Prospects in NSAID-derived chemoprevention of colorectal cancer

S. Chell, H.A. Patsos, D. Quailtrought, A.M. H-Zadeh, D.J. Hicks, A. Kaidi, I.R. Witherden, A.C. Williams and C. Paraskeva1

Cancer Research UK, Colorectal Tumour Biology Group, Department of Pathology and Microbiology, Bristol University, Bristol BS8 1TD, U.K.

Abstract

There is strong evidence for an important role for increased COX (cyclo-oxygenase)-2 expression and PG (prostaglandin) E2 production in colorectal tumorigenesis. PGE2 acts through four E-prostanoid receptors (EP1-4). COX-2 has therefore become a target for the potential chemoprevention and therapy of colorectal cancer. However, any therapeutic/preventive strategy has the potential to have an impact on physiological processes and hence result in side effects. General COX (COX-1 and -2) inhibition by traditional NSAIDs (non-steroidal anti-inflammatory drugs), such as aspirin, although chemopreventive, has some side effects, as do some conventional COX-2-selective NSAIDs. As PGE2 is thought to be the major PG species responsible for promoting colorectal tumorigenesis, research is being directed to a number of protein targets downstream of COX-2 that might allow the selective inhibition of the tumour-promoting activities of PGE2, while minimizing the associated adverse events. The PGE synthases and E-prostanoid receptors (EP1-4) have therefore recently attracted considerable interest as potential novel targets for the prevention/therapy of colorectal cancer. Selective (and possibly combinatorial) inhibition of the synthesis and signalling of those PGs most highly associated with colorectal tumorigenesis may have some advantages over COX-2-selective inhibitors.

The risk of CRC (colorectal cancer) and the need for preventive strategies

CRC is a major cause of cancer deaths in the U.K. Epidemiological studies suggest that dietary factors are very important and that between 50 and 80% of bowel cancers are preventable [1]. Changing dietary habits, including trends towards increasing obesity, suggest a potential for the further significant increase in the incidence of bowel cancer worldwide. As a consequence there is an urgent need to develop preventive strategies for CRC and to improve treatment either by adjuvants to current therapies or by identifying novel targets against which effective therapeutic approaches may be developed. As well as evidence that specific natural dietary factors may be preventative against bowel cancer, there is evidence that pharmacological agents, such as the NSAIDs (non-steroidal anti-inflammatory drugs) aspirin and sulindac, are chemopreventive against colorectal and other cancers [2,3]. CRC is an excellent example of the complex multistage process of carcinogenesis. Most CRCs develop from adenomas in what is often referred to as the adenoma–carcinoma sequence. This development and progression of small adenomas to large highly dysplastic adenomas, and ultimately carcinomas, can take decades and therefore offers a number of potential stages and targets for chemoprevention.

NSAIDs reduce CRC incidence by inhibiting COX (cyclo-oxygenase) activity

Epidemiological studies indicate that regular NSAID use can reduce the incidence of CRC in humans by 30–50%, as well as having chemotherapeutic properties both alone, and in combination with, conventional treatment strategies [4]. Furthermore, sulindac has been demonstrated to result in polyp regression in FAP (familial adenomatous polyposis) patients [5]. Traditional NSAIDs such as these are thought to exert most of their chemopreventive actions through the inhibition of COXs, key enzymes in PG (prostaglandin) biosynthesis [6–8]. There are at least two isoforms of COX. COX-1 is constitutively expressed in most tissues and has a role in normal tissue homeostasis, whereas COX-2 cannot be detected in the majority of normal tissues, but can be induced by a variety of cytokines and mitogens, and is elevated in 80–90% of CRCs, as well as a significant subset of adenomas (depending on their size) [9,10]. It is the inhibition of COX-1 by traditional NSAIDs that is thought to be responsible for side effects such as gastric bleeding, whereas the inhibition of COX-2 is thought to account for their chemopreventive activity. Therefore COX-2-selective NSAIDs were developed (these include drugs such as rofecoxib and celecoxib), with the expectation that their use would also be accompanied by a reduction in the incidence of upper gastrointestinal disease associated with the non-discriminate inhibition of COX-1 by traditional NSAIDs. This class of drug has been reported to retain many of the anti-neoplastic properties of traditional NSAIDs. For example, the COX-2-selective inhibitor celecoxib reduces the number, size and overall colorectal polyp burden in FAP patients [11]. This is similar to the observation that inactivating Cox-2 in ApcΔ716+/− mice [a murine model of FAP in which animals heterozygous for APC, adenomatous polyposis coli; COX, cyclo-oxygenase; CRC, colorectal cancer; EP, E-prostanoid; FAP, familial adenomatous polyposis; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; PGES, PGE2 synthase; Tx, thromboxane.

