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

G O’Callaghan¹,² and A Houston¹,³

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

Elevated expression of COX-2 and increased levels of PGE₂ 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 PGE₂ 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 PGE₂ are mediated by signalling through four distinct E-type prostanoid (EP) receptors – EP₁, EP₂, EP₃ and EP₄.

In recent years, extensive effort has gone into elucidating the function of PGE₂ 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 PGE₂, 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
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, PGE2. PGE2 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-PGE2 by the enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH also known as HPGD) (Tai et al., 2002). Elevated levels of PGE2 have been found in numerous cancers, with PGE2 shown to be responsible for many of the pro-tumorigenic effects seen following COX-2 dysregulation (Wu et al., 2010). However, PGE2 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 (PGI2) (Cannon and Cannon, 2012), resulting in an imbalance in the levels of PGI2 and thromboxanes in the body, increasing cardiovascular risk.

PGE2 signals through four pharmacologically distinct, G-protein coupled plasma membrane receptors, EP1, EP2, EP3 and EP4, which can each activate different downstream signalling pathways (Sugimoto and Narumiya, 2007). Differential suppression of PGE2 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...
EP2 receptors requires significant PGE2 with the ability to mediate highly varied effects. Variables can thus lead to differential receptor activation, providing PLC ultimately leads to an increase in intracellular Ca2+ (Sugimoto and Narumiya, 2007). Interactions between PGE2 represent high-affinity receptors and the potential role of the EP receptor family in many different tissue types and disease states.

The EP receptors

Following synthesis, PGE2 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 PGE2 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 EP2 and EP4 receptors represent high-affinity receptors, whereas activation of EP1 and EP3 receptors requires significantly higher levels of PGE2. These variables can thus lead to differential receptor activation, providing PGE2 with the ability to mediate highly varied effects on cell biology in many different tissue types and disease states.

The EP1 receptor is coupled to the Goα protein subunit that activates phosphoinositide-PLC (Figure 2A). Activation of PLC ultimately leads to an increase in intracellular Ca2+ 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 EP3 and EP4 receptors are linked to G-stimulatory (Gsα) 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/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 GSK3β/catenin pathway by the EP2 receptor occurs primarily through the activation of PKA, whereas the activation of the T cell factor (TCF)-β-catenin signalling by the EP4 receptor primarily involves the activation of the PI3K/Akt pathway (Fujino et al., 2002).

The EP3 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 EP3 receptor is capable of coupling with a number of G-protein subunits including Gαi, Gαo and G13, and is thus capable of stimulating or inhibiting cAMP (by stimulating or inhibiting adenylate cyclase), as well as stimulating Ca2+ mobilization, possibly via PLC (Figure 2D). The major EP3 splice variant though is thought to be coupled to an inhibitory (Gi) protein, and hence the major outcome of PGE2-EP3 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 EP1 (Zhang et al., 2014), EP2 (Sales et al., 2004) and EP4 (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. Abrupt 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 PGE2, acting through the EP receptors to transactivate the EGFR, may be responsible for the
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 PGE2 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, EP1 has the least affinity for PGE2 (Dey et al., 2006) and so is probably activated predominantly when COX-2 is upregulated and PGE2 synthesis is high, such as that which occurs during tumorigenesis. Some of the first studies implicating the EP1 receptor in malignancy used EP1 receptor knockout mice and the EP1 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 EP1 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 EP1 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).
The tumour-promoting role of PGE2-induced EP1 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 EP1 receptor by PGE2 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 EP1 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 EP1 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, PGE2-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).

PGE2-induced EP1 receptor activation has also been shown to help tumours to adapt to hypoxia. Hypoxia induces COX-2 expression and increases PGE2 levels via hypoxic-inducible factor (HIF)-1, with increased levels of PGE2, 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 EP1 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 EP1 receptor aids in the adaptation of cancer cells to hypoxic conditions in the tumour microenvironment.

Signalling through the EP1 receptor by PGE2 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 PGE2 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 PGE2 produced by the tumour cells in response to Fas ligation (Zhang et al., 2009). Thus, signalling through the EP1 receptor by PGE2, 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 EP1 receptor in numerous tumours of varying origin, studies in breast cancer suggest that the EP1 receptor may have an anti-metastatic function (Ma et al., 2010), with nuclear expression of EP1 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 EP1 receptor may activate anti-inflammatory pathways, in contrast to the signalling pathways activated by cytoplasmic EP1 receptor.

