Chapter 5

ROLE OF PROSTAGLANDIN SYNTHESIS AND CYCLOOXYGENASE-2 IN PROSTATE CANCER AND METASTASIS

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Abstract: Metabolites of arachidonic acid, inclusive of prostatglandins (PGs), have been implicated in cancer for a number of years. In this overview, the evidence of the role of PGs and their interaction with endogenous hormones and exogenous (environmental) influences, predominately dietary factors, in the development and metastasis of prostate cancer, the mechanisms of action and approaches toward facilitating the design of effective strategies for the prevention and intervention of prostate cancer are considered. Included herein and apart from their traditionally thought of metabolic effects, PGs have been shown to function as biological response modifiers as evidenced from their effects on proliferation, apoptosis and immune responses.

Key words: arachidonic acid, cyclooxygenases (COX-1, COX-2), eicosanoids, prostaglandins

1. INTRODUCTION

The involvement of prostaglandins (PGs) and other eicosanoids in the development of human cancer has been known for more than two decades (1). Early knowledge focused on the role of PGs as mediators of thyroid carcinomas and on its participation in hypercalcemia (1). It was also demonstrated that the elevation in PG synthesis may influence tumor growth in humans and experimental animals (2). Numerous studies were further carried out to describe the effect of PG synthesis on carcinogen metabolism and tumor cell proliferation and metastatic potential (3–5). Additionally, the efficacy of inhibiting PG synthesis was examined as a means for preventing tumor development (4, 5). The role of PGs in the genesis of cancer was elucidated from observations demonstrating that: a) there is a direct relationship

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between the level of PGs synthesized and cancer incidence in both humans and animal models; b) the influence of various cancer-causing agents can be linked to their effect on PG synthesis; and c) the inhibition of PG synthesis hinders the development of tumor in animal models and in some human cancers (see below).

Prostate cancer is a leading cause of death in the world. In 2004, it was estimated that about 230,000 new cases (30% of all new cancers in men) were diagnosed and more than 30,000 patients died of prostate cancer in the USA (6). In UK, about 12,000 new cases of prostate cancer are diagnosed each year and the incidence has jumped 66% in the past 15 years (7). Among all European countries, only Switzerland has a higher fatality rate of 44.2 deaths per 100,000 compared with 34 in the UK (7). Over the past few decades, therefore, extensive research was directed towards understanding the mechanism(s) of prostate cancer development and providing practical measures for cure, prevention, and early detection. This effort enabled researchers to identify several candidate molecules, genes, and proteins that are linked to prostate cancer. Among these, PGs have emerged as possible promoters or growth enhancers, furnishing a promising tool that could be implemented or, at least, integrated into an effective strategy for prevention.

The present chapter reviews the evidence suggesting a possible role of PGs in the genesis of prostate cancer and discusses the mechanisms by which these lipid molecules may contribute to tumor development. Understanding this relationship may ultimately indicate new avenues of approach that might facilitate designing effective strategies for prostate cancer prevention and intervention.

2. PG SYNTHESIS

Arachidonic acid (AA) metabolites such as PGs, prostacyclins, thromboxanes and various lipoxygenase products, collectively known as eicosanoids, are produced in many tissues and modulate diverse physiological and pathophysiological responses. These bioactive lipids are potent mediators of a number of signal transduction pathways that modulate cellular adhesion, growth and differentiation (8). Cyclooxygenase (COX), also known as prostaglandin *H*-synthase, is the rate limiting enzyme in the metabolic conversion of AA to PGs and related eicosanoids (Figure 1). AA released from membrane phospholipids by phospholipase A_2 is converted to PGH_2 through the action of COX. COX enzymes contain two moieties: a) the cyclooxygenase moiety, which introduces two molecules of oxygen into AA to form the hydroperoxy endoperoxide PGG_2 and b) the endoper-

