

## Cyclooxygenase-2 (COX-2): a molecular target in prostate cancer

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### Abstract

Epidemiological studies provided the first evidence that COX may be involved in the pathogenesis of cancer. In the process of carcinogenesis and in the route of intracellular signalling during carcinogenesis, COX-2 expression may be a universal phenomenon. In general, COX-2 is up-regulated throughout the tumorigenic process, from early hyperplasia to metastatic disease. COX-2 has been reported to be constitutively overexpressed in a variety of malignancies and is frequently constitutively elevated in prostate carcinoma. COX-2 was consistently overexpressed in premalignant lesions such as prostatic intraepithelial neoplasia, and carcinoma. Cases are described with evolution of proliferative inflammatory atrophy of the prostate and prostate carcinoma. The increase of evidence implicating COX-2 in cancer has stimulated clinical trials to investigate the efficacy of selective COX-2 inhibitors in individuals at risk for human cancer. Regarding prostate carcinoma there is much direct or indirect evidence to support the use of COX-2 inhibitors in this disease. Trials using these drugs in familial adenomatous polyposis (FAP) and other patients with a high risk of colorectal carcinoma are ongoing.

### Key words

Prostate cancer; Cyclooxygenase-2; Inhibitors; Pharmacological treatment; Molecular target

## **Introduction**

COX-1 and COX-2 are the two isoforms of cyclooxygenase, which convert arachidonic acid (AA) into several eicosanoids such as prostaglandin, thromboxanes and prostacyclin, which participate in several normal physiologic processes and inflammation. Whereas COX-1 is constitutively expressed in most tissues, COX-2 is an inducible enzyme, stimulated by cytokines, growth factors, oncogenes or tumour promoters during inflammation or malignancy. At present, the overexpression pattern of COX-2 in prostate carcinoma is unresolved. While some reports suggest no significant increase in COX-2 expression in prostate tumour tissue when compared to normal tissues, others have reported a direct association of prostate tumour grade and poorly differentiated prostate cancer with COX-2 levels.

Both autocrine and paracrine mechanisms may be responsible for the development and/or progression of androgen-independent prostate cancer either directly by stimulation of tumour cell proliferation or indirectly by enhanced prostaglandin-regulated local androgen synthesis.

One of the first clues that cyclooxygenase inhibition might be an effective approach to preventing cancer came from epidemiological studies. The fact that all non-steroidal anti-inflammatory drugs (NSAIDs) in clinical use are cyclooxygenase inhibitors provides a putative link between the inhibition of COX activity and the antitumour effect of NSAIDs.

The underlying hypothesis examined is whether COX-2 inhibition would enhance response to cytotoxic chemotherapy through inhibition of tumour angiogenesis, promotion of apoptosis or other alternative mechanisms.

## **COX-2 and prostate carcinoma**

The molecular pathogenesis of prostate cancer has been characterised by alterations of genes and proteins involved in proinflammatory pathways. Earlier studies have indicated that one of the prostate cancer precursor lesions, proliferative inflammatory atrophy (PIA), shares some molecular traits with prostatic intraepithelial neoplasia (PIN), and both of these two early precursor lesions of the prostate promote further cancer growth. At this juncture, it is worth recalling the findings from earlier studies showing a strong correlation between the levels of AA metabolites and accumulation of proinflammatory prostaglandins in prostate carcinogenesis.

COX-2 has been reported to be constitutively over-expressed in a variety of malignancies and is frequently constitutively elevated in prostate carcinoma. COX-2 appears to be induced in prostate adenocarcinoma cells in dogs [1].

Numerous investigators have evaluated the expression of COX-2 in human prostate cancer with conflicting results. Several studies have suggested that COX-2 is commonly overexpressed in prostate cancer [2–5]. In contrast, others have found that levels of COX-2 are extremely low or absent in the majority of prostate cancers [6, 7].

