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REVIEW

Prostaglandin E2 and the EP receptors in malignancy: possible therapeutic targets?

G O'Callaghan[1,2] and A Houston[1,3]

[1]Department of Medicine, [2]HRB Clinical Research Facility, and [3]Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland

Correspondence
Dr Aileen Houston, Department of Medicine, UCC, Clinical Sciences Building, Cork University Hospital, Cork, Ireland.
E-mail: a.houston@ucc.ie

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Elevated expression of COX-2 and increased levels of PGE_2 are found in numerous cancers and are associated with tumour development and progression. Although epidemiological, clinical and preclinical studies have shown that the inhibition of PGE_2 synthesis through the use of either non-steroidal anti-inflammatory drugs (NSAIDs) or specific COX-2 inhibitors (COXibs) has the potential to prevent and treat malignant disease, toxicities due to inhibition of COX-2 have limited their use. Thus, there is an urgent need for the development of strategies whereby COX-2 activity may be reduced without inducing any side effects. The biological effects of PGE_2 are mediated by signalling through four distinct E-type prostanoid (EP) receptors – EP_1, EP_2, EP_3 and EP_4. In recent years, extensive effort has gone into elucidating the function of PGE_2 and the EP receptors in health and disease, with the goal of creating selective inhibitors as a means of therapy. In this review, we focus on PGE_2, and in particular on the role of the individual EP receptors and their signalling pathways in neoplastic disease. As knowledge concerning the role of the EP receptors in cancer grows, so does the potential for exploiting the EP receptors as therapeutic targets for the treatment of cancer and metastatic disease.

Abbreviations

COXibs, specific COX-2 inhibitors; EMT, epithelial–mesenchymal transition; NSAIDs, non-steroidal anti-inflammatory drugs; YB-1, Y-box binding protein 1

Tables of Links

TARGETS	
GPCRs[a]	**Enzymes**[d]
DP_1 receptor	15-PGDH (HPGD)
EP_1 receptor	Adenylate cyclase
EP_2 receptor	COX-1
EP_3 receptor	COX-2
EP_4 receptor	DNMT1
TP receptors	DNMT3
Nuclear hormone receptors[b]	FAK
NR4A2	GSK3β
Catalytic receptors[c]	HIF-1
EGFR	MMP9
Fas (TNFRSF6)	mPGES-1
VEGFR-1	mPGES-2
VEGFR-3	mTOR
	PKA
	PKC
	PLCγ
	Src

LIGANDS	
AH6809	IL-2
AH23848	ONO-AE3-208
Arachidonic acid	ONO-AE3-240
β-catenin	ONO-8711
cAMP	ONO-8713
Celecoxib	PF-04418948
CREB	PGE2
EGF	PGH2
Erlotinib	PGI2
Fas ligand (FasL)	TGF-α
ICAM-1	TGF-β
IFN-γ	VEGF

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d]Alexander *et al.*, 2013a,b,c,d).

Introduction

Inflammation has been established in recent years as playing a major role in cancer, with cancer-promoting inflammation an enabling characteristic underlying many, if not all, of the six hallmarks of cancer (Hanahan and Weinberg, 2011). In some cancers, the inflammatory conditions precede the development of malignancy, for example, ulcerative colitis is a major risk factor for colon cancer (Gupta *et al.*, 2007). Alternatively, oncogenic mutations can drive tumour-promoting inflammation in tumours that are epidemiologically unrelated to overt inflammatory conditions (Del Prete *et al.*, 2011). One key inflammatory mediator deregulated in many cancers is the COX enzyme, COX-2 (Janakiram and Rao, 2014). COX-2 expression has been shown in many cancers to be inversely associated with patient survival (Gallo *et al.*, 2002; Peng *et al.*, 2013; Sicking *et al.*, 2014), with epidemiological studies suggesting that regular aspirin use decreases colorectal cancer incidence and mortality through the inhibition of COX-2 (Chan *et al.*, 2009). Thus, drugs that target COX-2 may have chemopreventative or chemotherapeutic functions. Although drugs that target the COX enzymes have entered the clinic, albeit for different diseases, inhibition of COX-2 using either non-steroidal anti-inflammatory drugs (NSAIDs) or specific COX-2 inhibitors (COXibs) is associated with various side effects including gastric ulceration and myocardial infarction (Ranger, 2014). Such toxicities have limited their clinical applications.

Dysregulation of COX-2 leads to elevated levels of its principle metabolic product, PGE_2. PGE_2 is produced from arachidonic acid through the actions of COX enzymes and PGE synthases (Figure 1) and is catabolized in turn to the inactive 15-keto-PGE_2 by the enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH also known as HPGD) (Tai *et al.*, 2002). Elevated levels of PGE_2 have been found in numerous cancers, with PGE_2 shown to be responsible for many of the pro-tumorigenic effects seen following COX-2 dysregulation (Wu *et al.*, 2010). However, PGE_2 is not the only product of COX-2, with the toxicities associated with COX-2 inhibition proposed to be due to the concurrent inhibition of prostacyclin (PGI_2) (Cannon and Cannon, 2012), resulting in an imbalance in the levels of PGI_2 and thromboxanes in the body, increasing cardiovascular risk.