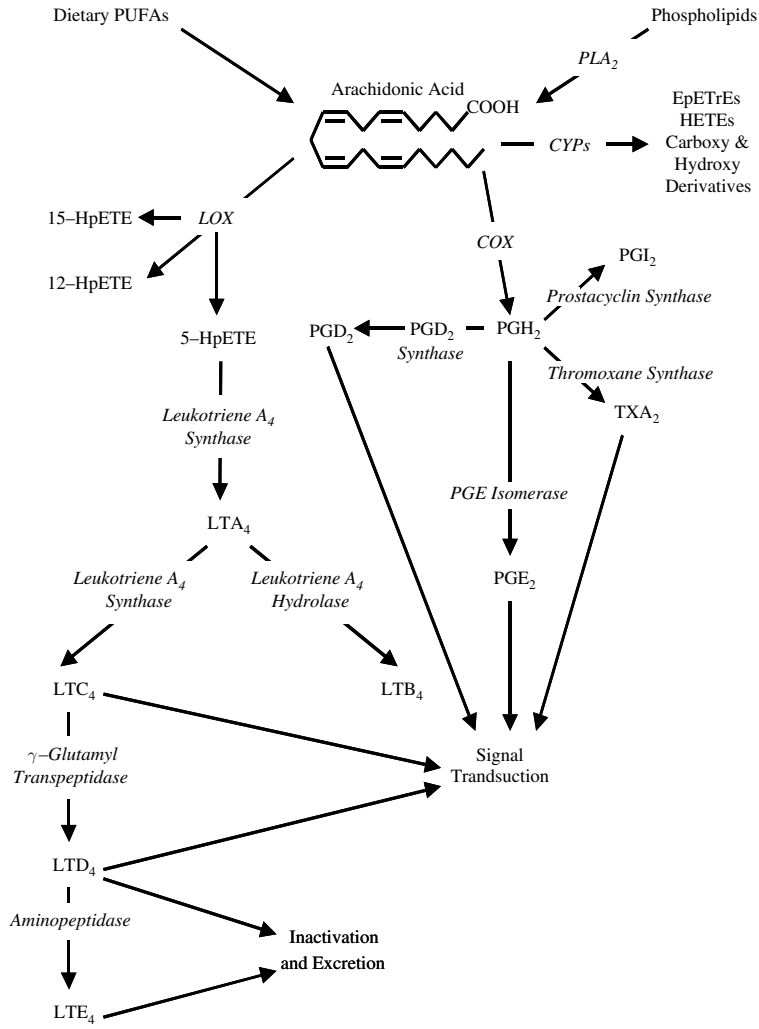

Figure 1. The metabolic conversion of arachidonic acid to prostaglandins and related eicosanoids. COX, cyclooxygenases (1 and 2); CYPs, cytochrome P450 enzymes; EpETrEs, epoxyeicosa-tetraenoic acids; HETEs, hydroxyeicosatetraenoic acids; HpETEs, hydroperoxyeicosa-tetraenoic acids; LOX, lipoxygenases; LT, leukotrienes; PG, prostaglnadins; PLA2, phospholipase A2; PUFAs, polyunsat-urated fatty acids; and TX, thromboxanes.

oxidase moiety, which reduces PGG_2 to the hydroxy endoperoxide PGH_2. Subsequently, PGH_2 is converted by cell-specific synthases to products such as PGE_2, $PGF_{2\alpha}$, PGI_2 or thromboxanes (9).

Table 1. Characteristics of cyclooxygenase-1 and cyclooxygenase-2

Characteristic	**COX-1**	**COX-2**
Expression	Constitutive	Inducible
Range of induction	2- to 4-fold	10- to 80-fold
Site of PG synthesis	ER[a]	ER & NE[a]
C terminus (18 aa cassette)[b]	(−)	(+)
Aspirin acetylation site	Ser^{530}	Ser^{516}
Chromosome (Human)	9	1
Protein size	72 kDa	72 & 74 kDa
Gene size	(single band)	(doublet)
mRNA size	22 kb (11 exons)	8.3 kb (10 exons)
	2.7 kb	4.5 kb

[a]ER: Endoplasmic Reticulum, NE: nuclear envelope
[b]Absent (−) or present (+). aa denotes amino acid.

Two COX isoforms, COX-1 and COX-2, have been identified (Table 1). COX-1 is constitutively expressed in many tissues (9, 10), although its expression can vary with the state of differentiation or following stimulation with cytokines or tumor promoters (10–13). PGs produced by COX-1 are thought to mediate "housekeeping" functions such as cytoprotection of the gastric mucosa, regulation of renal blood flow and platelet aggregation (14–16).

The recently discovered COX-2 message and protein (17), in contrast to COX-1, are normally undetectable in most tissues (for review see Ref. (18)). COX-2, however, is an inducible enzyme and it is expressed in response to proinflammatory agents, including cytokines, endotoxins, growth factors, tumor promoters and mitogens (19–22). COX-2 is expressed in a few specialized tissues such as brain, testes and macula densa of the kidney in the apparent absence of any activation. Because of its rapid induction by mitogens, the gene encoding COX-2 (*COX-2*) has been termed an immediate-early, or primary, response gene like c-*myc,* c-*fos* and c-*jun* ((20), 23–25).