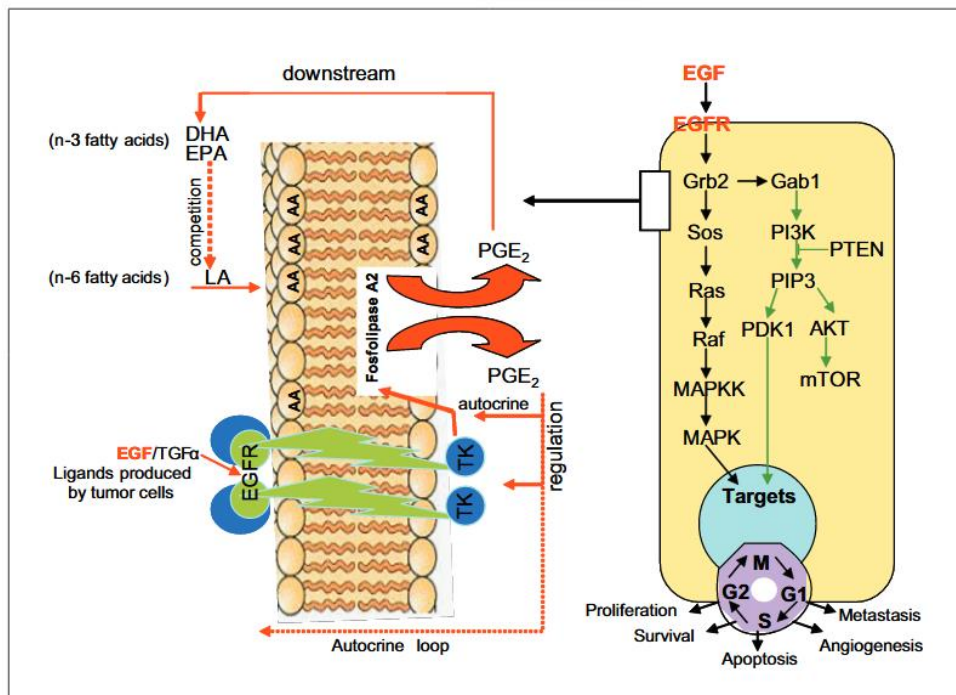
Several lines of evidence implicate 12-lipoxygenase (12-LOX) as a regulator of human prostate cancer [8], and are expressed in the prostate cancer cell lines DU-145 and PC3 [9]. Platelet-type 12-LOX has been shown to regulate growth, metastasis, and angiogenesis of prostate cancer: the 12-LOX pathway is a critical regulator of prostate cancer progression and apoptosis, by affecting various proteins regulating these processes.

Platelet-type 12-LOX expression was detected in human prostatic tumours and correlated to the clinical stage of disease. Human prostate cancer cell lines express the platelet-type isoform of 12-LOX at both the mRNA and protein levels, and immunohistochemistry revealed 12-LOX expression in human prostate tumours. The enzyme was localised to the plasma membrane, cytoplasmic organelles and nucleus in non-metastatic cells (PC-3 nm) and to the cytoskeleton and nucleus in metastatic cells (DU-145) [9].

A study involving the human prostate cancer cell lines PC-3 nm and LNCaP demonstrated up-regulated COX-2 mRNA expression and increased cell proliferation, total DNA content and endogenous prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production in response to the administration of exogenous dimethylprostaglandin E<sub>2</sub> [10]. PGE<sub>2</sub>, as a downstream metabolite of linoleic acid (LA), can increase cell growth as well as the enzyme responsible for its own synthesis. Growth of the androgen-unresponsive PC-3 nm human prostate cancer cells is stimulated *in vitro* by the addition of the omega-6 polyunsaturated LA. The growth effects of essential fatty acids appear to involve both PGs and leukotrienes, which interconnect with autocrine regulation through epidermal growth factor (EGF)-related polypeptides. Studies in many laboratories have indicated that the fatty acid composition of the diet, as well as the absolute amounts of fat consumed, may influence prostate cancer risk. Prostate cancer incidence rates have been increasing and arguments have been postulated that there might be a relationship between the rising cancer risk and an increase in the consumption of vegetable oils rich in LA.