Finally, although most studies investigating the role of the EP1 receptor in cancer examined the effect of EP1 receptor antagonists on cancer initiation, targeting the EP1 receptor using the EP1 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 EP1 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 PGE2-EP1 receptor signaling in cancer, this suggests that the EP1 receptor may be a valid therapeutic target in some cancers. However, all studies demonstrating in vivo efficacy of EP1 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

<table>
<thead>
<tr>
<th>EP receptor antagonists</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP2 receptor: ONO-8711</td>
<td>Reduced formation of ACF in azoxymethane-treated mice (Watanabe et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>Delayed occurrence of PhlPc-induced breast tumours (Kawamori et al., 2001)</td>
</tr>
<tr>
<td></td>
<td>Reduced incidence, multiplicity and volume of colon carcinomas in azoxymethane-treated rats (Niho et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Reduced incidence of tongue squamous cell carcinomas in 4-NQO-treated rats (Makita et al., 2007)</td>
</tr>
<tr>
<td>ONO-8713</td>
<td>Reduced formation of ACF in azoxymethane-treated mice (Watanabe et al., 2000)</td>
</tr>
<tr>
<td></td>
<td>Reduced number of skin tumours induced by ultraviolet light in mice (Tober et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Reduced growth of established colon tumour cells in syngeneic mice (O’Callaghan et al., 2013)</td>
</tr>
<tr>
<td>EP2 receptor: AH6809a</td>
<td>Used in multiple studies, but not selective for the EP2 receptor</td>
</tr>
<tr>
<td>PF-04418948</td>
<td>No studies to date in preclinical cancer models</td>
</tr>
<tr>
<td>EP3 receptor: ONO-AE3-240</td>
<td>No effect on breast cancer cell metastasis in syngeneic mice (Ma et al., 2006)</td>
</tr>
<tr>
<td>EP4 receptor: AH23848b</td>
<td>Reduced breast cancer cell metastasis in syngeneic mice (Ma et al., 2006)</td>
</tr>
<tr>
<td>ONO-AE3-208</td>
<td>Reduced lung cancer cell metastasis to the liver in syngeneic mice (Xin et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>Inhibited the growth and metastasis of breast cancer cells in syngeneic mice (Xin et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>Reduced metastasis of prostate cancer cells to the bone (Xu et al., 2014)</td>
</tr>
<tr>
<td>ONO-AE-227</td>
<td>Reduced formation of ACF in azoxymethane-treated mice and polypl number in APCminf mice (Mutoh et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>Reduced polypl size in APC1309f mice (Kitamura et al., 2003)</td>
</tr>
</tbody>
</table>

*Also an antagonist of the DP1 receptor (PGD2 receptor) and EP1 receptor.
*Also a potent antagonist of the TP receptors (thromboxane receptors).
*Chemical-induced model of breast cancer.
*Chemical-induced model of squamous cell carcinoma of the tongue.
*Develop multiple intestinal polyps due to a heterozygous nonsense mutation in the APC gene.
*Develop multiple intestinal polyps due to the introduction of a specific mutation into the murine APC gene.

**EP2 receptor**

The role of the EP2 receptor in malignancy appears most commonly ascribed to its induction of angiogenesis, with deletion of the EP2 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 EP2 receptor activation (Sales et al., 2004; Chang et al., 2005a), EP2 receptor signalling in endothelial cells regulates endothelial cell motility and survival, further contributing to tumour angiogenesis in vivo (Kamiyama et al., 2006).

