis <sup>[61]</sup>	Contraction <sup>[53]</sup> , migration <sup>[62]</sup>
EP4	Gas→AC→cAMP→PKA G <sup>α</sup> i AC→cAMP→PK	EGFR-transactivation, cell growth <sup>[63]</sup> , proliferation <sup>[64]</sup> , survival <sup>[65]</sup> , EMT <sup>[66]</sup> , metastasis <sup>[67]</sup> , chemokine expression <sup>[68]</sup>	Migration, tubulogenesis <sup>[69]</sup> , cell-cell adhesion <sup>[70]</sup> , CXCL8 induction <sup>[71]</sup> , ICAM1 expression, immunosuppression <sup>[70]</sup> , differentiation of EC precursors <sup>[72]</sup>	Inhibition of calcium release <sup>[59]</sup>

role in smooth muscle cells and pericytes is yet to be elucidated. *In vivo* it is likely that PGE<sub>2</sub> induces hyperproliferation of CRC cells via occupation of EP1, EP2 and EP3 simultaneously. Fujino *et al* report EP3 to be pro-metastatic for HCA-7 cells, although this is an indirect effect mediated by VEGFR1 expression on ECs<sup>[44]</sup>. All four EPs regulate various steps of angiogenesis and while EP2 and EP4 independently promote EC migration and survival, no studies as yet report a role for PGE<sub>2</sub> in proliferation. Instead, EC proliferation may be induced via the action of VEGF released by tumour cells, stromal cells and ECs themselves later in carcinogenesis. EP1 and EP3 may contribute to the autocrine action of VEGF on ECs through HIF-1 $\alpha$  induction<sup>[45]</sup>. All four EP receptors have been found expressed on vascular smooth muscle cells, which comprise the major cell type mediating vascular tone and providing mechanical support to the vessel. Recently, Fischer *et al*<sup>[46]</sup> demonstrated hyaluronan to be induced in smooth muscle cells via EP2 activation. This molecule is a potent angiogenic factor in its own right with roles in EC proliferation, migration and tubulogenesis<sup>[47]</sup>. Currently, no studies have identified expression of EP receptors on vascular pericytes, despite the important paracrine role of these cells in EC survival<sup>[48]</sup>. Relatively little remains known regarding the action of PGE<sub>2</sub> on pericytes, despite higher pericyte investiture for vasculature in advanced CRCs being a negative prognostic indicator<sup>[49]</sup>.

#### EP Receptors in Tumour Microenvironment-Indirect Angiogenesis

The tumour microenvironment (TME) describes the myriad of cells, soluble factors and ECM components that surround malignant epithelial cells and play a crucial role in regulating the tumour phenotype. Certain cells, including CD8<sup>+</sup> T cells, may promote tumour cell death, while other components often mediate tumour progression and metastasis. The roles of TAMs, CAFs and myeloid-derived suppressor cells (MDSCs) with regards to PGE<sub>2</sub> signalling and angiogenesis will be examined in more detail below.

#### Tumor-associated macrophages (TAMs), PGE<sub>2</sub> and Angiogenesis

TAMs isolated from colorectal tumours are found to have two distinct phenotypes, denominated M1 and M2. M1 TAMs are characterized by high expression of iNOS, TNF- $\alpha$ , IL-12 and MHC-1/2, while M2 TAMs abundantly express IL-10, TGF- $\beta$  and arginase1<sup>[73]</sup>. Owing to their cytokine expression pattern, M1 TAMs are reported to promote an anti-tumour immune response via their ability to activate and polarize CD4<sup>+</sup> T cells to a Th1 phenotype which in turn facilitate CD8<sup>+</sup> cytotoxic T cell function, while M2 TAMs actively suppress an anti-tumour immune response and are, by inference, the most abundant TAM subtype in the TME. Nevertheless, there is considerable plasticity in macrophage phenotype, with M1 TAMs undergoing reprogramming to M2 cells (and vice versa) under the influence of local mediators<sup>[74]</sup>.