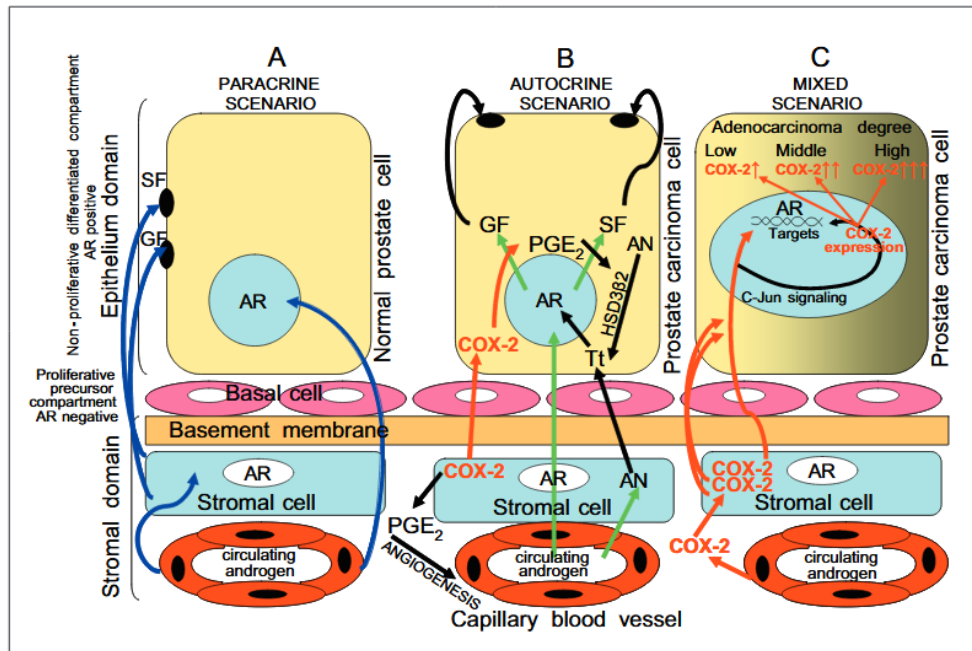
As a first approach to the question, a series of experiments *in vitro* are performed to examine the effects of both LA, an n-6 polyunsaturated fatty acid (PUFA), and docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), two n-3 fatty acids, on the growth of human prostate cancer cells [11]. The n-3 fatty acids compete with LA and its metabolic product AA for enzymes that regulate eicosanoid biosynthesis. When AA is formed from LA, it is largely incorporated into the cell membrane phospholipids where it provides a reserve of substrate for eicosanoid biosynthesis. Mobilisation of AA involves phospholipase A<sub>2</sub>, an enzyme that has been shown to be stimulated by EGF [12, 13] (Fig. 1). In addition, EGFR has been proposed to be localised with PI3K and ceramide molecules within the plasma membrane microdomains (caveolae/raft structures), which are enriched in sphingolipids and cholesterol [14, 15].

*In vitro*, like PC-3 nm cells, the DU145 human prostate cancer cell line does not require the presence of androgens for its growth; it grows readily in the absence of serum and synthesises both EGF and the related polypeptide transforming growth factor  $\alpha$  (TGF- $\alpha$ ). Both PC-3 nm and DU145 cells possess EGF/TGF- $\alpha$  receptors, and it has been suggested that an autocrine loop exists for the regulation of prostate cancer cell growth [11] (Fig. 1).



**Fig. 1.** Interaction of n-6 fatty acid (LA) and EGF in eicosanoid biosynthesis. DHA and EPA, both n-3 fatty acids, compete with LA and its metabolic product AA. Mobilisation of AA involves phospholipase A<sub>2</sub>, an enzyme that stimulates PGE<sub>2</sub> synthesis and which has been shown to be stimulated by EGF

The expression of COX-1 is found in prostate tissues as well as in prostate carcinoma samples [16]. The expression of COX-2 was modulated by the degree of adenocarcinoma differentiation (Fig. 2): in the low and middle groups of adenocarcinoma the expression was strong and there was a very strong expression level in the high group of adenocarcinomas. The expression of COX-2 also was high in the blood vessels and stromal tissues of prostate carcinoma samples. However, the expressions of COX-2 in the blood vessels and stromal tissues from benign prostatic hypertrophy, PIN and normal prostate samples were at basic levels [16]



**Fig. 2A.** In normal prostate gland, growth and maintenance of prostate epithelium depends on growth factors (GF) and survival factors (SF) produced by stromal cells. GF and SF are produced as a result of androgen signalling through AR. **B.** During transformation to cancer, the paracrine mechanism of AR action is replaced by an emergent autocrine mechanism, whereby cancer cells are less dependent on stromal cell factors. Hypothetical COX-2-derived PGE<sub>2</sub> potentiates androgen biosynthesis by the 3β-hydroxysteroid dehydrogenase type 2 (HSD3β2) enzyme and promotes angiogenesis, cell proliferation survival and metastasis. AN, androstenedione; T<sub>t</sub>, testosterone. **C.** Both autocrine and paracrine mechanisms may be responsible for the development and/or progression of androgen-independent prostate cancer by enhanced prostaglandin-regulated local androgen synthesis. COX-2 is a potent stimulator of AR function and the induction of c-Jun expression/phosphorylation plays an important role. C-jun exhibits an inhibitory effect on AR protein activity; c-Jun can inhibit the transcription activities of the AR promoter

Moreover, elevated 12-LOX mRNA levels were detected in cancer cells and the expression associated with poor differentiation and invasiveness of prostate cancer: approximately one-third of patients showed elevated levels of 12-LOX mRNA in prostate cancer tissues compared with the matching normal tissues [12]. The increase of 12-LOX expression in prostate cancer was found to correlate with advanced stage, poor differentiation and invasive potential. More importantly, elevation in the expression of this marker in prostate cancer patients may predict the disease aggressiveness in this subset of patients [17].