PGE_2 signals through four pharmacologically distinct, G-protein coupled plasma membrane receptors, EP_1, EP_2, EP_3 and EP_4, which can each activate different downstream signalling pathways (Sugimoto and Narumiya, 2007). Differential suppression of PGE_2 biological activity could thus potentially retain the anticancer benefits of COX-2 inhibition, whilst circumventing the adverse side effects. Numerous studies in recent years have focused on identifying the specific EP receptors and signalling pathways that mediate

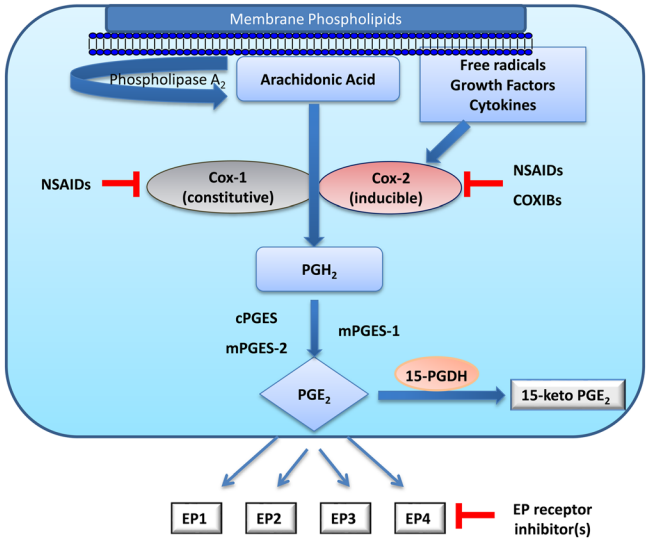

Figure 1

Prostaglandin E_2 biosynthesis. Following its release from cellular membranes through the actions of phospholipase A_2 family members, arachidonic acid is converted to PGH_2 through the activity of the COX enzymes. COX-1 is constitutively expressed at basal levels in many cells, generating low levels of PGs that are cytoprotective and maintain homeostasis. In contrast, COX-2 is normally absent from most cells but is induced in response to a variety of stimuli including growth factors and cytokines. PGH_2 is rapidly converted to PGE_2 by one of three PGE_2 synthases – cPGES, mPGES-1 or mPGES-2. PGE_2 is degraded, in turn, into 15-keto PGE_2 by 15-PGDH. PGE_2 signals through four GPCRs, EP_1, EP_2, EP_3 and EP_4. NSAIDs and COXibs, which block the activity of the COX enzymes, and inhibitors of the PGE_2 synthases can potentially suppress the pro-tumorigenic effects of PGE_2 by reducing its synthesis. Alternatively, targeting the individual EP receptors may suppress the activity of PGE_2.

the pleiotropic activities of PGE_2 in an attempt to identify the receptor(s) that represents the best target(s) for anticancer therapy. In this review, the emphasis is on outlining recent findings on the signalling pathways activated by the individual EP receptors and the potential role of the EP receptor antagonists in malignancy.

The EP receptors

Following synthesis, PGE_2 exits the cell where it acts in either an autocrine or paracrine manner via one of its four receptors (Sugimoto and Narumiya, 2007). Interactions between PGE_2 and its receptors are thought to be dependent on tissue/cell type and location, specific receptor expression and variation in binding affinities (Narumiya *et al.*, 1999). The EP_3 and EP_4 receptors represent high-affinity receptors, whereas activation of EP_1 and EP_2 receptors requires significantly higher levels of PGE_2. These variables can thus lead to differential receptor activation, providing PGE_2 with the ability to mediate highly varied effects on cell biology in many different tissue types and disease states.

The EP_1 receptor is coupled to the $G\alpha_q$ protein subunit that activates phosphoinositide-PLC (Figure 2A). Activation of PLC ultimately leads to an increase in intracellular Ca^{2+} and activation of PKC, inducing gene transcription through the activation of nuclear factor of activated T cells (NFAT), nuclear factor-kappaB (NFκB) and the MAPK pathways (Sugimoto and Narumiya, 2007).

Both the EP_2 and EP_4 receptors are linked to G-stimulatory ($G\alpha_s$) proteins and activate adenylate cyclase, increasing cAMP levels in the cell, which results in the activation of PKA (Figure 2B and C). PKA directly phosphorylates and activates transcription factors such as the cAMP-responsive element binding protein (CREB). Both receptors can also activate the GSK3β/β-catenin pathway, which in turn increases the transcription of many genes implicated in cancer, such as c-myc, cyclin D1 and VEGF. However, activation of the GSK3/β-catenin pathway by the EP_2 receptor occurs primarily through the activation of PKA, whereas the activation of the T cell factor (TCF)–β-catenin signalling by the EP_4 receptor primarily involves the activation of the PI3K/Akt pathway (Fujino *et al.*, 2002).

The EP_3 receptor is unique in that it exists as alternative spliced variants, characterized by differences in the cytoplasmic C-terminal tail (Namba *et al.*, 1993). As a result, the EP_3 receptor is capable of coupling with a number of G-protein subunits including G_i, G_s and G_{13}, and is thus capable of stimulating or inhibiting cAMP (by stimulating or inhibiting adenylate cyclase), as well as stimulating Ca^{2+} mobilization, possibly via PLC (Figure 2D). The major EP_3 splice variant though is thought to be coupled to an inhibitory (G_i) protein, and hence the major outcome of PGE_2-EP_3 receptor signalling is inhibition of adenylate cyclase and activation of the Ras/Raf and MAPK signalling pathway (Woodward *et al.*, 2011).