The discovery of *COX-2* has stimulated a great deal of research in the field, much of which was to rationalize the redundancy between *COX-1* and *-2* and to understand the role of the *COX-2* gene in cancer development. COX-1 and COX-2 are encoded by separate genes, but the enzymes are structurally similar (Figure 2). The amino acid sequence of COX-2 is 61% identical to the COX-1 protein in humans (17). As indicated in Figure 2, the N-terminal signal peptide region is shorter in COX-2 than in COX-1. However, the two N-linked glycosylation sites at residues 53 and 130, the two heme ligands His-295 and His-374, the putative transmembrane

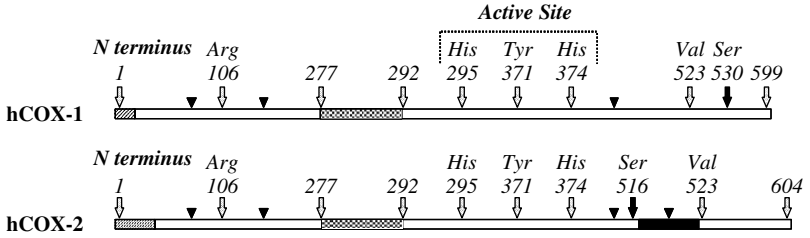

Figure 2. A diagram for COX-1 and COX-2 enzymes. The diagram highlights the putative N-terminal signal peptide region (//); the transmembrane domain residue 277–292 (✣); the 18-amino acid cassette insertion at the C-terminus (■); the putative N-linked glycosyl-ation sites (▼); the serine amino acid residue, the aspirin acetylation site (⇓); the axial (His-295) and the distal (His-374) heme ligands and the active site residue, Tyr-371, (17, 26, 27).

domain residues 277–292, the active-site residue Tyr-371, and the sequence surrounding the serine residue, the aspirin-acetylation site, at the C-terminus are conserved between COX-1 and COX-2 (17). There is an 18-amino acid insertion containing a putative N-glycosylation site at the C-terminal region of hCOX-2 (Figure 2 and Table 1). Apparently the important difference between the two isoenzymes is the substitution of Ile-523 in COX-1 by Val–523 in COX-2 (26). The presence of this smaller Val residue in COX-2 creates a larger active site (27) and allows for the appearance of a new pocket in the channel that accommodates the sulfur-containing side chains of selective COX-2 inhibitors (26).

Although COX-1 and -2 catalyze the conversion of AA to PGH_2 with similar kinetics (25), they utilize different phospholipase systems or lipid stores of AA (29). Moreover, COX-1 produces PGs only on the endoplasmic reticulum, while COX-2 forms products within or on the nuclear envelope as well as on the endoplasmic reticulum ((30), Table 1). It has been suggested that the COX-1 pathway is part of an acute signaling system because of its generalized constitutive expression (31). On the other hand, the COX-2 pathway, because of its inducible nature and the time lag required for expression, produces PGs that are likely to be employed in the secondary elaboration of various physiological events such as inflammation and mitogenesis (31).

During the metabolism of AA by COX-1 and -2, many chemicals, including carcinogenic agents, are oxidized. The oxidation of these xenobiotics occurs via either the peroxidase activity of the COX enzymes, the peroxyl radicals generated during AA oxygenation, or a combination of these two mechanisms (32). In many cases these reactions result in the formation of reactive intermediates that have mutagenic and carcinogenic activity (see 33). There is no adequate evidence to support a definitive role

of xenobiotic metabolism in the genesis of prostate cancer. Nevertheless, carcinogen activation that occurs during AA oxygenation can participate in the initiation of carcinogenesis in various extra-hepatic tissues (including prostate) where the mixed function monooxygenase enzymatic system exists in a relatively low capacity (see below).

The COX isoforms are the primary targets of the non-steroidal anti-inflammatory drugs (NSAIDs) which act by inhibiting the activity of the two isoenzymes. The best known of these are aspirin, indomethacin, ibuprofen, piroxicam and sulindac. Aspirin inhibits the cyclooxygenase (but not the endoperoxidase) activity of COX-1 or -2 by acetylating a particular serine residue (Ser-530 in COX-1 and Ser-516 in COX-2) and thus blocking the channel that leads to the active site. This acetylation results in an irreversible inhibition of PG synthesis (34). Indomethacin forms a tight, slowly dissociable complex with COX that induces an inhibitory conformational change (35). Ibuprofen and piroxicam, on the other hand, compete with AA for the active site (36, 37). In general, most of these "classical" NSAIDs are better inhibitors of COX-1 than COX-2, although some, like flurbiprofen and ibuprofen, have nearly equal IC_{50} values (38). Since PGs produced by COX-2 are formed particularly at the sites of inflammation while PGs synthesized by COX-1 are required for the protection of mucosal membranes, inhibiting both COX-1 and COX-2 by the classical NSAIDs may lead to gastrointestinal and genitourinary toxicity. Therefore, reducing the inflammatory effects of COX-2 while retaining the cytoprotective actions of COX-1 is a desirable outcome that is achieved by specific COX-2 inhibitors (39). A variety of these inhibitors have become available recently such as NS-398, which blocks COX-2 expression and completely inhibit PG synthesis in inflammatory cells with no influence on PGs synthesized from COX-1 (39, 40). Generally, COX-2 inhibitors cause conformational changes in the protein and irreversible loss of activity (41).