The immunohistochemical analysis of human prostate carcinoma reveals that COX-2 expression is multi-focal and moderate to strong in the majority of cases. Staining for COX-2 is mild in benign tissue as compared to prostate carcinoma tissue. Expression of COX-2 in prostate carcinoma tissue is predominantly granular and mostly localised in tumour cells. Interestingly, COX-2 expression in prostate carcinoma tissues is greater in the surrounding stromal cells or infiltrating inflammatory cells than in tumour cells. This observation raises interesting possibilities about the localisation of COX-2-expressing cells in prostate carcinoma. Therefore, stromally derived COX-2 may promote tumour growth by producing bioactive prostaglandins which affect carcinoma cells in a paracrine fashion. COX-2 may thus be acting as a “landscaping tumour promoter” according to the landscaping model [18].

Tumour cell invasion and metastasis are complex processes involving multiple host–tumour interactions (stromal-epithelium). [12(*S*)-Hydroxy eicosatetraenoic acid] [12(*S*)-HETE] can enhance the invasive and metastatic potentials of tumour cells by modulating several steps of this complex process. There is evidence to suggest that the ability of tumour cells to generate 12(*S*)-HETE is correlated with their metastatic potential [19]. A growing body of recent evidence suggests that 12(*S*)-HETE may act as a second messenger in stimulus–response coupling in some cells.

There are conflicting data regarding whether COX-2 is increased in the epithelial or in the stromal component of tumours. COX-2 has been found to be increased in the epithelium of adenomas (i.e., colonic epithelium). It has been found that COX-2 expression is increased in the stromal component of adenomas [20, 21].

### Mechanisms of cyclooxygenase inhibitors

The molecular mechanisms by which NSAIDs exhibit anticancer activities are not completely understood (Table 1) and it is plausible that COX-2-inhibitor-mediated apoptosis works through both COX-2-activity-dependent and -independent pathways.

**Table 1.** The molecular mechanisms of cyclooxygenase inhibitor (celecoxib) at cellular levels via COX-2 independent mechanisms against prostate cancer

The molecular mechanisms of celecoxib
<ul style="list-style-type: none"> <li>• Caused an increase in the number of cells at the G<sub>1</sub>–S-phase transition point with a trend toward accumulation of more cells in the G<sub>1</sub> phase and a corresponding decrease in the percentage of cells in the S phase [29, 30].</li> <li>• Induces cell cycle arrest at the G<sub>1</sub>–S-phase transition point and modifies cell cycle regulatory proteins such as cyclin D1, retinoblastoma (Rb), phosphorylated Rb, Cyclin E, p27<sup>KIP1</sup> and p21<sup>WAF1/CIP1</sup> [74].</li> <li>• Perturb intracellular calcium by inhibiting endoplasmic reticulum Ca<sup>2+</sup> -ATPases [75].</li> <li>• Blocks Akt signalling by inhibiting PDK-1 kinase activity [25–29].</li> <li>• Enhanced expression and phosphorylation of c-Jun in LNCaP cells. Overexpression of c-Jun inhibited the AR promoter [33].</li> <li>• Inhibited the expression of androgen up-regulated genes [33].</li> <li>• Inhibited AR-induced gene expression and AR promoter activity at the transcription level [36].</li> <li>• Overexpressed c-Jun by the NSAIDs not only inhibited the function of AR but also directly repressed AR expression at the transcription level [34–37].</li> <li>• Induced apoptosis in prostate cancer cells is caspase-3 and -9 mediated [63].</li> <li>• Induced apoptosis was associated with down-regulation of COX-2, nuclear factor κBp65, and with activation of peroxisome proliferator-activated receptor α, apoptosis activating factor-1 and caspase-3 [31, 32].</li> <li>• Inhibited cancer cell growth and mediated protein–protein interactions between heat shock protein 70 (HSP70) and p53 at low doses [76].</li> </ul>

The biochemical mechanism underlying COX-2-inhibitor-induced apoptosis, however, remains elusive. At least three distinct mechanisms have been proposed: first, NSAID treatment of cells resulted in an increase in AA that, in turn, stimulated the production of ceramide, a known death signal, by activating neutral sphingomyelinase [22]; second, the COX-2 inhibitors have been reported to sensitise prostate cancer cells to apoptosis by down-regulating the anti-apoptotic protein Bcl-2 [23, 24]; third, a study shows the involvement of a COX-2-independent pathway during the apoptosis signalling [25].