Crosstalk with other signalling pathways

Numerous studies have recently shown that significant crosstalk exists between the EP receptors, in particular EP_1 (Zhang *et al.*, 2014), EP_2 (Sales *et al.*, 2004) and EP_4 (Oshima *et al.*, 2011), and the EGF receptor (EGFR) signalling pathway, adding a further level of complexity to the EP receptor signalling pathways (Figure 3). The EGFR is located on the cell surface and is activated by binding of its specific ligands, including EGF and TGFα (Jorissen *et al.*, 2003). Transactivation of the EGFR by the EP receptors involves the activation of c-Src, which either activates EGFR directly by phosphorylation (Zhang *et al.*, 2014) or indirectly by inducing a matrix metalloproteinase activity that releases membrane-bound TGFα (Pai *et al.*, 2002). Activation of the EGFR leads to the activation of several signal transduction cascades, principally the MAPK, PI3K/Akt, STAT and PLC signalling pathways, resulting in cell proliferation, differentiation, migration and survival. The EGFR has also been shown to be involved in the pathogenesis and progression of numerous tumour types. Aberrant activation of the EGFR promotes uncontrolled cell proliferation and metastasis (Normanno *et al.*, 2006), with EGFR inhibitors approved for the treatment of non-small-cell, pancreatic, breast and colon cancer (Wykosky *et al.*, 2011).

Despite the dramatic response seen to these inhibitors, most patients ultimately become resistant to the therapy (Wykosky *et al.*, 2011). The ability of PGE_2, acting through the EP receptors to transactivate the EGFR, may be responsible for the

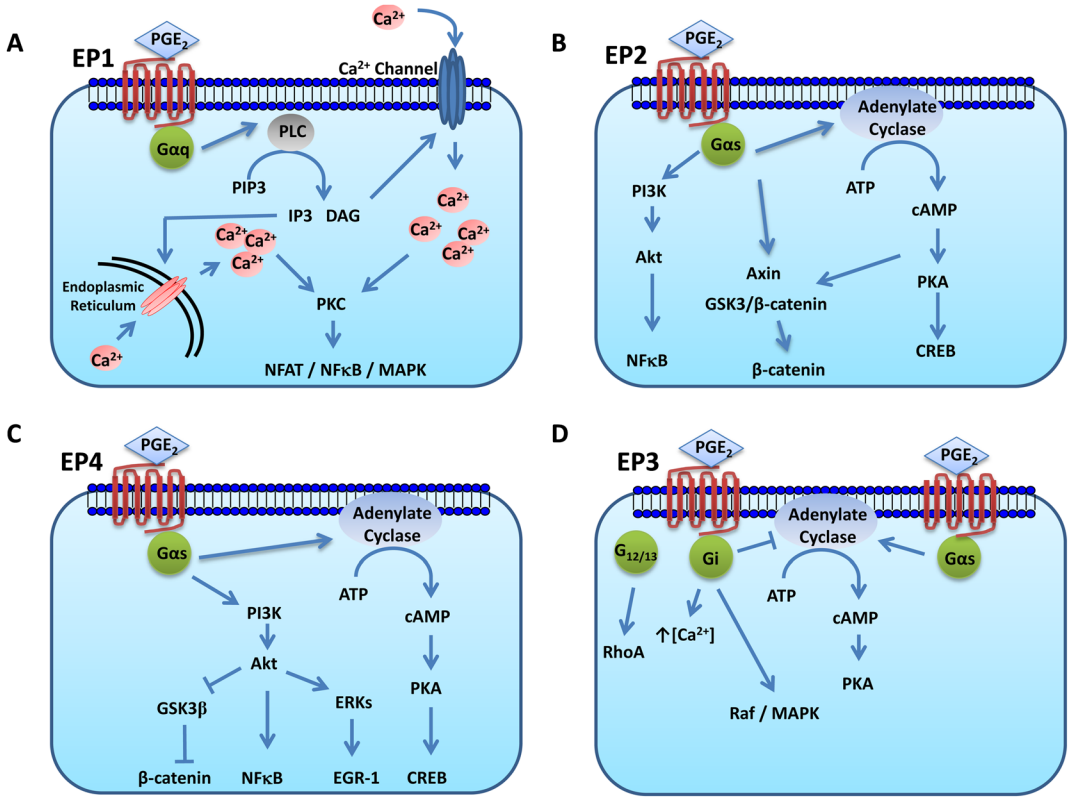

Figure 2

Canonical signalling pathways activated by the EP receptors. The EP receptors are high-affinity GPCRs characterized by the activation of different signalling pathways. (A) EP_1 receptors couple to Gαq protein and mediate signalling events by activation of PLC. This results in the elevation of cytoplasmic signalling intermediates including IP3 and DAG, an increase in intracellular Ca^{2+}, leading to the activation of PKC. (B) and (C) EP_2 and EP_4 receptors are linked to Gαs proteins and function by inducing the adenylate cyclase (AC) system, increasing the level of the secondary messenger cAMP and activating PKA. The receptors can also activate the PI3K signalling pathway by phosphorylation induced by GPCR kinases. This ultimately results in the triggering of NF-κB-mediated transcription programmes. (D) The EP_3 receptors are unique in their ability to couple to multiple G proteins. Activation of Gi proteins results in the inhibition of adenylate cyclase, whereas signalling through Gs results in cAMP production. EP_3 receptors can also be coupled to $G_{12/13}$ proteins, resulting in the activation of the small G protein Rho.

development of resistance in some cancer patients. However, clinical trials combining COX-2 specific inhibitors, including celecoxib and aproxicoxib, with EGFR inhibitors, such as erlotinib, have showed limited success, and in some patients, increased toxicity (Csiki *et al.*, 2005; Gitlitz *et al.*, 2014).

