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PROSTAGLANDINS AND CANCER

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1. ABSTRACT

Prostaglandins (PGs) are lipids produced enzymatically and nonenzymatically from 20 carbon fatty acids, particularly arachidonic acid. Prostaglandins and related metabolites (collectively referred to as eicosanoids), including the thromboxanes, prostacyclins, hydroperoxy and hydroxy fatty acids, and leukotrienes are produced by most tissues of the body by oxidation of arachidonic acid, although the amount and class of product varies with cell type. The biological action of many of these eicosanoids as key regulators of cell processes that range from proliferation to adhesion and migration are currently being elucidated. An association of high levels of PGs and their synthetic enzymes, the PG synthases/cyclooxygenases, have been noted for many of the major types of cancer. Prostaglandins may contribute to the cancer processes through one or more of several mechanisms including increased proliferation, apoptosis, enhanced carcinogen metabolism or modulation of the immune system. The recent understanding of the regulation of substrate availability and of the regulation (or dysregulation in many neoplasias) of the synthetic enzymes has opened avenues leading to the design of isozyme specific inhibitors and better cancer prevention strategies. However, the apoptosis caused by these inhibitors as well as other drugs has raised some question concerning the relative importance of PGs. This is an issue that remains to be resolved.

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2. INTRODUCTION

The concept that PGs or other eicosanoids derived from fatty acids may play a role in the development of cancer is not a new one. A review written over twenty years ago (1) focused on three areas of involvement: *i*) humoral PGs as mediators of the symptoms of medullary carcinoma of the thyroid; *ii*) the role of PGs in hypercalcemia and bone resorption associated with neoplasia; and iii) the effects of inhibitors of PG synthesis, particularly indomethacin, on tumor growth. Subsequent work, reviewed by Karmali (2), was concerned with demonstrating an elevation of PGs in human and experimental tumors and showing that this affected tumor growth. Since this time, the literature has blossomed with reports on the effect of PGs on carcinogen metabolism, tumor cell proliferation, metastatic potential and the efficacy of inhibitors of PG synthesis in preventing tumor development (3, 4, 5). Not all of the reports are in agreement, however, leading to the conclusion that we do not yet know the exact role of PGs in cancer development and progression. Overall, however, several general observations can be made: i) there

appears to be a direct relationship between the level of PGs synthesized, in humans or experimental animal models, and cancer incidence; and ii) for several experimental models and in some types of human cancer, particularly colon, inhibitors of PG synthesis are protective against cancer development. Although these latter observations have suggested that PGs (or other eicosanoids) can promote the development of tumors, there is also evidence that the inhibitors of PG synthesis commonly employed have anti-tumor functions independent of PG synthesis (4).

This review focuses on how PG synthesis is regulated in animal and human studies and on the role of PGs in the major types of cancer, including skin, colon and breast. The several possible mechanisms by which high levels of PG synthesis may contribute to tumor development are also discussed. The ultimate goal of understanding the function and contribution of PGs to cancer is the use of this knowledge in designing better cancer prevention or intervention strategies.

3. THE ARACHIDONATE CASCADE

3.1. Metabolic Pathways

Arachidonic acid (5,8,11,14 eicosatetraenoic acid; 20:4n-6) is found esterified in membrane phospholipids and triglycerides in all mammalian tissues. In this bound form, arachidonic acid is not usually a substrate for metabolizing enzymes. The esterification of arachidonic acid in phospholipids has been postulated to serve both the function of sustaining membrane fluidity and of substrate storage (6). Oxidative metabolism of arachidonic acid to PGs, hydroxy-fatty acids and leukotrienes, collectively referred to as eicosanoids, depends on the availability of free, nonesterified fatty acid. Arachidonic acid is readily released as the free fatty acid by one or more of the phospholipase A_2s (PLA_2), which by definition hydrolyze the ester linkage at the *sn*-2 position of a phospholipid. In many respects, PLA_2s are among the more important enzymes involved in eicosanoid synthesis because the availability of free arachidonic acid is believed to be one of the rate-limiting steps for the formation of all the eicosanoids. Because eicosanoids are involved in a number of patho-physiological conditions in addition to carcinogenesis, an understanding of the mechanisms by which PLA_2 activity is regulated is of great interest, and is discussed briefly below.