Celecoxib induces apoptosis, in part, by blocking the activation of the antiapoptotic kinase Akt (also called protein kinase B). The evidence from laboratories has demonstrated the involvement of molecular targets other than COX-2 in celecoxib-mediated in vitro antiproliferative effects in prostate [25–28]; the 3-phosphoinositi- tide-dependent protein kinase-1 (PDK-1)/Akt signalling pathway represents a major COX-2-independent mechanism by which celecoxib exerts in vivo antiproliferative effects against prostate tumours [29]. Among various putative pathways reported in the literature, blockade of Akt signalling is especially noteworthy [26–28].

It is well documented that PDK-1/Akt signalling plays a central role in regulating cancer cell survival and proliferation by affecting the phosphorylation status of a plethora of downstream effectors that are either PDK-1 or Akt substrates [30]. Consequently, blockade of this signalling cascade leads to growth inhibition by cell cycle arrest and/or apoptosis in malignant cells.

Moreover, celecoxib inhibits adenocarcinoma of the transgenic adenocarcinoma of mouse prostate (TRAMP) in a dose-dependent manner, and thus limits the growth of metastatic prostate cancer: immunohistochemical detection of proinflammatory protein, NF- $\kappa$ Bp65 and COX-2 indicate down-regulation by celecoxib. Suppression of the above proinflammatory proteins was associated with a higher rate of apoptosis and reduced tumour growth [31].

Earlier studies on immunohistochemical detection of NF- $\kappa$  in tissues from human prostatectomy demonstrated overexpression of active subunit of NF- $\kappa$ Bp65 [32].

Observations strongly indicate a correlation in the down-regulation of COX-2 with targets such as NF- $\kappa$ Bp65 that makes cells susceptible to the actions of apoptosis-inducing mechanisms that are believed to be simultaneously activated by celecoxib.

In the other way, some reports strongly suggest that NSAIDs are potent inhibitors of androgen receptor (AR) function and that the induction of c-Jun expression/phosphorylation [33] plays an important role in mediating their effects. Previous studies [34–37] demonstrated that c-Jun exhibits an inhibitory effect on AR protein activity. In addition, c-Jun can inhibit the transcription activities of the AR promoter [33]. c-Jun is a component of AP-1 protein complex and a member of the basic leucine zipper family of sequence-specific dimeric DNA-binding proteins. Stimulated overexpression of c-Jun protein can inhibit the function of the AR [34–37]. The transactivation functions of the AR as well as other steroid receptors can be affected by c-Jun. The interaction between the AR and c-Jun is mediated through the DNA- and ligand-binding domains of AR and the leucine zipper region of c-Jun that may affect transcriptional function of the AR [36]. Other studies indicate that the transcriptional interference between AR and c-Jun is partly mediated through competition for intracellular cAMP-responsive element binding protein [37], which serves as an integrator/coactivator for AR trans-activation activities.

### **Clinical prospects and future directions for COX-2 inhibitors**

The increasing evidence implicating COX-2 in cancer has stimulated clinical trials to investigate the efficacy of selective COX-2 inhibitors in individuals at risk for human cancer. Treatment with COX-2 inhibitors has been shown to reduce the size and number of polyps in familial adenomatous polyposis (FAP) patients, and is currently being evaluated for efficacy in preventing sporadic colorectal adenomas [38, 39]. There is enough reviewed evidence that aberrant COX-2 expression is also associated with breast cancer: selective COX-2 inhibitors have proved effective in preventing experimental breast cancer [40].

Some, but not all, studies suggest that COX-2 is overexpressed in primary human prostate cancers and in human prostate cancer cell lines [41–45]. Selective COX-2 inhibitors induce apoptosis of human prostate cancer cells *in vitro* and *in vivo*.

Attending to prostate carcinoma, there is much direct or indirect evidence to support the use of COX-2 inhibitors in this disease. Inhibition of cyclooxygenase by traditional NSAIDs and other selective inhibitors of the proinflammatory COX-2 isoform may also prove to have chemopreventive efficacy in prostate.