Studies such as these underscore the importance of improving our understanding of tumour biology to individualize cancer therapies and the identification of novel biomarkers to predict patient cohorts most likely to respond to therapy. Moreover, given that PGE_2 acts through multiple receptors, characterising the role of the individual receptors in carcinogenesis may identify a receptor, or combination of receptors, which offers a better target(s) for anticancer therapy.

The EP receptors and tumorigenesis

The availability of mouse strains with genetic ablation of each EP receptor subtype and the development of selective EP antagonists (Table 1) has greatly advanced our knowledge of the pathways activated by the EP receptors and their role in malignancy.

EP1 receptor

Of the four receptors, EP_1 has the least affinity for PGE_2 (Dey *et al.*, 2006) and so is probably activated predominantly when COX-2 is upregulated and PGE_2 synthesis is high, such as that which occurs during tumorigenesis. Some of the first studies implicating the EP_1 receptor in malignancy used EP_1 receptor knockout mice and the EP_1 receptor selective antagonists, ONO-8711 and ONO-8713 (Watanabe *et al.*, 1999; Watanabe *et al.*, 2000). In a chemically induced model of colon cancer, mice lacking the EP_1 receptor were found to develop approximately 60% fewer azoxymethane-induced colonic preneoplastic lesions than wild-type mice, as well as significantly reduced colon cancer incidence. A role for these EP_1 receptor antagonists in preventing tumour development was subsequently confirmed in other cancers, including breast (Kawamori *et al.*, 2001) and skin cancer (Tober *et al.*, 2006) (Table 1).

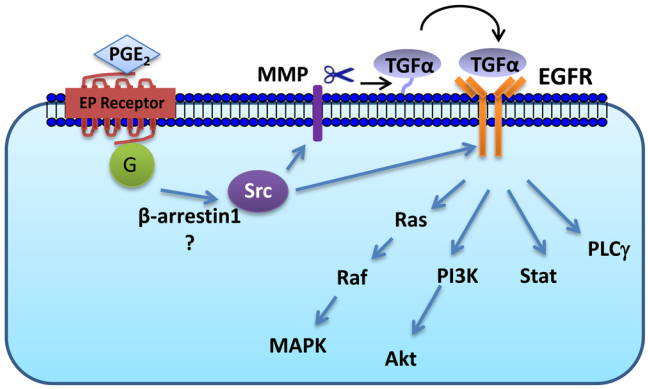

Figure 3

Transactivation of the EGFR by PGE_2. Signalling through the EP_1, EP_2 or EP_4 receptors by PGE_2 activates c-Src, which in turn activates the EGFR, either directly via phosphorylation or indirectly through the induction of MMP activity. Cleavage of the pro-form of TGFα releases the active TGFα, the ligand for EGFR. Activation of c-Src by the EP_2 and EP_4 receptors may involve the recruitment of β-arrestin1 to the receptor. This triggers the dephosphorylation of β-arrestin, thus allowing its association with c-Src, with β-arrestin1 subsequently activating c-Src.

The tumour-promoting role of PGE_2-induced EP_1 receptor signalling appears to predominantly involve activation of signalling pathways mediating cell migration and invasion (Yang *et al.*, 2010; Kim *et al.*, 2011b; Zhang *et al.*, 2014). The ability to migrate and invade is a key requirement for cells to metastasize, with metastatic spread responsible for most of the mortality caused by cancer. Signalling through the EP_1 receptor by PGE_2 has also been shown to enhance integrin expression (Zhang *et al.*, 2014) and induce the phosphorylation and activation of focal adhesion kinase (FAK) in cancer cells (Bai *et al.*, 2013). FAK is a non-receptor cytoplasmic tyrosine kinase that plays a key role in the regulation of cell proliferation and migration, with FAK phosphorylation shown to involve EP_1 receptor-mediated activation of the PKC/c-Src and EGFR signalling pathways (Bai *et al.*, 2013). Subsequent studies in hepatocellular carcinoma cells revealed that c-Src activation and transactivation of the EGFR by the EP_1 receptor also induced the expression of the transcription/translation regulatory protein Y-box binding protein 1 (YB-1) (Zhang *et al.*, 2014). YB-1 is overexpressed in a number of human malignancies, with expression shown to be associated with poor prognosis and disease recurrence (Kosnopfel *et al.*, 2014). YB-1 has been shown to regulate the expression of genes involved in epithelial–mesenchymal transition (EMT) (Kosnopfel *et al.*, 2014). EMT is a multi-step morphogenetic process during which epithelial cells down-regulate their epithelial properties and up-regulate mesenchymal characteristics, and is a key process in metastasis. Consistent with this, PGE_2-induced YB-1 up-regulated the expression of Snail, a key inducer of EMT, and greatly enhanced hepatocellular carcinoma cell invasion (Zhang *et al.*, 2014).

PGE_2-induced EP_1 receptor activation has also been shown to help tumours to adapt to hypoxia. Hypoxia induces COX-2 expression and increases PGE_2 levels via hypoxic-inducible factor (HIF)-1, with increased levels of PGE_2, in turn, potentiating HIF-1 transcriptional activity (Kaidi *et al.*, 2006). HIF-1 is a major transcription factor that activates the transcription of genes that participate in numerous cellular processes, such as the promotion of survival under conditions of low oxygen availability. Moreover, the EP_1 receptor itself was also shown to be induced in colorectal tumour cells under hypoxic conditions (Kim *et al.*, 2011b). This suggests that signalling through the EP_1 receptor aids in the adaptation of cancer cells to hypoxic conditions in the tumour microenvironment.