A second pathway for arachidonic acid release is via phospholipase C, which hydrolyzes the head group function of phospholipids and thus yields diacylglycerol (DAG). A DAG lipase can then release arachidonic acid in a subsequent reaction. In many tissues including murine keratinocytes both phospholipases A_2 and C appear to be operative following treatment with irritating agents such as tumor promoters (7).

The levels of free arachidonic acid are normally very low since the liberated fatty acid is rapidly metabolized. Free arachidonic acid is a substrate for two distinctively different enzymatic pathways, one leading to the synthesis of PGs, the other to the hydroperoxy- and hydroxy-eicosatetraenoic acids referred to as HPETEs and HETEs, respectively. The two pathways have in common the insertion of molecular oxygen into the fatty acid, although PGs have, in addition, a cyclopentane ring. Details of the enzymatic reactions involved in arachidonic acid metabolism have recently been reviewed (8). Briefly, the enzymes responsible for the production of PGs are referred to as PG synthetases, which contain two active sites. The cyclooxygenase (COX) moiety introduces two molecules of oxygen into arachidonic acid to form the hydroperoxy endoperoxide, PGG_2, which is then reduced by the endoperoxidase moiety of the enzyme to the hydroxy endoperoxide, PGH_2. Prostaglandin synthetases or cyclooxygenases, as they are commonly called, are the target of many PG synthesis inhibitors, particularly the nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin (8). These inhibitors have been particularly useful in demonstrating an active role for eicosanoids in many physiological and pathological conditions. Their use in studies on the involvement of eicosanoids in tumor development will be described below.

The intermediate endoperoxide generated by PG synthetase, PGH_2, is the substrate for several enzymes. The PG endoperoxide E and D isomerases produce PGE_2 and PGD_2, respectively (9, 10). Additional dehydration and isomerization of PGE_2 produce PGA_2, PGB_2 and PGC_2. PGF_{2alpha} arises either via nonenzymatic reduction of PGH_2 or from PGE_2 by way of a 9-keto-reductase enzyme (8). The endoperoxides can also give rise to the thromboxanes by way of thromboxane synthetase and to prostacyclin by way of an oxy-cyclase (11).

The second major arachidonic acid metabolic pathway involves the lipoxygenases. These enzymes give rise to hydroperoxy products which can be reduced by glutathione peroxidase to hydroxy forms. A half dozen or so different lipoxygenases have been described as reviewed recently by Funk (12), each responsible for the insertion of molecular oxygen at a particular carbon. Hence, the 5-lipoxygenase generates the 5-HPETE, the 12-lipoxygenase, 12-HPETE, and so forth. The 5-HPETE is noteworthy in that it is the only HPETE that can be further metabolized to the leukotrienes, which historically have been referred to as the slow-reacting substances of anaphylaxis (13). Mammalian lipoxygenases are of the (S) type, i.e., they generate (S) hydroperoxy fatty acids (14). This feature has been used in many cases to distinguish enzymatic from nonenzymatic metabolism in which the (R) form is also produced.

Arachidonic acid may also be metabolized by cytochrome P-450 type enzyme systems to produce a number of hydroxy and carboxy products, including the 19-hydroxy, 19-oxo, 20-hydroxy and 20-carboxy metabolites (15). With the possible exception of the formation of the 12 (R)-HETE in psoriatic scales, cytochrome P-450 derived products are probably of minimal importance in such tissues as skin (8), in part, because of the extremely low levels of cytochrome P-450 in normal skin and the suppression of carcinogen-induced

cytochrome, P-450, *cyp1a-1*, by tumor promoting phorbol esters (16). However, in tissues such as the kidney and cornea, cytochrome P450 metabolism results in biologically active products that play a role in the physiology of these tissues.