Key words: adenoma, carcinoma, chemoprevention, colon, cyclo-oxygenase 2 (COX-2), non-steroidal anti-inflammatory drug (NSAID).

Abbreviations used: APC, adenomatous polyposis coli; COX, cyclo-oxygenase; CRC, colorectal cancer; EP, E-prostanoid; FAP, familial adenomatous polyposis; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; PGES, PGE2 synthase; Tx, thromboxane.

1To whom correspondence should be addressed (email c.paraskeva@bristol.ac.uk).
loss of APC (adenomatous polyposis coli) function develop a high number of intestinal polyps, or treating this mouse with a COX-2-selective inhibitor, results in dramatic reductions in the size and number of intestinal polyps [12]. These studies provide evidence that COX-2 promotes tumour development in intestinal tissues and confirms it to be an attractive target for chemoprevention [13].

**COX enzymes represent the committed step in PG biosynthesis**

COX enzymes catalyse the conversion of arachidonic acid into the five primary prostanoids, PGE₂, PGF₂α, PGD₂, PGI₂ and TXA₂ (thromboxane A₂) (the major aspects of PG synthesis and signalling are outlined in Figure 1). Each prostanoid has important biological functions, for example, TXA₂ and PGI₂ regulate blood pressure through constriction and dilation of blood vessels respectively, as well as promoting and inhibiting blood clotting [14]. Similarly, PGE₂ can induce fever and promote blood vessel dilation, whereas PGF₂α can promote blood vessel constriction and PGD₂ has been found to oppose inflammatory responses [15]. PGD₂ is also one of the major prostaglandins in the brain, promoting sleep, whereas PGE₂ signalling has been associated with wakefulness [16]. Prostanoid signalling has therefore been implicated in physiological processes as diverse as immunity, reproduction and pregnancy, apoptosis, nerve growth and development, bone metabolism and adipogenesis [17].

It is therefore interesting to note that, despite the positive association of COX-2 with intestinal tumorigenesis, some more recent findings are beginning to put the suitability of highly selective COX-2 inhibition as a long-term chemopreventive strategy into question. For example, whereas COX-2 is expressed in most colorectal carcinomas and in many colorectal adenomas, the level of COX-2 expression in colorectal tumours can vary [20]. In addition, COX-1 is also being implicated in tumorigenesis [21–24]. Furthermore, roles for constitutive COX-2 expression are also being discovered in the biology of tissues such as that from human kidney [25] and brain [26], as well as rat periodontal tissue [27]. Perhaps most importantly, COX-2 inhibitors are not free from side effects, as was first hoped. In fact, despite reduced gastrointestinal toxicity, COX-2-selective NSAIDs are beginning to show the potential to cause adverse cardiovascular effects. This was first highlighted by the VIGOR (Vioxx in Gastrointestinal Outcomes Research) study [28], and has resulted in the recent withdrawal of rofecoxib (Vioxx) because it almost doubled the risk of events leading to heart attack or stroke [29]. Proposed mechanisms for these adverse events include the selective inhibition of PGI₂ over TXA₂, leading to thrombosis, as well as inhibition of PGE₂ and PGI₂ within the kidney, leading to sodium and water retention and consequently blood-pressure elevation [30].