Signalling through the EP_1 receptor by PGE_2 has also been shown to induce Fas ligand (FasL) expression in cancer cells (O'Callaghan *et al.*, 2008, 2013), with FasL, in turn, inducing the production of PGE_2 upon binding to its receptor Fas (Zhang *et al.*, 2009). Cancer cells have been shown to depend on the constitutive activity of Fas, stimulated by cancer-produced FasL, for optimal growth (Chen *et al.*, 2010), with suppression of either FasL (O'Callaghan *et al.*, 2013) or Fas (Chen *et al.*, 2010) significantly delaying tumour formation and growth *in vivo*. Stimulation of tumour-expressed Fas by FasL has also been shown to be associated with increased infiltration of the tumours with regulatory T (Treg) cells (O'Callaghan *et al.*, 2013) and myeloid-derived suppressor cells (MDSCs) (Zhang *et al.*, 2009), immune cells with potent immunosuppressive activity. This infiltration of tumours by MDSCs was shown to be mediated by PGE_2 produced by the tumour cells in response to Fas ligation (Zhang *et al.*, 2009). Thus, signalling through the EP_1 receptor by PGE_2, through the induction of FasL on tumour cells, may play a role in the immune suppression that promotes tumour progression *in vivo*.

In contrast to the tumour-promoting activity of the EP_1 receptor in numerous tumours of varying origin, studies in breast cancer suggest that the EP_1 receptor may have an anti-metastatic function (Ma *et al.*, 2010), with nuclear expression of EP_1 receptors correlating with good prognostic markers (Thorat *et al.*, 2008). Although the reasons for these disparate findings are unclear, they may be due to the highly tissue-specific functional activities of the EP receptors. Alternatively, the nuclear EP_1 receptor may activate anti-inflammatory pathways, in contrast to the signalling pathways activated by cytoplasmic EP_1 receptor.

Finally, although most studies investigating the role of the EP_1 receptor in cancer examined the effect of EP_1 receptor antagonists on cancer initiation, targeting the EP_1 receptor using the EP_1 receptor specific antagonist ONO-8713 has also shown to be effective post cancer initiation (Watanabe *et al.*, 2000; O'Callaghan *et al.*, 2013). Moreover, the EP_1 receptor is expressed in tumour cells in several cancers, including skin squamous cell carcinoma (Lee *et al.*, 2005), colon (Gustafsson *et al.*, 2007) and hepatocellular (Bai *et al.*, 2014) cancer. Given the multiple functions ascribed to PGE_2-EP_1 receptor signalling in cancer, this suggests that the EP_1 receptor may be a valid therapeutic target in some cancers. However, all studies demonstrating *in vivo* efficacy of EP_1 receptor antagonists have been performed in preclinical animal models, and it is not known whether any therapeutic benefit will be seen in human cancer.

Table 1

Activity of EP receptor antagonists used in preclinical cancer studies

EP receptor antagonists	Activity
EP_1 receptor: ONO-8711	Reduced formation of ACF in azoxymethane-treated mice (Watanabe *et al.*, 1999)
	Delayed occurrence of PhIP[c]-induced breast tumours (Kawamori *et al.*, 2001)
	Reduced incidence, multiplicity and volume of colon carcinomas in azoxymethane-treated rats (Niho *et al.*, 2005)
	Reduced incidence of tongue squamous cell carcinomas in 4-NQO[d]-treated rats (Makita *et al.*, 2007)
ONO-8713	Reduced formation of ACF in azoxymethane-treated mice (Watanabe *et al.*, 2000)
	Reduced number of skin tumours induced by ultraviolet light in mice (Tober *et al.*, 2006)
	Reduced growth of established colon tumour cells in syngeneic mice (O'Callaghan *et al.*, 2013)
EP_2 receptor: AH6809[a]	Used in multiple studies, but not selective for the EP_2 receptor
PF-04418948	No studies to date in preclinical cancer models
EP_3 receptor: ONO-AE3-240	No effect on breast cancer cell metastasis in syngeneic mice (Ma *et al.*, 2006)
EP4 receptor: AH23848[b]	Reduced breast cancer cell metastasis in syngeneic mice (Ma *et al.*, 2006)
ONO-AE3-208	Reduced breast cancer cell metastasis in syngeneic mice (Ma *et al.*, 2006)
	Reduced lung cancer cell metastasis to the lung and colon cancer cell metastasis to the liver in syngeneic mice (Yang *et al.*, 2006)
	Inhibited the growth and metastasis of breast cancer cells in syngeneic mice (Xin *et al.*, 2012)
	Reduced metastasis of prostate cancer cells to the bone (Xu *et al.*, 2014)
ONO-AE-227	Reduced formation of ACF in azoxymethane-treated mice and polyp number in APCmin[e] mice (Mutoh *et al.*, 2002)
	Reduced polyp size in APC1309[f] mice (Kitamura *et al.*, 2003)

[a]Also an antagonist of the DP_1 receptor (PGD_2 receptor) and EP_1 receptor.
[b]Also a potent antagonist of the TP receptors (thromboxane receptors).
[c]Chemical-induced model of breast cancer.
[d]Chemical-induced model of squamous cell carcinoma of the tongue.
[e]Develop multiple intestinal polyps due to a heterozygous nonsense mutation in the APC gene.
[f]Develop multiple intestinal polyps due to the introduction of a specific mutation into the murine APC gene.