Several studies report the potential of PGE<sub>2</sub>, in conjunction with other cytokines, to polarize monocytes towards M2 TAMs<sup>[75,76]</sup>, and in downregulating TNF- $\alpha$  expression<sup>[77]</sup>. Together, these data suggest an indirect immunomodulatory mechanism for PGE<sub>2</sub>, although the EP receptors mediating these effects have not been characterised. There has also been considerable interest in the role of TAMs in angiogenesis. Recent work by Jetten and colleagues was found to promote EC proliferation and tubulogenesis *in vivo*, which were dependent on macrophage-derived FGF-2 and PIGF<sup>[78]</sup>. Contemporaneous work by Zajac *et al*<sup>[79]</sup> further demonstrated that M2-macrophage induced angiogenesis is also dependent on expression the zymogen pro-MMP9 which enables ECM

degradation. PGE<sub>2</sub> has also been demonstrated to increase MMP9 in colorectal tumour cell lines<sup>[80]</sup>, thereby indicating a synergistic effect of PGE<sub>2</sub> and TAMs in promoting EC migration. Furthermore, Wu *et al* demonstrated PGE<sub>2</sub> to be a potent inducer of VEGF in M2 TAMs an effect that is exacerbated under hypoxia<sup>[81]</sup>. It is clear from the above data that PGE<sub>2</sub> is a major player in the reciprocal signaling between tumour cells, TAMs and ECs. Understanding the EP signaling pathways regulating M2 macrophage activity may assist in developing drugs which target these pathways to deplete M2 cells while increasing levels of the M1 variety.

#### Cancer-associated fibroblasts (CAFs), PGE<sub>2</sub> and EP receptors

There is considerable interest in understanding the biology of CAFs as they are increasingly being recognized as a central element in the TME, with known roles in inflammation, tumour survival, metabolic reprogramming and angiogenesis<sup>[82-84]</sup>. Normal fibroblasts play a key role in ECM remodelling and wound healing and are not normally located in tumours. It is not clear if CAFs originate from normal fibroblasts or differentiate from mesenchymal stem cells or other stromal cells<sup>[85]</sup>.

CAFs derived from primary CRCs have been demonstrated to abundantly express COX-2 and this has been implicated in promoting tumour EMT via involvement of downstream NF $\kappa$ B and HIF1 activation<sup>[86]</sup>. PGE<sub>2</sub> expression in CAFs has also been noted, indicating a potential interaction with ECs<sup>[87]</sup>. In stromal fibroblasts derived from mice with gastric cancer high levels of VEGF secretion were induced under the influence of PGE<sub>2</sub>, although the involvement of EP receptors was not examined<sup>[88]</sup>. Few studies have investigated EP receptor expression on CAFs, although Odaka *et al*<sup>[89]</sup> identified EP2 and EP4 to be expressed on lung fibroblasts. Owing to the tissue-specific nature of EP expression, it is not clear whether these findings apply to CRC stromal fibroblasts. As CAFs comprise the predominant stromal cell type, further work is necessary to elucidate the interactions between PGE<sub>2</sub>, fibroblasts and tumour cells.

#### MDSCs, PGE<sub>2</sub> and EP receptors

When MDSCs were initially discovered in the 1970's they were named 'natural suppressor' or null cells owing to a lack of classical monocyte and lymphocyte phenotypic markers. They were later found to have potent immunosuppressive properties and contribute to tumour immune evasion. MDSCs can broadly be defined as the precursors to innate immune components (principally granulocytes, macrophages and dendritic cells) which undergo aberrant clonal expansion in the bone marrow. It is now known that MDSC-specific markers in mice include GR1<sup>+</sup> and CD11b<sup>+</sup> while in humans they are CD14<sup>-</sup> CD11b<sup>+</sup> and release immunomodulatory factors such as Arg1, IL-10 and iNOS<sup>[90]</sup>. The last decade has seen an explosion of research on MDSCs and investigation of their roles in a number of cancer hallmarks.