Arachidonic acid is not the only fatty acid that can be metabolized by PG synthetase and/or the lipoxygenases. Since fish oils that contain the n-3 fatty acids, eicosapentaenoic and docosahexaenoic acids, have been shown to reduce tumor development at several organ sites (18, 19), there has been considerable interest in the nature and biological activity of their metabolites. In the skin, eicosapentaenoic acid has been shown to compete with arachidonic acid for incorporation into cell membrane phospholipids (20). This results in both competitive inhibition of arachidonic acid metabolism as well as the production of such metabolites as PGE_3, PGD_3, and 12- and 15-hydroxyeicosapentaenoic acids (HEPES) (20). These metabolites are biologically less active than the corresponding arachidonic acid metabolites and presumably compete with them for receptor occupancy.

The 15-lipoxygenase, distributed widely among various types of tissues, converts arachidonic acid to 15-HPETE which can then be reduced by glutathione peroxidase to 15-HETE (8). However, the preferential endogenous substrate is believed to be linoleic acid (18:2n-6) leading to the production of 13-hydroxyoctadecadienoic acid (13-HODE). For some organs this is significant because, for example, the upper layers of the epidermis contain high levels of linoleate-rich sphingolipids that contribute to the barrier function of the skin (21). Murine keratinocytes are also capable of synthesizing 9-HODE from linoleic acid (unpublished data); the function or activity of this eicosanoid is unknown at this time. Unlike eicosapentaenoic acid, linoleic acid cannot be metabolized by COX to PG-like products (22). The significance of the production of non-arachidonate eicosanoids to the function of skin or other organs is not well understood; however, the ability of some of them to modulate tumorigenesis indicates that they may, be at least as important as the arachidonate metabolites. This is an area in need of additional investigation.

In addition to enzymatically generated products, arachidonic acid can also be converted to PG-like compounds referred to as isoprostanes (see reference 23 for review). Isoprostanes are generated by free radical catalyzed peroxidation of lipids and are found *in vivo* in both esterified and unesterified forms. Because isoprostanes are isomeric to COX-derived PGs it was thought that they should exert biological effects. Recently it has been shown that 8-iso-PGE_2 and 8-iso-PGF_{2alpha} are in fact potent vasoconstrictors in the renal vascular bed and are associated with a number of pathophysiological processes in this and other tissues (23).

3.2. Regulation of Metabolism

3.2.1. Phospholipase A_2

The regulatory mechanisms governing PLA_2 activation are not well understood; this is largely because of the number of different mechanisms that can elicit activity, and because of the recent identification of several different types of PLA_2s. An understanding of the mechanisms of activation and regulation of PLA_2 activity is crucial because this determines the types and amounts of fatty acid released, which in turn is rate-limiting for the production of biologically active eicosanoids. Several families of PLA_2 have been identified in mammalian tissues: the low-molecular weight (14kD) types I and II PLA_2, referred to as secretory or $sPLA_2$, and the high-molecular weight (85kD) cytosolic PLA_2 ($cPLA_2$) (24, 25). The $sPLA_2$s differ from the $cPLA_2$ in several significant ways. $sPLA_2$s are found either in the membrane or extracellularly and, with regards to both phospholipid and *sn*-2 fatty acid, have a broad range of substrate preferences (24). In tissues where they have been studied, activation of $sPLA_2$ has been observed to occur through at least two distinct mechanisms: one mediated by elevated cAMP and the other by the inflammatory cytokines, interleukin-1 and tumor necrosis factor-alpha (26). It has been suggested that cell-associated $sPLA_2$ functions primarily in maintaining cell membrane homeostasis, extracellular $sPLA_2$ plays a role in inflammatory diseases, while $cPLA_2$ may be involved in the initiation of the inflammatory response (24).

Due to its preference for arachidonic acid over other fatty acids, the activation of $cPLA_2$, in particular, may be critical for subsequent eicosanoid biosynthesis. Among the PLA_2s, the mechanisms by which $cPLA_2$ is activated is the best understood. Several kinds of regulatory mechanisms have been reported, including phopsphorylation, interaction with calcium, and receptor-induced interaction with GTP-binding proteins (27). Growth factor stimulation of cells has been reported to result in increased phosphorylation of $cPLA_2$ on serine residues by either p42 MAP kinase or protein kinase C (28-30).