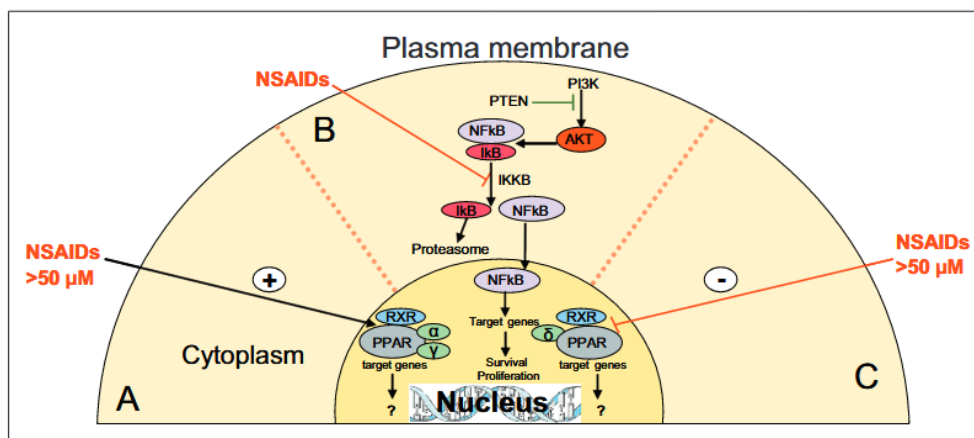
The results of case-control studies suggest that men who regularly took NSAIDs may have a reduced relative risk of prostate cancer, particularly for advanced cases of the disease [46]. Limited epidemiological studies suggest that NSAID use is associated with reduced risk of prostate cancer [46–48]. Elevated levels of PGE<sub>2</sub> and AA metabolism are seen in benign and malignant human prostate tumour cells, and NSAIDs inhibit prostate tumour cell growth [49–52].

Two NSAID derivatives that have lost COX inhibitory but retained pro-apoptotic activity are also promising chemopreventive agents in prostate: the sulphone metabolite of sulindac most probably stimulates apoptosis via inhibition of cyclic GMP phosphodiesterase. This drug was found to inhibit the rise of (prostate-specific antigen) PSA in patients with recurring prostate cancer after radical prostatectomy [53].

In addition, it has also been shown that the 12-LOX pathway of AA metabolism regulates cell growth, through inhibition of cell proliferation, protein regulating apoptosis, transition in the cell cycle and Rb protein family members. Inhibition of 12-LOX led to growth inhibition associated with a specific G1 arrest, followed by induction of apoptosis through caspase and Bcl-mediated mechanisms [54].

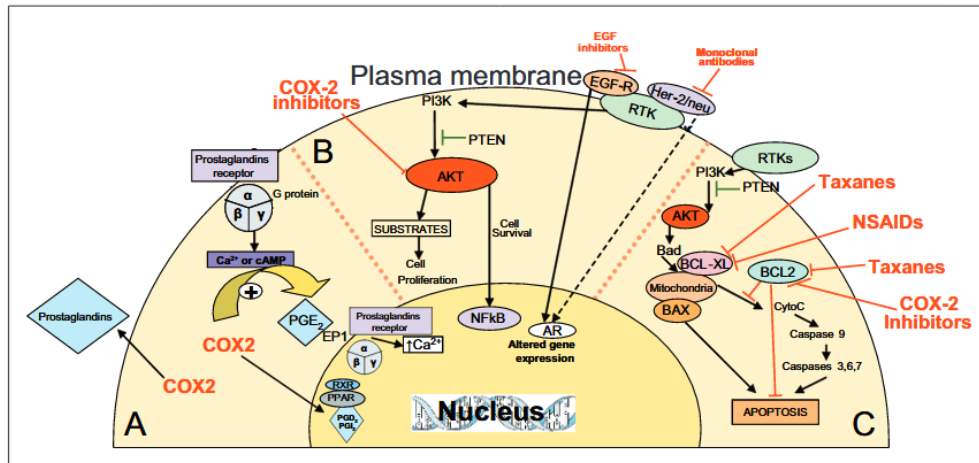
As COX-2 is overexpressed in HER-2/neu-positive breast cancer, selective COX-2 inhibitors should be evaluated as therapy in the prostate cancer population. Because COX-2-derived PGs may enhance aromatase activity, a therapeutic regimen combining a selective COX-2 inhibitor with an aromatase inhibitor should be considered. Actually, in some hormone-resistant prostate tumours, glucocorticoid was added to complement the treatment with good success. Hormonal therapy with glucocorticoid may constitute a basic or first treatment for some prostate carcinoma tumours in patients with an adenocarcinoma that has a Gleason score  $\geq 8$  by including glucocorticoid therapy as soon as the diagnosis is confirmed. Transcription of COX-2 is inhibited markedly by glucocorticoid therapy.

NSAIDs have been seen to inhibit NF- $\kappa$ B signalling (Fig. 3) by directly blocking the activity of I $\kappa$ B kinase  $\beta$  [55, 56]. Additionally, indomethacin, a non-selective COX inhibitor, can act as a direct ligand for peroxisome proliferator activating receptor (PPAR)  $\alpha$  and  $\gamma$ , and thus may directly regulate gene transcription [57]. The ability of PPARs to bind eicosanoids suggested that PPAR  $\delta$  might be a target of chemopreventive nonsteroidal anti-inflammatory drugs [58–60]. The findings suggest that PPAR  $\delta$  represents a  $\beta$ -catenin/Tcf-4 target with particular importance for chemoprevention. Whereas APC or  $\beta$ -catenin mutations can result in increased PPAR  $\delta$  activity, NSAIDs can compensate for this defect by suppressing PPAR  $\delta$  activity and promoting apoptosis. This suppression of PPAR  $\delta$  is mediated in part by the ability of some NSAIDs to directly inhibit the DNA-binding activity of PPAR  $\delta$  (Figs. 3 and 4).