Yang *et al*<sup>[91]</sup> noted that MDSCs comprise approximately 5% of tumour volume in mice bearing MC-26 CRC cells. Subsequent work by this group reveals MDSCs to be associated with enhanced MVD and vascular stability. While early work showed prostaglandins to be MDSC inducers, Sinha and colleagues were the first group to characterize the expression of EP receptors on these cells<sup>[92]</sup>. They found expression of all four EPs on MDSCs in BALB/c mice, with EP2 critical for MDSC induction and immunosuppressive function. EP2 knockout was associated with a corresponding decrease in tumour size. Other work has since indicated both EP2 and EP4 signalling may be involved in MDSC induction<sup>[93]</sup>. In comparison, there is little evidence to indicate the importance of EP1 and EP3

expression on MDSCs.

MDSCs express a number of pro-angiogenic factors including VEGF, MMPs 2 and 9 and chemokines CXCL2 and CXCL5<sup>[90, 94]</sup>. MDSCs are also frequently found to overexpress COX-2 and mPGES-1<sup>[93, 95]</sup>, providing a number of drug targets. Interestingly, Xiang *et al.* noted that released tumour exosomes containing PGE<sub>2</sub> and other bioactive molecules were able to fuse with MDSCs to induce expression of VEGF from these cells<sup>[96]</sup>. While our understanding of exosome release from tumour and stromal cells is in its infancy, it is clear that this represents an important cross-talk mechanism with therapeutic potential. The role of the TME and PGE<sub>2</sub> in angiogenesis is summarized in figure 2 below:

## INHIBITION OF THE PGE<sub>2</sub>-EP RECEPTOR AXIS IN ANTIANGIOGENIC THERAPY

Given the important role of PGE<sub>2</sub> in angiogenesis, specific targeting of this prostanoid may yield results in inducing vessel regression, stabilizing existing vasculature and inhibiting tumour growth and metastasis. Such an approach would also attenuate inflammation, chronic levels of which are conducive to tumorigenesis.