3. PGS, COX-2 AND PROSTATE CANCER DEVELOPMENT AND METASTASIS

The role of PGs in the development of prostate cancer has been substantiated from several experimental evidence in both human and animal models (Section 3.1) as well as from epidemiological associations between a lower risk of prostate cancer with the intake of inhibitors of PG synthesis (Section 3.2). Moreover, various evidence concerning a role of PGs in the development of prostate cancer was derived from the possible involvement

of these lipid molecules in the carcinogenic effects of fats and hormones on the gland (Section 3.3).

3.1 Experimental Evidence

Prostate exhibits the highest levels of COX-2 mRNA among other human tissues (10). Additionally, in the adult rat male reproductive system, COX-2 is the predominant isoform. It is heavily localized in the epithelium of the distal *vas deferens*, whereas COX-1 expression was many-fold greater than COX-2 in the other body organs (42). Intracellular PGE_2 has been shown to be involved in the mitogenic effects of estradiol (43) and testosterone (44) in the rat seminal vesicles but not in the ventral prostate (43, 44). It was suggested recently that PGE_2 plays a major role in the growth of prostate cancer cells through the activation of *COX-2* gene expression (45, 46). Elevated levels of PGs have been widely reported in malignant human prostate tumors (47–51) as well as in carcinogen-induced rat and mouse prostate cancers (52–54). *In vitro* studies with tissue explants or primary cultures of prostate tumor cells have also demonstrated higher PG production in malignant tissue compared to benign or normal (46, 50). Increased synthesis of PGs was associated with advancing prostate cancer and the concentrations raised as the degree of tumor differentiation progressed, *i.e.,* worse prognosis (50). We have found that PG synthesis and COX-2 mRNA and protein expression are significantly higher in prostate cancer tissues compared to controls (Figure 3, Badawi Personal Communication) Although the pathophysiologic significance of this correlation in the context of a role for PGs and COX-2 in prostate cancer is unclear, several studies with murine prostate cancer models indicate that PGs may indeed play a multifunctional role in controlling growth, metastasis, aggressiveness and host immune responses (47–53, 55).

Various studies of the inhibitory effects of NSAIDs on prostate carcinogenesis further implicate a function of PG synthesis in the development of prostate cancer. Rose and Connolly (56) reported inhibition in the growth of androgen-responsive and -unresponsive human prostate cancer cells by indomethacin. They concluded that NSAIDs have a significant protective activity when administered either during the early stage (initiation) or late stage (promotion) of prostate carcinogenesis. Similar results were obtained in Nb rats bearing subcutaneous implants of an androgen-insensitive prostate adenocarcinomas (57) and in estradiol-induced rat prostate tumor (43).

In *COX-2* stably transfected rat intestinal epithelial cells, over-expression of the gene has been accompanied by several phenotypic changes such as

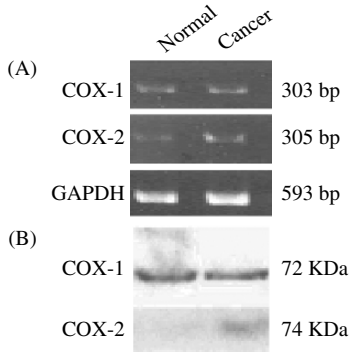

Figure 3. Expression of COX-2 mRNA and protein in human prostate cancer. Levels of COX-2 mRNA (A) in the prostate tissue were measured by RT-PCR. Single stranded cDNA was reverse transcribed from total RNA and used for PCR amplification with COX-1 (upper panel), COX-2 (middle panel) and GAPDH (lower panel) primers. Expression of COX-1 and COX-2 protein (B) was carried out by Western blot analysis. The blots represent $n = 20$ specimens in each group.

elevated levels of E-cadherin leading to increased adhesion to the extracellular matrix proteins and elevated bcl-2 protein expression with resistance to apoptosis (58). Furthermore, cells transformed with the Ha-*ras* oncogene showed an enhanced expression of *COX-2* accompanied by an increased production of PGE_2 (59). Similar changes may occur in prostate epithelial cells and would enhance the growth or reduce the loss of initiated or preneoplastic cells. Indeed, LNCaP, the human prostate cancer cell line that overexpresses *COX-2*, exhibited induction in apoptosis and down-regulation in *bcl-2* gene expression when treated with NS-398 (60).