EP2 receptor

The majority of studies to date investigating the role of the EP_2 receptor in malignancy have relied on gene deletion studies and gene knockout mice because of the lack of a selective antagonist (Table 1). Although AH6809 is commonly used as an EP_2 receptor antagonist, in addition to blocking the EP_2 receptor, AH6809 also acts as an EP_1 and DP_1 receptor antagonist (Abramovitz *et al.*, 2000; Woodward *et al.*, 2011). However, a recently developed selective EP_2 receptor antagonist, PF-04418948, may aid in the elucidation of the role of the EP_2 receptor, complementing the gene knockout studies (af Forselles *et al.*, 2011; Birrell and Nials, 2011). Studies utilising EP_2 receptor knockout mice have demonstrated a role for the EP_2 receptor in malignancy, with EP_2 receptor deficient mice developing significantly less lung (Keith *et al.*, 2006), skin (Sung *et al.*, 2005) and breast (Chang *et al.*, 2005b) tumours following exposure to carcinogenic promoters. Genetic ablation of the EP_2 receptor also decreased both the size and number of intestinal polyps in adenomatous polyposis coli (APC) 1309 mice, which are genetically susceptible to intestinal polyp development (Sonoshita *et al.*, 2001). Moreover, the EP_2 receptor has been shown to be expressed by tumour cells in several cancers, including colon (Gustafsson *et al.*, 2007), prostate (Jain *et al.*, 2008) and breast (Chang *et al.*, 2004) cancer.

The role of the EP_2 receptor in cancer appears most commonly ascribed to its induction of angiogenesis, with deletion of the EP_2 receptor impairing the induction of the pro-angiogenic factor, VEGF, and tumour angiogenesis (Sales *et al.*, 2004; Chang *et al.*, 2005a; Kamiyama *et al.*, 2006). In addition to the induction of VEGF upon EP_2 receptor activation (Sales *et al.*, 2004; Chang *et al.*, 2005a), EP_2 receptor signalling in endothelial cells regulates endothelial cell motility and survival, further contributing to tumour angiogenesis *in vivo* (Kamiyama *et al.*, 2006).

PGE_2-induced EP_2 receptor signalling also plays an important role in suppressing the antitumour immune response (Kalinski, 2012). Indeed, most of the immunomodulatory effects of PGE_2 on immune cells occur as a result of signalling through the EP_2 and EP_4 receptors (Nataraj *et al.*, 2001; Kalinski, 2012). This is probably due to the fact that signalling through both these receptors is transduced by the same Gαs stimulatory protein, and upon activation leads to an increase in the intracellular concentration of cAMP. This

increase in cAMP was shown to be responsible for the inhibition of T helper (T_H)1 cells and the associated reduction in IL-2 and IFNγ (Betz and Fox, 1991; Harris *et al.*, 2002), which is important given that CD4 + T_H cells represent a key effector arm of the adaptive immune system required for cancer control. PGE_2 also inhibits, in an EP_2 and EP_4 receptor-mediated fashion, the activity of NK cells and cytotoxic T cells (CTL) (Martinet *et al.*, 2010; Holt *et al.*, 2012), two cell types that can also form part of the antitumour immune response. In addition to directly suppressing the activity of immune cells, signalling through the EP_2 and EP_4 receptors promotes the development of Treg cells (Sharma *et al.*, 2005). Treg cells are potent inhibitors of the immune system, suppressing the activity of numerous immune cells, including dendritic cells (DCs) (Lakshmi Narendra *et al.*, 2013). DCs play a central role in the initiation of the tumour-specific immune response, with the presence of DCs in tumours correlating with improved prognosis (Gulubova *et al.*, 2012). Signalling through the EP_2 (and EP_4) receptors not only blocked the activity of DCs through the induction of Treg cells but also blocked their generation from monocytes, resulting instead in the development of the immunosuppressive MDSCs from monocytes (Sinha *et al.*, 2007; Obermajer and Kalinski, 2012; De Keijzer *et al.*, 2013).

Despite these studies demonstrating an immunosuppressive function for the EP_2 (and EP_4) receptors, PGE_2 is also a potent pro-inflammatory factor (Yao *et al.*, 2009). In contrast to its inhibitory effect on the generation of DCs, PGE_2 promotes the maturation of immature DCs and enhances their T cell stimulatory capacity (De Keijzer *et al.*, 2013). Moreover, PGE_2 can either inhibit (Betz and Fox, 1991; Harris *et al.*, 2002) or promote (Yao *et al.*, 2009) T_H1 cell differentiation, with promotion requiring a strong T cell receptor (TCR) activation signal, together with a low concentration of PGE_2. Whether the pro-inflammatory or anti-inflammatory effects of PGE_2 prevail appears to depend to a large degree on the presence and type of activated cells, their maturation status, the concentration of PGE_2 and on the local balance of pro-inflammatory and anti-inflammatory factors present in the microenvironment (Sreeramkumar *et al.*, 2012). Thus, in the tumour microenvironment, it is likely that the anti-inflammatory and pro-tumorigenic function of PGE_2 prevails because of the low level of chronic inflammation present, coupled with the immunosuppressive microenvironment.