**Fig. 3A.** There is evidence that PGs can modulate cellular pathways by acting directly within the nucleus. Certain NSAIDs can activate peroxisome proliferator activated receptor subtypes (PPAR $\alpha$  and PPAR $\gamma$ ). **B.** NSAIDs can inhibit the activity of I $\kappa$ B kinase  $\beta$  (IKK $\beta$ ); this leads to antagonism of the NF- $\kappa$ B signalling pathway by blocking the destruction of the NF- $\kappa$ B inhibitor I $\kappa$ B, preventing NF- $\kappa$ B from translocating to the nucleus and regulating key target genes. **C.** Other NSAIDs have been shown to inhibit the DNA-binding activity of peroxisome proliferator activated receptor subtype (sulindac)





**Fig. 4A.** The different classes of PGs exert their effects by binding to a G-protein-coupled cell-surface receptor leading to changes in the cellular levels of cAMP and  $\text{Ca}^{2+}$ , or by acting directly within the nucleus.  $\text{PGE}_2$  works by its receptor EP1 (localised to the nuclear envelope) and activation of the receptor leads to change in nuclear levels of  $\text{Ca}^{2+}$ , retinoid X receptor (RXR). Metabolites of  $\text{PGD}_2$  can transactivate members of the PPAR family of nuclear hormone receptors. **B.** Epidermal growth factor receptor (EGFR), aberrantly expressed in prostate carcinoma, can activate cell growth through the RTK/PI3K/AKT pathway. COX-2 inhibitors induce apoptosis by blocking AKT activation in prostate cancer cells independently of Bcl-2. Otherwise, overexpression of Her2/neu has been implicated in the activation of the AR and in inducing androgen-resistant prostate cancer cell growth. **C.** NSAIDs can decrease the levels of the anti-apoptotic gene bcl-xL, thereby increasing the cellular ratio of BAX/bcl-xL. There is also evidence that micro-tubule-interfering agents stimulate COX-2 transcription; otherwise, microtubule-interfering agents inhibit Bcl-2 activity by inducing Bcl-2 phosphorylation

There is also recent evidence that microtubule-interfering agents, including taxanes, stimulate COX-2 transcription [61]. This could modify the efficacy of this class of drugs. Thus, coadministration of a selective COX-2 inhibitor with cytotoxic drugs, such as taxanes, might enhance their anti-cancer activity. Induced expression of COX-2 is at least partly responsible for the increased resistance to chemotherapeutic drugs. Furthermore, systemic inhibition of COX-2 activity may abrogate this resistance, thus opening a new avenue for increasing the efficacy of antitumour drugs against hormone refractory prostate cancer (HRPC). Activation of prosurvival pathways mediated through up-regulation of inducible enzyme COX-2 due to inflammation or stress appears to be one of the limiting factors in reducing the efficacy of chemodrugs and increasing metastatic potentials of established tumours. A taxane-containing regimen will be selected for clinical study, because prior work [61] found that this chemotherapeutic agent induced COX-2 and prostaglandin biosynthesis. A selective COX-2 inhibitor would be expected to prevent the possible negative action of this chemotherapeutic agent. Modulation of the Bcl-2 pathway may also contribute to the antitumour effects noted with the nonsteroidal anti-inflammatory drugs and COX-2-specific inhibitors [62]. Several agents with preclinical activity in prostate cancer have now been demonstrated to modulate Bcl-2. The taxanes inhibit Bcl-2 activity by inducing Bcl-2 phosphorylation.

Cytotoxicity of single or combination treatment increased apoptosis, which was associated with caspase-3 and -9 activation, increased BAD protein, but decreased protein levels of X-linked inhibitor of apoptosis protein (XIAP) and Bcl-xL. Overall, a synergistic enhancement of antitumour activity in combination treatment was observed in vitro and an additive effect in vivo [63]. These results indicate that a combination of cytotoxic drug as taxanes with COX-2 inhibitor has a synergistic effect and therefore improves the cytotoxicity of either drug (Fig. 4).