3.2 Epidemiologic Evidence

In contrast to the experimental findings, the epidemiologic evidence for a protective effect of NSAIDs (*e.g.,* aspirin) in prostate cancer development is, at the moment, equivocal (61). The association between prostate cancer risk and the use of NSAIDs was investigated in a population-based case-control study in Auckland, New Zealand (62). A total of 317 newly diagnosed prostate cancer cases and 480 age-matched controls were recruited. The study reported a trend toward reduced risks of advanced prostate cancer associated with regular use of total NSAIDs (relative risk, $RR = 0.73$; 95% confidence intervals, $CI = 0.50 - 1.07$) and total aspirin ($RR = 0.71$; 95% $CI = 0.47 - 1.08$). Although these associations were statistically

non-significant, the authors concluded that the findings support an etiological role for COX enzymes in prostate cancer progression (62).

A more recent study examined the effect of NSAIDs on tumor prevalence in abusers of analgesics, including aspirin (63). A group of 618 patients were compared to matched controls without evidence of aspirin abuse. The study concluded that the use of aspirin and other analgesics was associated with an overall risk of 0.4-fold of having developed malignancy relative to the control individuals. However, no statistically significant effect was found for patients with prostate cancer. Another prospective study that was carried out on 73 patients with chronic prostatitis showed that therapy with ibuprofen proved effective in alleviating the symptoms in about 70% of the patients (64).

Generally, the epidemiologic evidence for a protective effect of NSAIDs in prostate cancer development is inconsistent and still inconclusive. These inconsistencies are perhaps attributed to the nature and the limitations of epidemiologic studies. Conflicting observations may be due to a number of factors, such as the characteristics of the evaluated population, the choice of the control groups, the sample size, or various other biases that may result in random or systematic inaccuracies. Changes in the profile of NSAIDs use over time particularly with recent use, exclusion of the socioeconomic status that influences cancer risk, and the lack of information concerning NSAIDs other than aspirin all may combine and consequently contribute to misinterpretation of the protective role of NSAIDs in prostate cancer. Indeed, the promising experimental evidence and the inconclusive epidemiologic findings reflect the gaps in our understanding of the protective effects of NSAIDs.

One explanation for the inconsistent epidemiologic findings may relate to individual differences in NSAID metabolism due to genetic polymorphisms in enzymes (*e.g.*, cytochrome P450 2C9; CYP2C9) known to be involved in NSAID metabolism. Recently, we indicated that the failure to examine these molecular biomarkers of individual susceptibility and response may have contributed to the contradictory epidemiologic findings on the effects of NSAIDs in prostate cancer (69).

3.3 Evidence Derived from the Carcinogenic Action of Hormones and Dietary Fats

It is known that prostate cancer results from an interplay between endogenous hormones (65–69) and exogenous (environmental) influences that include, most prominently, dietary factors ((56), 69–71). The influence of hormones and diet, particularly fat consumption, on prostate cancer can

be mediated, at least in part, by their effects on PG synthesis in the gland. Recent studies in the rat mammary gland suggest that the influence of hormones (72, 73) and dietary fat (73–75) on cancer development may be mediated, at least partly, by their effects on *COX-2* and PG synthesis (72–75). These effects can also be operative in the case of prostate cancer.

3.3.1 Hormones and PGs

Hormones such as androgens are crucial for the normal development of the prostate gland and the maintenance of its functional state in the adult. However, prolonged presence of androgens may be a risk factor in the development of prostate cancer (66, 76). For example, it is evident that both androgens and estrogens play an integral role in the growth of benign prostatic hypertrophy (77). Moreover, testosterone was found to be positively associated with human prostate cancer (78) and various epidemiologic studies suggest that the racial differences in the susceptibility and incidence of prostate carcinomas are partially related to hormonal influences (79). In animal studies, chronic exposure of adult mice and hamsters and prenatal exposure of mice to estrogenic compounds increased the incidence of prostate cancer (for review see Ref. (65)). Stimulation of prostate cancer growth was also evident in a variety of animal models following treatment with pituitary hormones such as prolactin (65). Further support for the role of hormones in prostate cancer was derived from studies showing androgen withdrawal as an effective approach in prostate cancer therapy (80, 81).