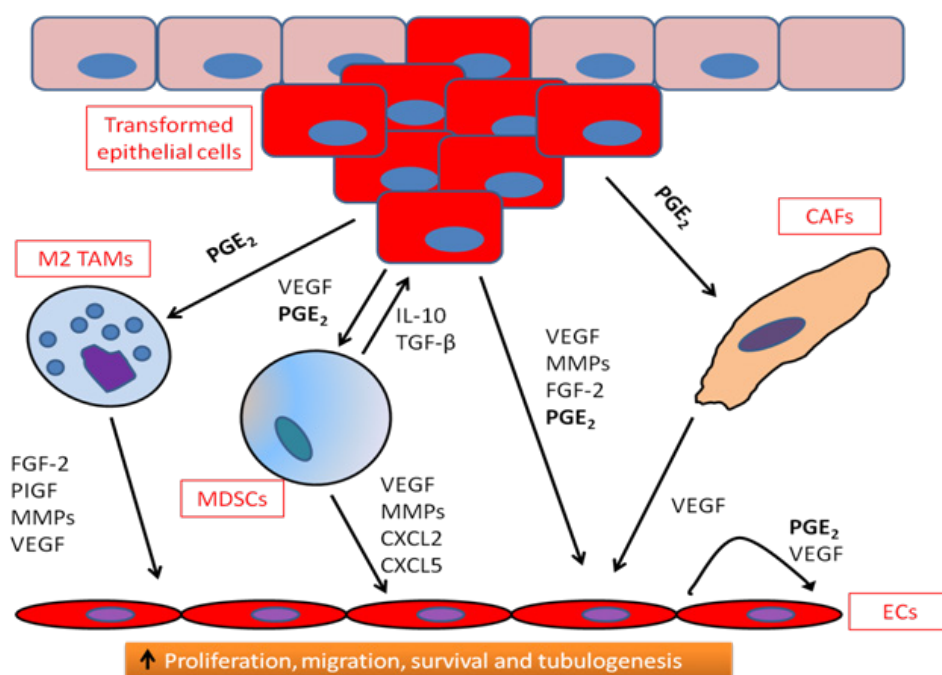
### mPGES-1 inhibition

As the enzyme directly upstream of PGE<sub>2</sub>, the potential of inhibiting mPGES-1 has been extensively studied. A number of studies have shown mPGES-1 to be overexpressed in resected colorectal tumours and to be negatively linked to prognosis independent of COX-2 levels. Nakanishi *et al.*<sup>[97]</sup> demonstrated significantly reduced intestinal cancer growth in APC knockout mice which were also null for mPGES1. Interestingly, mPGES1 deletion was also associated with impaired vascular morphogenesis. This study implies a role for PGE<sub>2</sub> in both in cancer cell growth and proper vascularisation of the primary tumour. Lack of PGE<sub>2</sub> may reduce tumour size by a loss of both processes. While the data for mPGES1 in tumour growth has been corroborated by other studies<sup>[98, 99]</sup>, experiments by Elander *et al.*<sup>[100]</sup> generated conflicting results showing mPGES1 deletion increases polyp size and frequency in APC knockout mice.

This observation may reflect the shunting of PGH<sub>2</sub> into parallel pathways that generate alternative eicosanoid species with similar pro-tumour effects<sup>[101]</sup>. Nevertheless, kamata and colleagues highlight the importance of mPGES-1 expressed in mouse bone marrow-derived cells. Gene knockout significantly impaired angiogenesis in a sponge implantation model, which was strongly correlated with decreased VEGF concentrations in the tumour stroma<sup>[102]</sup>. This study underlines the interdependence between PGE<sub>2</sub> and VEGF in tumour angiogenesis. Recent work by Finnetti *et al* also shows synergism between mPGES-1 and EGFR in angiogenesis, as pharmacological inhibition with AF3485 (a benzamide derivative) displayed inhibition of mPGES-1 with an approximate 50% decrease in MVD that was also dependent on decreased EGFR signaling<sup>[103]</sup>. The release of VEGF and FGF-2 in cell lines was also reduced, likely due to a decrease in PGE<sub>2</sub>. 15-deoxy-Delta(12,14)-prostaglandin J<sub>2</sub>, the FLAP inhibitor MK886 and the NSAID sulindac have all shown mPGES-1 inhibitory potential<sup>[104]</sup>. The data with regards to the anti-angiogenic potential of 15-deoxy-Delta(12,14)-prostaglandin J<sub>2</sub> are conflicting. While able to induce EC apoptosis via P53 upregulation<sup>[105]</sup>, it also apparently promotes angiogenesis through upregulating VEGF expression from tumour cells *in vivo* through PPAR $\gamma$  modulation<sup>[106]</sup>. It is clear that the effects of this compound are cell-type specific. The role of this compound in regulating angiogenesis in the complex tumour milieu requires further investigation in animal models.

The only study to assess the anti-tumour activity of MK886 in CRC cell lines indicates that MK886 in addition to COX-2 inhibitors are necessary to inhibit cell proliferation and angiogenesis via dual block on 5-LOX and COX-2 pathways<sup>[107]</sup>. Again, further investigation is needed.

Interestingly, DPE, a compound present in olive oil, has been found to decrease expression of mPGES-1 and HIF1- $\alpha$ . This impaired vessel maturation and morphology rather than MVD *in vivo*<sup>[108]</sup>. Despite a number of natural compounds displaying mPGES-1 inhibitory activity, including curcumin, garcinol and hyperforin, extended administration of these compounds has also shown extended inhibition of the 5-LOX pathway which may limit the biosynthesis of the leukotrienes, essential components of a coordinated inflammatory response<sup>[109, 110]</sup>.



**Figure 2** Transformed epithelial cells release soluble factors, including PGE<sub>2</sub>, which bind to a range of stromal cells and endothelial cells to generate a tumour microenvironment conducive to angiogenesis.

### Anti-angiogenic Potential of EP Receptor Modulation

The critical role of EP receptors controlling angiogenesis makes them ideal therapeutic targets. Many studies referenced above have utilized compounds that competitively bind these receptors to examine their functions. It is unlikely that highly selective EP antagonists by themselves will have therapeutic utility owing to the redundancy in molecular pathways emanating from these receptors. Nevertheless, selective EP blockade combined with chemotherapy or other anti-angiogenic therapies may prove useful. Dual EP receptor inhibitors (e.g. EP2 and EP4 inhibitors) may also show promise.