In addition to being associated with angiogenesis and immune suppression in malignancy, a recent study showed that EP_2 receptor activation by PGE_2 markedly enhanced hepatocellular carcinoma cell invasion and migration ability by up-regulating the expression level of Snail, a key inducer of EMT (Cheng *et al.*, 2014). The EP_2 receptor has also been linked to metastasis in breast cancer, in part through its ability to alter the response of cells to TGF-β (Tian and Schiemann, 2010). TGF-β plays an essential role in maintaining tissue homeostasis by inducing cell cycle arrest, differentiation and apoptosis. However, during tumorigenesis, genetic and epigenetic events convert TGF-β from a tumour suppressor to a promoter of cell growth, invasion and metastasis (Siegel and Massague, 2003). The altered response to TGF-β was because of the suppression of TGF-β-induced Smad2/3 nuclear localisation and signalling by PGE_2, thus uncoupling TGF-β from activating Smad3, with TGF-β instead stimulating breast cancer cell invasion and metastasis (Tian and Schiemann, 2010).

EP3 receptor

The role of the EP_3 receptor in tumorigenesis is unclear, with studies reporting conflicting effects on tumorigenesis following targeting of the EP_3 receptor. Genetic deletion of the EP_3 receptor had no effect on colon tumour formation in $APC^{\Delta 716}$ mice, which spontaneously develop numerous polyps in the intestinal tract (Sonoshita *et al.*, 2001). Similarly, treatment of breast cancer cells with the EP_3 antagonist ONO-AE3-240 had no effect on breast cancer metastasis (Ma *et al.*, 2006) (Table 1).In contrast, azoxymethane-induced colon cancer development was enhanced in EP_3 receptor knockout mice compared with wild-type mice, suggesting an anti-tumorigenic function for the receptor in this model (Shoji *et al.*, 2004). In the skin, EP_3 receptor deficiency either had no effect (Sung *et al.*, 2005; Rundhaug *et al.*, 2011) or was shown to contribute to squamous cell carcinoma (SCC) development, but not progression (Shoji *et al.*, 2005). Consistent with the EP_3 receptor not playing an important role in tumorigenesis, EP_3 receptor expression has been shown to be down-regulated in colonic tumour cells relative to normal mucosa epithelial cells (Shoji *et al.*, 2004). Similar findings of a down-regulation of the EP_3 receptor in cancer was seen in the skin with regards to SCC (Lee *et al.*, 2005) and in breast cancer (Chang *et al.*, 2004).

Some studies suggest an indirect pro-tumorigenic function for the EP_3 receptor, whereby signalling through host stromal EP_3 receptor plays a role in tumour development by promoting angiogenesis and lymphangiogenesis. The growth and metastasis of implanted tumours was shown to be suppressed in EP_3 receptor knockout mice, with suppression associated with a reduction in VEGF and matrix metalloproteinase-9 (MMP9) expression in the stroma, concomitant with a reduction in tumour-associated angiogenesis (Amano *et al.*, 2003; Amano *et al.*, 2009; Ogawa *et al.*, 2009). Consistent with this, overexpression of the EP_3 receptor in HEK cells increased expression of VEGF and its receptor VEGFR1 (Taniguchi *et al.*, 2008). EP_3 receptor signalling by host cells was also shown to play an important role in tumour-associated lymphangiogenesis (Kubo *et al.*, 2010). The expression of a potent pro-lymphangiogenic growth factor, VEGF-C, and its receptor, VEGFR3, in the stromal compartment of the tumour tissues was also found to be significantly reduced in EP_3 receptor knockout mice, as was expression of podoplanin, a marker for lymphatic endothelial cells (Kubo *et al.*, 2010).

Such discrepancies may be due to differences in the expression of the isoforms of the EP_3 receptor. As the EP_3 receptor is capable of stimulating or inhibiting cAMP (by stimulating or inhibiting adenylate cyclase), as well as stimulating Ca^{2+} mobilization, differences in isoform expression may account for the differing responses seen in these tumours. Alternatively, the function of the EP_3 receptor in tumorigenesis may be determined by its cellular location in the tumour microenvironment, with stromal, and not tumour cell, expression of the EP_3 receptor important in promoting tumorigenesis. The existence of these isoforms, as well as the differing outcomes seen following suppression

of EP_3 receptor signalling suggest that the EP_3 receptor is unlikely to be a promising target for anticancer therapy.

EP4 receptor

Of the four EP receptors, the EP_4 receptor is probably the one that is best characterized in terms of its involvement in cancer. PGE_2-induced EP_4 receptor activation has been implicated in a number of diverse cellular processes. As outlined earlier (see EP_2 receptor), signalling through the EP_4 receptor by PGE_2 promotes the development of a pro-tumorigenic immune response, inducing the development of Treg cells (Sharma *et al.*, 2005) and MDSCs (Sinha *et al.*, 2007; Obermajer and Kalinski, 2012; De Keijzer *et al.*, 2013), as well as suppressing NK and CTL activity (Martinet *et al.*, 2010; Holt *et al.*, 2012).

The EP_4 receptor can also play a role in tumour cell migration and metastasis (Buchanan *et al.*, 2006; Xia *et al.*, 2014). Several different signalling pathways have been shown to mediate this effect. For instance, PGE_2 was shown to significantly upregulate c-Myc expression in hepatocellular carcinoma cells through the activation of the CREB transcription factor (Figure 2C), thus promoting cell growth and invasion (Xia *et al.*, 2014). Alternatively, in colon cancer cells, activation of the EP_4 receptor increased cell proliferation and VEGF production, with mTORC1 acting as a signalling intermediary (Dufour *et al.*, 2014). EP_4 receptor activation was also shown to promote the migration and metastasis of colon cancer cells via the formation of an EP_4/β-arrestin/c-Src signalling complex that transactivated the EGFR, resulting in the downstream activation of the PI3K/Akt signalling pathway (Figure 3) (Buchanan *et al.*, 2006; Vo *et al.*, 2013). Activation of the PI3K/Akt pathway can also lead to upregulation of Snail expression (Lau and Leung, 2012), important for EMT. Consistent with this, suppression of the EP_4 receptor blocked PGE_2-induced Snail expression (Kim *et al.*, 2011a).