There is some evidence linking hormonal effects on prostate carcinogenesis to PG synthesis (48–50, 55). Administration of testosterone to male dogs results in a significant increase in PGs synthesized in the prostate (82). Androgen induced elevation in PGE_2 levels in the mouse genitourinary tract *in vivo* (83). Furthermore, PGs were suggested to be acting as second messengers for prolactin effects on prostate cancer development (84) and to mediate the mitogenic potential of testosterone in rat prostate (46). The acute effect of testosterone on the prostate was presumed to be related to its ability to increase the synthesis of $PGF2_\alpha$ in the gland (82). PGE_2 was also suggested to play a role in the mitogenicity of estradiol (43) and testosterone (44) in the rat seminal vesicles but not in the ventral prostate (43, 44). Finally, elevated levels of PGE_2 were associated with the aggressive, metastatic, androgen receptor-negative tumors (52, 53).

3.3.2 Dietary Fats and PGs

Dietary fatty acids are known to modulate prostate gland carcinogenesis in experimental animals (48, 85, 86) and may have similar effects in humans

(69, 70, 86). Numerous studies in rodents have shown that vegetable oils rich in n-6 polyunsaturated fatty acids (PUFAs) promote prostate cancer, whereas similar levels of marine oils rich in n-3 PUFAs inhibit (48, 85, 86, see below). n–6 PUFAs also stimulate, and n-3 inhibit, human prostate cancer cells in culture (56, 70). Feeding diets rich in marine oils suppresses the growth of human prostate cancer cells as solid tumors in athymic nude mice (70). Concerning the levels of fatty acids in human prostatic tissue, it has been shown that patients with malignant prostatic disease have significantly lower levels of AA concentration in the tissue phospholipids compared to the normal glands (87). The production of PGs by prostate cancer tissue was 10-fold higher than normal (87). Therefore, it can be suggested that the decreased levels of AA in prostate tumors are not due to its lower rate of formation but rather its elevated conversion to PGs. The relationship between prostate cancer incidence and dietary fat intake was estimated by international comparisons and epidemiological studies, which suggest that marine oil is an effective preventive factor (88).

Evidence linking the effects of dietary fat on prostate carcinogenesis to PG synthesis derives mainly from the observation that NSAIDs generally inhibit the promoting effects of diets rich in n-6 PUFAs (56). Furthermore, n-6 PUFAs are precursors of the PGs 2 series that are known to be mitogenic in both human and rodent prostate models (70). On the other hand, n-3 PUFAs are precursors of the PGs 3 series that lack a mitogenic effect (70). In support, PGs 3 series are 10-fold higher in the urine of volunteers after ingestion of n-3 PUFAs for 12 weeks compared to controls (89), suggesting their contribution to the observed favorable effects of marine oils on renal pathogenesis (88, 89).

4. FUNCTION OF PGS AND COX-2 IN PROSTATE CANCER DEVELOPMENT AND METASTASIS

Various theories were proposed to explain the exact mechanism(s) by which high rates of PG synthesis foster prostate cancer development and growth. These theories include the effects of PGs on cell proliferation, apoptosis, host immune response, and carcinogen metabolism.

4.1 Proliferation

PGs appear to be functioning as endogenous biological modifiers in different tissues and cells. PGs act, at least in part, through specific

G-protein-linked receptors to modulate the levels of the second messengers cAMP and Ca^{++} (8, 9) and, therefore, are involved in a variety of biological functions. The role of PG synthesis in controlling cell growth was substantiated from studies showing that these lipid molecules are not mitogens *per se* but act as permissive factors allowing the mitogenic action of various growth factors. For example, the epidermal growth factor-dependent proliferation of Balb/c 3T3 cells is inhibited by the COX inhibitor indomethacin (90). Moreover, $PGF_{2\alpha}$ stimulated the proliferation of the MC3T3-E1 osteoblast cells by increasing the number of insulin-like growth factor (IGF)-I binding sites (91). Additionally, human keratinocytes (92) and colon cancer cells (93) required PGE_2 for normal cell growth and proliferation. This proliferation was inhibited by NSAIDs in a manner that was overcome by the addition of PGE_2 (92).