Furthermore, increased resistance to cytotoxic anti-tumour drugs is also known to be dependent on associated stromal cells in many tumours. Data presented recently [63] demonstrate that stromal factor induced overexpression of COX-2 and thereby increased release of cytoprotective prostacyclin  $\text{PGI}_2$  in prostate carcinoma cells, and may be one of the factors responsible for limiting the efficacy of chemodrugs on prostate carcinoma cells. Stromal cells and factors derived from them have been known to influence therapeutic response in vivo and reduce cytotoxicity in vitro in a variety of tumours and cell types [64]. Some reports document that stromal cells alter expression of cell adhesion molecules, integrins, cytokines, chemokines and also cell cycle-related events. In fact it was demonstrated that



coculture of prostate carcinoma cells (LNCaP) with osteoblasts cells caused an increase in the number of prostate cells in G<sub>0</sub>-G<sub>1</sub> and a decrease in S and G<sub>2</sub>-M phases of cell cycle. This stromal-induced enhanced G<sub>0</sub>-G<sub>1</sub> checkpoint control affected the chemosensitivity of prostate cancer cell line to chemodrugs [65].

In the other regard, the EGF receptor is frequently aberrantly expressed in prostate carcinoma [64] and has been proposed as a target for treatment of prostate carcinoma [66, 67]. The EGF/EGFR transduction pathway has been recognised to play a crucial role in metastatic prostate cancer cells [68, 69]. EGFR and its ligands EGF/TGF  $\alpha$  appear to participate through autocrine and paracrine loops in prostatic tumour growth invasiveness and angiogenic processes by activating MAPK/PI3K and PLC $\gamma$  intracellular cascades and so increasing the cellular expression of PKA signalling [70, 71]. The activation of EGFR might lead to the negative modulation of apoptotic signals by activating PI3K/AKT survival cascade and inhibiting ceramide accumulation [72]. In vitro, simultaneous EGFR inhibitor and nitric oxide re- leasing compound might lead to a synergy in the ceramide and reactive oxygen species production, which might cause cellular membrane damage of metastatic prostate carcinoma cells [73]. Clinical benefits might derive from a regimen that combined a COX-2 inhibitor with an EGF receptor inhibitor (Fig. 4). COX-2 and EGFR are gaining significant attention as potential chemopreventive agents [77]. Targeting COX-2 and EGFR separately has shown promising antitumour activity. As both COX-2 and EGFR are involved in prostate carcinogenesis, COX-2 and EGFR inhibitors can be used as therapeutic strategies to treat prostate carcinoma; combinations of both inhibitors have been reported to show synergistic additive effects in preclinical studies [78].

The current trials advance prior preclinical work that implicates synergistic effects of combining COX inhibitors with cytotoxic chemotherapy and/or pharmacologic agents. In this way, at cellular levels via COX-2 independent mechanisms, celecoxib are capable of inducing cell cycle arrest at the G<sub>1</sub>-S-phase transition [74], perturbing intracellular calcium by inhibiting endoplasmic reticulum Ca<sup>2+</sup> -ATPases [75], inhibiting cancer cell growth and mediating protein-protein interactions between heat shock protein 70 (HSP70) and p53 at low doses [76]. Recently, there has been evidence of modest biologic activity for celecoxib in men with rising PSA levels after radical prostatectomy and/or radiation therapy for prostate cancer. A recent study suggests that celecoxib with weekly docetaxel and estramustine is an effective and safe treatment for patients with hormone-refractory prostate cancer, but it does not seem to add any benefit to docetaxel [79].

## Conclusions

One of the first clues that cyclooxygenase inhibition might be an effective approach to preventing cancer came from epidemiological studies. Preclinical and clinical evidence has established COX-2 as an attractive therapeutic or chemopreventive target in the prostate. Inducible COX-2 affects synthesis of prostaglandins from AA and is frequently activated during inflammation and carcinogenesis. COX-2 is also overexpressed in preneoplastic prostate lesions and inducible prostaglandin synthase is overexpressed in prostate carcinoma. It is also notable that epidemiologic data demonstrated a role for aspirin in suppressing prostate carcinogenesis.

Taken together, these findings clearly indicate that chronic elevation of COX-2 is pathological and suggest that inhibition of COX-2 via pharmacological means or regulation of its expression can limit the development or progression of human prostate cancer.

The investigations should be commended for designing and conducting trials which are excellent examples of translational research, by addressing whether NSAIDs can be administered in combination with chemotherapy. Undoubtedly the potential use of selective COX-2 inhibitors for the treatment of prostate cancer will also be investigated.

The ultimate goal of cancer treatment is to specifically prevent the growth of precancerous or cancerous cells without affecting normal cells. This is particularly important in chemoprevention and treatment of early disease, which typically involves long-term treatment of healthy subjects.

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