PGE_2, signalling through the EP_4 receptor, has recently been shown to also play a role in promoting aberrant DNA methylation in colon tumours (Xia *et al.*, 2012). Aberrant DNA methylation is considered to be one of the major mechanisms by which key genes involved in the tumorigenic process, such as tumour-suppressor genes and DNA repair genes, are silenced. Signalling by PGE_2 through the EP_4 receptor induced the expression of two DNA methytransferases, *DNMT1* and *DNMT3B*, in colon cancer cells (Xia *et al.*, 2012). Moreover, treatment of $APC^{min/+}$ mice with PGE_2 induced the expression of *DNMT1* and *DNMT3B* in colonic tumours and accelerated the growth of intestinal adenomas, whereas treatment with a de-methylating agent reversed the effect of PGE_2 on intestinal growth (Xia *et al.*, 2012). In cancer, gene silencing through methylation occurs at least as frequently as mutations or deletions. Thus, PGE_2, through its ability to contribute to the dysregulated hypermethylation seen in numerous cancers, may help to drive the tumorigenic process.

Metabolic changes are an emerging hallmark of cancer (Hanahan and Weinberg, 2011) required to meet the energetic and biosynthetic demands of growing tumours. Although cancer cells have traditionally been thought to rely on the glycolytic pathway to generate ATP, recent studies suggest that cancer cells can shift to the fatty acid oxidation pathway as an alternative energy source. PGE_2 was recently shown to induce the expression of NR4A2 in colon cancer cells via the EP_4 receptor, with NR4A2 in turn, increasing fatty acid oxidation by inducing the expression of multiple proteins in the fatty acid oxidation pathway (Holla *et al.*, 2006, 2011). Enhanced expression of NR4A2 is also associated with increased resistance to chemotherapy and enhanced tumour cell survival (Han *et al.*, 2013). Thus, PGE_2, acting through the EP_4 receptor, may promote tumorigenesis by acting as a regulator of the adaptive shift in tumours to energy utilization via fatty acid oxidation.

Consistent with the many roles identified for the EP_4 receptor in tumorigenesis, blocking the EP_4 receptor, using either EP_4 knockout mice and/or a selective EP_4 antagonist, was shown to suppress tumour development and progression in numerous tumour types. Several EP_4 receptor specific antagonists are available, including ONO-AE3-208, ONO-AE2-227 and AH23848 (Table 1), and they were shown to suppress tumour cell migration, invasion and metastasis in colon (Mutoh *et al.*, 2002; Chell *et al.*, 2006; Yang *et al.*, 2006), breast (Ma *et al.*, 2006; Xin *et al.*, 2012) and prostate (Xu *et al.*, 2014) cancer. EP_4 receptor knockout mice also showed a reduction in the formation of azoxymethane-induced colon aberrant crypt foci (ACF), with ONO-AE2-227 administered in the diet at the time of azoxymethane administration also capable of reducing the formation of ACF (Mutoh *et al.*, 2002). Consistent with a role for the EP_4 receptor in tumorigenesis, expression of the EP_4 receptor was up-regulated in numerous cancers, including colon (Chell *et al.*, 2006), breast (Kundu *et al.*, 2014) and prostate (Jain *et al.*, 2008) cancer.

Conclusions

Extensive preclinical and epidemiological studies support the targeting of the COX pathway for the prevention and treatment of malignancy. However, the use of COXibs over prolonged periods of time is not recommended because of the significant gastrointestinal and renal toxicities associated with them. As PGE_2 mediates most, if not all, of the carcinogenic effects of COX-2 overexpression, extensive efforts have focused on identifying the signalling pathways activated by the EP receptors, with the hope that targeting EP receptor signalling may circumvent the toxic effects associated with COX inhibition, whilst simultaneously retaining the anticancer properties. EP receptor antagonists, in particular those targeting the EP_1, EP_2 and EP_4 receptors, have been used successfully in preclinical models to suppress the development and growth of tumours. However, whether they will prove effective, and less toxic, in clinical studies is unknown. One limitation may be the effectiveness of these antagonists as compared with NSAIDs. Whilst COXibs inhibit all prostaglandins downstream of the COX, EP receptor antagonists target only one pathway. Thus, more than one antagonist may be required to suppress and/or treat malignant disease. For instance, the use of both EP_1 and EP_4 antagonists were shown to yield additive effects on colon tumour development and growth, compared with treatment with either antagonist alone, in a preclinical model (Kitamura *et al.*, 2003). Moreover, given the extensive crosstalk between the EP receptors and the EGF signalling pathways, combined targeting of individual EP receptors and the EGFR pathway

may yield improved chemotherapeutic benefits and improved clinical outcome in cancer. Whether combinations of specific antagonists represent a more efficient therapeutic option is currently unclear. In conclusion, whilst extensive studies have elucidated many of the signalling pathways activated by the EP receptors, future studies are required to determine whether the EP receptors represent possible therapeutic targets in malignancy.

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Conflict of interest

The authors declare no conflict of interest.

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