12-*O*-tetradecanylphorbol-13-acetate (TPA) is a cancer-promoting agent that is usually used in mouse skin cancer models and induces a considerable PG synthesis at the site of administration (94). Topical application of TPA to mice caused epidermal hyperproliferation that was inhibited by indomethacin, an inhibition that was reversed by topical application of PGE_2 (94–96). In this model, PGE_2 was not a mitogen *per se* but rather acted as a co-mitogenic factor when applied with TPA (97). The marked increases in DNA, RNA and protein synthesis observed in rat skin treated topically with PGE_2 (98) suggest that PGs are regulatory factors in cell growth. These growth stimulating effects of PGs appear to be linked to biological modifiers such as polyamines. Elevated polyamines result from induced activity of ornithine decarboxylase (ODC) and are associated with increased DNA synthesis required for tumor growth. It is likely that tumor promoters enhance ODC activity in a PGE_2-dependent fashion (99), since inhibitors of AA metabolism inhibit both ODC activity and tumor development (58). This observation implies a possible link between PG synthesis, proliferation and tumorigenesis.

4.2 Apoptosis

COX-2 overexpression is associated with cell resistance to apoptosis, as observed in rat intestinal epithelial cells stably transfected with the gene (58) and in human prostate cancer cells (60). Several recent studies established a direct role of PGs in rendering cells resistant to apoptosis (100–104). For example, PGs effectively inhibited apoptosis in various animal (100, 101) and human (102) neuronal cell lines. This antiapoptotic effect of PGs was achieved by inhibitory signals following uptake through the PG transporter (101) or due to its role as a cAMP-elevating agent (102). Moreover, in rat

hepatocytes, PGI_2, PGD_2 and PGE_1 decreased the frequency of apoptotic nuclei in a dose-dependent manner by up to 80% and suppressed internucleosomal DNA fragmentation (104). In human colon cancer cells, PGE_2 inhibited programmed cell death caused by the selective COX-2 inhibitor SC-58125 (104). Therefore, decreased apoptosis caused by PGs could lead to enhanced growth and/or decreased loss of initiated or preneoplastic cells and may explain how NSAIDs prevent cancer. The biochemical basis for the anti-proliferative characteristics of NSAIDs was generally attributed to their ability to reduce PG synthesis. More recently, however, these characteristics were partially linked to the ability of NSAIDs to induce apoptotic cell death (105–107). Although it can be argued that NSAIDs, by inhibiting COX expression, can mediate the induction of apoptosis, recent evidence refutes this presumption and suggests that the two mechanisms are not related (108). In fact, the anti-proliferative effect of NSAIDs can be ascribed to their influence on a variety of membrane processes that influence apoptosis and may not be linked to PG synthesis, such as the inhibition of superoxide anion generation and the coupling of mitochondrial oxidative phosphorylation (for review see 108).

The relationship between PG synthesis and apoptosis was examined by adopting three main approaches. *a*) Using human colon cancer cells that express *COX-2* and synthesize PGs (*e.g.*, HT-29) and cells that do not exhibit these characteristics (*e.g.*, HT-15). In these studies NSAIDs inhibited the proliferation of both cell lines regardless of their ability to synthesize PGs (93); *b*) Using sulindac and its metabolites: the reduced form that inhibits COX (*i.e.*, sulindac sulfide) (109, 110) and the oxidized form that is not known to inhibit COX (110), lipoxygenase or phospholipase A2 (111) (*i.e.*, sulindac sulfone). This approach showed that in spite of their ability to inhibit PG synthesis, both sulindac metabolites induced apoptosis (112) and inhibited tumor growth (115, 112, 113). *c*) Using chiral NSAIDs which exist in *S* and *R* enantiomeric forms (*e.g.*, flurbiprofen and carprofen) that inhibit or do not affect COX activity, respectively. These studies demonstrated that regardless of the ability of the enantiomeric forms to inhibit COX, they had equal anti-proliferative activity (4). Taken together, it can be concluded that the anti-proliferative potency of NSAIDs (*via* apoptosis) is likely to be a PG-independent mechanism although *COX-2* over-expression is associated with resistance to apoptosis (58, 60, 114).

4.3 Immune Suppression

The growth of various tumors is frequently associated with reduced immune response (115, 116). Suppression of immune surveillance (117)

as well as inhibition of natural killer cell activity (118) were found to be mediated by high levels of PGE_2. It was suggested that PGs regulate immune function by acting as a negative feedback inhibitor for various processes including T cell proliferation, lymphokine production and macrophage and natural killer cell cytotoxicity (116–120). Moreover, colony stimulating factor, released by tumor cells, can cause monocytes and macrophages to synthesize PGE_2 as a contributory factor to the tumor-associated immune suppression (116, 121). This elevation in PGE_2 synthesis inhibited both the blastogenesis of the T cells and the activity of the natural killer cells (116, 121). Therefore, inhibition of COX activity may be associated with an enhanced immune response (118, 119) and reduced tumorigenesis. In support, indomethacin reduced the size of bone tumors in Moloney sarcoma virus infected mice (122). Moreover, administering PGE_2 to syngeneic mice bearing transplanted squamous cell carcinomas enhanced tumor transplantability (123). This inhibitory effect of NSAIDs on transplanted tumors is usually lost as growth progresses. In this case, immunosuppression results from the production of bone marrow-derived monocyte-like suppressor cells rather than from PG production (124).