PGE2-induced EP2 receptor signalling also plays an important role in suppressing the antitumour immune response (Kalinski, 2012). Indeed, most of the immunomodulatory effects of PGE2 on immune cells occur as a result of signalling through the EP2 and EP4 receptors (Nataraj et al., 2001; Kamiyama, 2012). This is probably due to the fact that signalling through both these receptors is transduced by the same Gs stimulatory protein, and upon activation leads to an increase in the intracellular concentration of cAMP. This 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.
increase in cAMP was shown to be responsible for the inhibition of T helper (Th1) cells and the associated reduction in IL-2 and IFNγ (Betz and Fox, 1991; Harris et al., 2002), which is important given that CD4+ T11 cells represent a key effector arm of the adaptive immune system required for cancer control. PGE2 also inhibits, in an EP2 and EP4 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 EP2 and EP4 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 EP2 (and EP4) 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 EP2 (and EP4) receptors, PGE2 is also a potent pro-inflammatory factor (Yao et al., 2009). In contrast to its inhibitory effect on the generation of DCs, PGE2 promotes the maturation of immature DCs and enhances their T-cell stimulatory capacity (De Keijzer et al., 2013). Moreover, PGE2 can either inhibit (Betz and Fox, 1991; Harris et al., 2002) or promote (Yao et al., 2009) T11 cell differentiation, with promotion requiring a strong T-cell receptor (TCR) activation signal, together with a low concentration of PGE2. Whether the pro-inflammatory or anti-inflammatory effects of PGE2 prevail appears to depend to a large degree on the presence and type of activated cells, their maturation status, the concentration of PGE2 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 PGE2 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 EP2 receptor activation by PGE2 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 EP2 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 Schieman, 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 localization and signalling by PGE2, 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 EP3 receptor in tumorigenesis is unclear, with studies reporting conflicting effects on tumorigenesis following targeting of the EP3 receptor. Genetic deletion of the EP3 receptor had no effect on colon tumour formation in APC<sup>min</sup> mice, which spontaneously develop numerous polyps in the intestinal tract (Sonoshita et al., 2001). Similarly, treatment of breast cancer cells with the EP3 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 EP3 receptor knockout mice compared with wild-type mice, suggesting an antitumorigenic function for the receptor in this model (Shoji et al., 2004). In the skin, EP3 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 EP3 receptor not playing an important role in tumorigenesis, EP3 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 EP3 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 EP3 receptor, whereby signalling through host stromal EP3 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 EP3 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 EP3 receptor in HEK cells increased expression of VEGF and its receptor VEGFR1 (Taniguchi et al., 2008). EP3 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 EP3 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 isofoms of the EP3 receptor. As the EP3 receptor is capable of stimulating or inhibiting cAMP (by stimulating or inhibiting adenylate cyclase), as well as stimulating Ca<sup>2+</sup> mobilization, differences in isoform expression may account for the differing responses seen in these tumours. Alternatively, the function of the EP3 receptor in tumorigenesis may be determined by its cellular location in the tumour microenvironment, with stromal, and not tumour cell, expression of the EP3 receptor important in promoting tumorigenesis. The existence of these isoforms, as well as the differing outcomes seen following suppression
of EP₃ receptor signalling suggest that the EP₃ receptor is unlikely to be a promising target for anticancer therapy.

**EP4 receptor**

Of the four EP receptors, the EP₄ receptor is probably the one that is best characterized in terms of its involvement in cancer. PGE₂-induced EP₄ receptor activation has been implicated in a number of diverse cellular processes. As outlined earlier (see EP₂ receptor), signalling through the EP₄ receptor by PGE₂ 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 EP₄ 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, PGE₂ 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 EP₄ receptor increased cell proliferation and VEGF production, with mTORC1 acting as a signalling intermediary (Dufour et al., 2014). EP₄ receptor activation was also shown to promote the migration and metastasis of colon cancer cells via the formation of an EP₄/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; Vö 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 EP₄ receptor blocked PGE₂-induced Snail expression (Kim et al., 2011a).

PGE₂ signalling through the EP₄ 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 PGE₂ through the EP₄ receptor induced the expression of two DNA methyltransferases, DNMT1 and DNMT3B, in colon cancer cells (Xia et al., 2012). Moreover, treatment of APCmin/+ mice with PGE₂ 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 PGE₂ on intestinal growth (Xia et al., 2012). In cancer, gene silencing through methylation occurs at least as frequently as mutations or deletions. Thus, PGE₂, 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. PGE₂ was recently shown to induce the expression of NR4A2 in colon cancer cells via the EP₄ 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, PGE₂ acting through the EP₄ 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 EP₄ receptor in tumorigenesis, blocking the EP₄ receptor, using either EP₄ knockout mice and/or a selective EP₄ antagonist, was shown to suppress tumour development and progression in numerous tumour types. Several EP₄ 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. EP₄ 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 EP₄ receptor in tumorigenesis, expression of the EP₄ 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 PGE₂ 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 EP₁, EP₂ and EP₄ 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 NSAIIDs. 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 EP₁ and EP₄ 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.

**Acknowledgements**

We would like to acknowledge Science Foundation Ireland for financial support (grant number 10/RFP/CAN2894 and UCC (TRAP award AS0884).

**Conflict of interest**

The authors declare no conflict of interest.

**References**