**PGE₂ is the predominant PG driving colorectal tumorigenesis**

Increased levels of PG production have been observed in benign adenomatous polyps and in cancerous colon tissue when compared with histologically normal tissue [34]. However, it is predominantly PGE₂ that is thought to be responsible for promoting colorectal tumorigenesis, with elevated levels reported in benign and malignant human and rodent colorectal tumours in vivo, compared with histologically normal mucosa [9,31,32]. PGE₂ levels are also reported to increase in a size-dependent manner in the adenomas of FAP patients in vivo [33] and in the adenomas of ApcMin mice [34], whereas the PGE₂ content of venous blood drained from human colorectal tumours also increases in a size-dependent manner [35]. In vivo studies also reveal that human adenoma regression is more effective when tissue-PGE₂ levels are dramatically reduced through NSAID treatment [33], and more specifically, inhibition of PGE₂ (by monoclonal antibody) has been

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**Figure 1** | **PGs and their receptors**

PGs are formed by the oxidative cyclization of the five central carbons within 20-carbon polyunsaturated fatty acids such as arachidonic acid by three groups of enzymes [phospholipases A (PLAs), COX and the terminal PG synthases], each acting sequentially. Arachidonic acid is first liberated from the plasma membrane by PLA₂. COX enzymes then catalyse the formation of PGH₂ (an unstable bicyclo-endoperoxide intermediate and universal prostanoid precursor) from liberated arachidonic acid. PGH₂ is then converted by any one of several terminal prostanoid synthases to form the major prostanoids produced in vivo, PGE₂, PGE₂, PGF₂, PGF₂, PGI₂ (prostacyclin) and TXA₂ [18]. There are also currently eight known PG receptors and subtypes coded by separate genes [as well as a number of splice variants of EP3, FP (F-prostanoid) and TP (T-prostanoid)] that mediate the effects of prostanoids in normal cells and tumour cells. There are therefore clearly a number of intervention points for the modulation of specific PG levels and signalling. This depends upon the enzymatic machinery and receptors present in the tissue or cell type in question. DP, D-prostanoid; IP, l-prostanoid.
Figure 2 | Specific synthesis and signalling of PGE$_2$

Signalling via the EP1 receptor subtype activates IP$_3$ [Ins(1,4,5)P$_3$] and mobilizes intracellular Ca$^{2+}$, through an as-yet-uncharacterized G-protein. The EP2 and EP4 receptors activate adenylate cyclase activity through binding Gs proteins. This leads to the stimulation of cAMP production, and, in turn, the activation of the cAMP-dependent kinase, PKA (protein kinase A). The EP4 receptor is also known to have P3K (phosphoinositide 3-kinase)-dependent effects on Akt and ERK (extracellular-signal-regulated kinase)-1/2 activation. The predominant EP3 receptor splice variant (of which four have been identified in humans) induces the inhibition of adenylate cyclase, and hence inhibits cAMP activation [19,40]. Arrows indicate activation by phosphorylation, whereas blocked arrows indicate inhibition of activation by phosphorylation. AC, adenylate cyclase; PDE, phosphodiesterase; PLC, phospholipase C.

PGE synthases and EP (E-prostanoid) receptors

COX-2-selective NSAIDs are thought to inhibit all COX-2-mediated PG production. However, as it is predominantly PGE$_2$ that is thought to be responsible for promoting colorectal tumorigenesis, there remain intervention points further down the PG biosynthesis and signalling pathway that may convey the anti-neoplastic effects of NSAIDs while not exhibiting any of the associated side effects. For example, PGE$_2$ is formed by the isomerization of PGH$_2$ by three specific PGESs (PGE$_2$ synthases), after which it is able to signal through any one of four G-protein-coupled EP receptors, and ultimately, four different signal transduction pathways ([18], and Figure 2). PGE$_2$ is also cleared from the extracellular environment by a specific PG transporter [38] and metabolized to ligands with diminished biological activity by further catabolic enzymes [39].