4.4 Xenobiotic Metabolism

The effect of PGs and its precursor AA on xenobiotic oxidation catalyzed by human recombinant CYP enzymes and by human liver microsomes has been investigated recently (125). AA significantly inhibited CYP1A1- and 1A2-dependent *O*-deethylation and CYP1A2-, 2C8- and 2C19-dependent hydroxylation. AA also inhibited xenobiotic oxidation catalyzed by CYP1B1, 2B6, 2C9, 2D6, 2E1 and 3A4 in recombinant systems. Additionally, AA inhibited the activity of alkaline phosphatase in rat chondrocytes due to PG, but not to leukotriene, production (126).

Although the intracellular AA and the resultant PGs may inhibit xenobiotic metabolism, the peroxidase component of COX itself can oxidize a wide range of chemical carcinogens (*e.g.*, heterocyclic and aromatic amines and polycyclic aromatic hydrocarbons) besides metabolizing AA *via* a co-oxidation reaction (5, 127, 128). This reaction is inhibited by NSAIDs by preventing the COX-catalyzed generation of hydroperoxide substrate. Inhibiting carcinogen activation by NSAIDs hindered the growth of bladder cancer induced by *N*-[4,5-nitro-2-furyl]-2-thiazole] formamide (129) and colon cancer initiated by heterocyclic aromatic amines (130). This observation further supports the notion that inhibiting COX by NSAIDs may have a direct preventive effect on tumorigenesis independent of its effect on PG synthesis.

Peroxy radicals are generated during AA metabolism. These stable oxy radicals cause epoxidation for a variety of chemical carcinogens to their ultimate reactive forms. Carcinogen active metabolites produced by COX activity can bind to the cellular macromolecules, including DNA, to form a wide range of DNA adducts leading to mutagenesis and carcinogenesis (127, 128). There is a stereospecific difference between DNA adducts formed as a result of COX activation and those formed *via* CYP-catalyzed pathway. Analysis of these two types of DNA adducts may allow a precise characterization for the contribution of each metabolic pathway to the process of chemical carcinogenesis in extra-hepatic tissues (32). COX-mediated carcinogen activation offered a valid explanation for the influence of cigarette smoking in susceptibility to human bladder cancer (33) and may be relevant to carcinogen-induced prostate cancer both in human and animal models.

5. CONCLUDING COMMENTARY

Endogenous hormones and dietary fatty acids that are known to promote the development of prostate adenocarcinogenesis in rats following carcinogen treatment may induce the expression of the *COX-2* gene in the prostate gland (58, 59). However, the possibility that the induction of *COX-2* gene expression and the resulting increase in PG synthesis can enhance susceptibility of the prostate gland to cancer remains to be clarified. Further studies should be carried out to examine this hypothesis in rodent models and to determine whether a similar mechanism could be operative in the development of human prostate cancer. Studies utilizing transgenic animal models can be employed to examine prostate transformation under the influence of *COX-2* over-expression. The transgenic animal model has been used for a number of years and has yielded valuable information regarding the process of cancer development (131, 132). Moreover, mice with a targeted disruption of the *COX-2* gene have been constructed from the C57B116 background (133, 134) that is susceptible to prostate carcinogenesis (132, 135). Using this model will determine whether prostate tumorigenesis in animals lacking the *COX-2* is promoted by factors that are known to influence the formation of prostate cancer to an extent different from that in normal animals. The role of *COX-2* gene expression in prostate cancer can also be examined in human prostate epithelial cells that are stably transfected with and constitutively express the gene. Generating these cells will further clarify whether constitutive expression of *COX-2* is associated with increased susceptibility to neoplastic transformation and whether there are accompanied cellular

and molecular changes that might be linked to the enhanced tumorigenic potential.

Elucidating the relationship between PG synthesis, *COX-2* expression, and prostate cancer development is critical for understanding the molecular basis of the disease and may suggest new views concerning scheduling chemotherapy. Comprehending the extent to which *COX-2* is involved in prostate cancer development may shed some light on the possibility of including NSAIDs that specifically inhibit COX-2 as an integral part of a reliable and effective strategy for cancer prevention.

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