While the EP1 antagonist ONO-8713 used by Watanabe *et al* was shown to reduce CRC growth, there is no data to indicate its potential effects on angiogenesis<sup>[111]</sup>. The EP1 antagonist SC-51089 has demonstrated anti-neoplastic potential in glioma, but thus far no work has examined this compound in relation to angiogenesis<sup>[112]</sup>. The paucity of angiogenesis research using EP-1 antagonists probably reflects the limited understanding of this receptor in neovascularisation.

In contrast, a number of interesting EP2 antagonists have emerged for research purposes but there are currently no clinical trials with these compounds. While a number of publications have reported the use of AH6809 to examine the sole impact of EP2 blockade on angiogenesis, this compound is relatively non-specific for EP2 and is known to interact with DP receptors as well as EP1<sup>[113]</sup>. This lack of specificity has led to conflicts in the literature between studies that used EP2 knockout and those that used AH6809<sup>[114]</sup>. Recent work by Forselles *et al* at Pfizer led to the development of PF-04418948. In cell-based assays this compound showed over 2000-fold higher selectivity for EP2 than other EP subtypes, with robust and reproducible findings *in vivo*<sup>[115,116]</sup>. While PF-04418948 is clearly a promising research tool in the field of tumour biology, its *in vivo* pharmacokinetic and safety profiles must be established prior to entering clinical trials. Another antagonist TG4-155 developed by Jiang *et al*<sup>[117]</sup> shows similar EP-2 selectivity and may prove useful in this area.

Development of EP3 antagonists began with development and modification of biaryl-acylsulphonamides<sup>[118]</sup>. This led to development of L-826266 which has been used extensively in cardiovascular research<sup>[119,120]</sup>. However, the high lipophilicity of this compound renders it unsuitable for clinical use. The compound ONO-AE3-240 was used by Amano *et al* in characterizing the pro-angiogenic role of EP3 in tumour cells and host stromal cells with proven efficacy *in vivo*. In spite of this, EP3 antagonists may replicate some of the cardiovascular issues associated with COX inhibitors as other work has shown that firing through the EP3 receptor may be cardioprotective<sup>[121]</sup>.

Antagonism of EP4, like that of EP2, has been extensively explored and the role of EP4 in angiogenesis is perhaps the best understood of all the prostanoid receptors. Zhang *et al* (2011) used the EP4 antagonist azogrel (AH23848) to demonstrate that EP4 signaling is critical to angiogenesis, a finding that was reproduced in a recent study also using this EP4 antagonist<sup>[57]</sup>. Nevertheless, all commercially available formulations of this compound also display TP1 inhibitory activity and may lead to adverse effects *in vivo*. A novel EP4 antagonist developed by RaQualia Pharmaceuticals, RQ-15986, has shown efficacy in murine models of breast cancer<sup>[122]</sup>. While the pharmacologic parameters of this compound must be investigated further, a structurally similar compound RQ7 has shown high safety and selectivity in Phase IIa clinical trials for osteoarthritis pain<sup>[123]</sup>. Thus RQ-15986 merits further investigation *in vivo*.

### PGT and 15-PGDH modulators

Following its synthesis, PGE<sub>2</sub> is exported from the cell predominantly by MRP4, a C group member of the ATP-binding cassette (ABC) family<sup>[124]</sup>. Extracellular PGE<sub>2</sub> is catabolized by a two-step process involving carrier-mediated influx by membrane prostaglandin transporter (PGT) and finally degradation by cytoplasmic 15-hydroxyprostaglandin dehydrogenase (15-PGDH)<sup>[125]</sup>. There is evidence to indicate that while MRP4 is upregulated in CRC, both PGT and 15-PGDH are suppressed, leading to accumulation of PGE<sub>2</sub> in the tumour microenvironment<sup>[126]</sup>. Thus, pharmacological targeting of these three components may enhance degradation of PGE<sub>2</sub> and impede angiogenesis together with other tumour hallmarks.