Various studies have suggested roles for PGES and EP1-4 receptor species in cancer. For example, increased murine PGES-1 expression has been associated with a number of human epithelial cancers that also associate with increased COX-2 expression, including CRC [41]. Furthermore, co-transfection of epithelial kidney cells with Cox-2 and mPges-1 promotes colony formation in soft agar culture, and tumour formation when injected subcutaneously into athymic nude mice [42], whereas cells expressing both mPGES-1 and COX-2 also produce more PGE$_2$, grow faster, and exhibit abnormal morphology [43]. Studies using EP-knockout mice have also suggested a role for each receptor subtype in murine intestinal tumour formation ([44–47], and Table 1). Indeed, through specific receptor signalling, PGE$_2$ can exert many tumour-promoting effects, for example, increased proliferation, migration/invasion and angiogenesis, as well as inhibition of apoptosis [3]. For example, studies within our laboratory suggest that increased EP4-receptor expression in an anchorage-dependent colorectal adenoma cell line is sufficient to convey an anchorage-independent growth phenotype (S. Chell and C. Paraskeva, unpublished work). However, the net effect
of PGE2 on colorectal tumour cells is dependent on not only the concentration, duration and site of ligand production, but also the type and level of the receptor species present on the plasma/nuclear membranes of proximal cells, as well as the intracellular signalling cascades to which the PGE2 engaged receptor(s) may couple.

**Conclusions**

There is strong evidence for the important role of increased COX-2 expression and PGE2 production in colorectal tumorigenesis. COX-2 has therefore become a target for the potential chemoprevention and -therapy of CRC. However, any chemo-preventive/therapeutic strategy using inhibitors of protein function has the potential to impact on physiological processes and hence result in side effects. The key is to target those aspects of aberrant tumour growth that will least compromise the viability of the individual. General COX inhibition by traditional NSAIDs has side effects, as do conventional COX-2-selective NSAIDs, and the benefits of COX inhibition may outweigh the associated adverse events. The PGESs and EP receptors have therefore recently attracted considerable interest as potential novel targets for the prevention/therapy of CRC. Selective (and possibly combinatorial) inhibition of the synthesis and signalling of those PGs most highly associated with colorectal tumorigenesis may have advantages over COX-2-selective inhibitors by reducing the side effects with which COX-2 inhibitors are becoming increasingly associated.

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**References**


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**Table 1 | EP receptor signalling and its relevance to murine tumour progression [44–47]**

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<thead>
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<th>Receptor</th>
<th>G-protein</th>
<th>Signalling</th>
<th>Relationship to cancer development</th>
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<tr>
<td>EP1</td>
<td>Unknown</td>
<td>PtdIns response</td>
<td>EP1&lt;sup&gt;−/−&lt;/sup&gt; mice are resistant to AOM induced ACF</td>
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<tr>
<td>EP2</td>
<td>G&lt;sub&gt;s&lt;/sub&gt;</td>
<td>cAMP increase</td>
<td>EP2&lt;sup&gt;−/−&lt;/sup&gt;/Apc&lt;sup&gt;Δ716&lt;/sup&gt; mice have less polyps than Apc&lt;sup&gt;Δ716&lt;/sup&gt; alone</td>
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<tr>
<td>EP3</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>cAMP decrease</td>
<td>EP3&lt;sup&gt;−/−&lt;/sup&gt; mice have reduced tumour-associated angiogenesis and growth</td>
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<tr>
<td>EP4</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>cAMP increase</td>
<td>EP4&lt;sup&gt;−/−&lt;/sup&gt; mice are resistant to AOM-induced ACF</td>
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AOM, azoxymethane; ACF, aberrant crypt foci.

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