Several NSAIDs, including indomethacin and ketoprofen, are shown to inhibit MRP4 as part of their mode of action<sup>[127]</sup>. Studies have also used the small molecule MK571<sup>[128,129]</sup>, although this is non-specific for MRP4 and also inhibits MRP1. More specific inhibitors for MRP4 may be required, although evidence suggests there is a small risk of cholestatic liver injury in patients given concomitant bile salt export pump (BSEP) inhibitors<sup>[130]</sup>.

15-PGDH has been found across several studies to function as a tumour suppressor. Loss of 15-PGDH expression is associated with increased colorectal tumorigenesis in two mouse models of CRC; namely, Min and C57BL/6J<sup>[131]</sup>. Indeed, this study also found 15-PGDH downregulation early in neoplasia in patients with hereditary predisposition to CRC, thus enabling unrestrained PGE<sub>2</sub> biosynthesis. Moreover, 15-PGDH expression levels have been proposed as a biomarker of CRC chemopreventive efficacy for both aspirin and the COX-2-targeted celecoxib, indicating these drugs function primarily by mitigating local PGE<sub>2</sub> accumulation in combination with 15-PGDH<sup>[132,133]</sup>. Evidence also suggests that inhibition of EGFR signaling is able to restore pre-neoplastic 15-PGDH levels<sup>[134]</sup>. As several studies implicate co-operation between EGFR and the EP4 receptor in CRC progression and angiogenesis, it is feasible that dual inhibition of EGFR and EP4 signalling may effectively reduce PGE<sub>2</sub> levels through enhanced 15-PGDH expression<sup>[135,136]</sup>.

The quest to restore normal PGT and 15-PGDH activity will undoubtedly be assisted by understanding the epigenetic mechanisms leading to silencing of these genes. While epigenetic silencing of PGT is postulated<sup>[126]</sup>, no evidence has yet been published to indicate that chromatin remodeling factors or microRNAs play a role in its regulation. By contrast, the genetic and epigenetic mechanisms regulating 15-PGDH have been extensively characterized. Snail, a member of the zinc finger transcription factors activated in EMT, is capable of binding conserved E-box elements in the promoter of 15-PGDH and repressing its expression<sup>[137]</sup>. Backlund *et al*<sup>[138]</sup> used various histone deacetylase inhibitors (HDACIs) in CRC cell lines to suggest HDAC2 plays a role in suppression of 15-PGDH. These findings were extended by Wang *et al*<sup>[139]</sup> who recently showed that reactivation of 15-PGDH in lung cancer using HDACs was paralleled by increased COX-2 expression. However, HDACIs together with COX-2 selective inhibitors or EP2/4 antagonists reversed PGE<sub>2</sub> accumulation and nullified angiogenesis *in vitro*. While recapitulation of these results is needed *in vivo* for CRC, it is clear that a multi-pronged approach targeting various components of the PGE<sub>2</sub> axis is desirable.

## CONCLUSIONS AND FUTURE DIRECTIONS

It is clear that PGE<sub>2</sub> and EP receptors play an important role in angiogenesis, with their effects being best characterized on ECs and tumour cells. However, given the complexity of signaling in the TME

it is necessary to elucidate the role and tissue expression patterns of prostanoid receptors in TAMs, MDSCs and CAFs. It is also unclear what role EP receptors play in the smooth muscle cells and pericytes which support tumour vasculature. Targeting EP receptors may reduce the pro-angiogenic potential of one cell type but may have adverse consequences on other cell types and whole organ systems. Therefore more studies *in vivo* in CRC models are necessary, perhaps examining combinations of EP antagonists and FP antagonists together with PGT and 15-PGDH enhancers. Synergism of these compounds with existing chemotherapies and molecular targeted therapies should also be investigated. The development of small molecules which are specific for particular prostanoid receptors will undoubtedly assist in furthering our understanding of prostanoid signaling, and following pharmacokinetic profiling and chemical modification may display potent therapeutic effects *in vivo*. Progress in this area may also be applied more broadly to other solid malignancies and autoimmune diseases.

## CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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