Specific regulators of PLA_2 have also been investigated; lipocortins (members of the annexin family of molecules) have been touted as negative modulators, while PLA_2-activating-protein (PLAP) has been suggested to be a positive regulator. Glucocorticoids have been linked to an increase in the amount of lipocortin, a protein with anti-PLA_2 activity (31). Glucocorticoids are known to suppress both arachidonic acid release and inflammation; this is believed to, at least, partially explain their mechanism of action as anti-tumor promoters in the mouse skin carcinogenesis model (32). However, the anti-inflammatory activity of lipocortin has not always been conclusive and appears to occur only in *in vitro* assays (31). Based on sequence, six members of this protein super-family have now been identified. A physiological role, however, has not been established for any of them and it was concluded that there is not sufficient evidence for a negative regulatory role for lipocortins (31).

PLAP, which has homology to melittin, has recently been isolated and identified (33). PLAP increases PLA_2 activity by increasing the V_{max} of a phosphatidylcholine-specific PLA_2 (33). The importance of PLAP to eicosanoid synthesis was recently demonstrated by showing that

stimulation by leukotriene D_4 of smooth muscle and endothelial cells resulted in increased PLAP mRNA and that the antisense cDNA for PLAP effectively blocked the activation of PLA_2 (34).

3.2.2. Prostaglandin Synthases/Cyclooxygenases

Although originally thought to be one enzyme, it has recently been shown that there are two PG synthetases, referred to as PG synthetase-1 and -2, or COX-1 and COX-2, respectively (24, 35-37). These two distinct gene products exhibit similar COX and peroxidase activities although they are differentially regulated (24, 35-37). While a variety of factors, including serum, growth factors and phorbol esters, can upregulate the mRNA levels for both COX-1 and COX-2, generally COX-2 responds in a much more dramatic fashion and thus has been referred to as a phorbol ester-inducible immediate early gene product. The molecular biology, including DNA and amino acid sequences of the PG synthetases, has recently been well reviewed and described (24, 35-37).

While it has long been a tenet that the availability of free arachidonic acid controls PG synthesis, recent evidence suggests that significant regulation also occurs at the level of COX gene expression (36). For example, treatment of mouse skin or cultured keratinocytes with phorbol ester tumor promoters results in high PG production which could be due to either elevated substrate levels (phorbol esters activate $cPLA_2$) or increased expression of COX-1 or COX-2. Using cultured keratinocytes, phorbol esters were shown to significantly increase mRNA and protein levels for COX-2 but not for COX-1 (38). When COX-2 activity was inhibited with the selective inhibitor, NS-398, PG synthesis was reduced to control levels. Thus, induction of COX-2 appears to be the primary determinant of the increased PGE_2 in stimulated keratinocytes. These observations are similar to those reported for endothelial cells where phorbol esters were shown also to induce *de novo* synthesis of COX. This induction appears to be required for continued high levels of PG synthesis in the face of high levels of autoinactivation (39).

3.2.3. Lipoxygenases

The lipoxygenase products are considered to be biologically at least as important as the PGs because of their mediation of many aspects of inflammation. There are only limited reports on the regulation of specific lipoxygenases and in the context of cancer development this has been restricted primarily to the mouse skin model. Here, phorbol ester treatment of mouse skin was shown to have little effect on the 5-, 12- or 15-lipoxygenase pathways; however, a cytosolic 8-lipoxygenase activity was strongly induced (40). The 8(S)-lipoxygenase was recently cloned, allowing the demonstration that expression at the mRNA and protein level was greatly enhanced by phorbol esters (Alan Brash, Vanderbilt University; personal communication). While the exact function of 8-HPETE or 8-HETE is unknown, its importance in tumor promoter-elicited events is suggested by the finding that (*i*) an induction of 8-lipoxygenase or hyperplasia is not observed in the phorbol ester-resistant C57BL/6J mouse, but is in sensitive mice (40, 41) and (*ii*) application of the lipoxygenase inhibitor eicosatetraynoic acid maximally inhibits tumor promotion when applied at the time (18 h) of maximum induction of 8-lipoxygenase (42). Although papillomas and carcinomas show dramatically elevated levels, compared to normal skin, of both 8-HETE and 12-HETE, it is not yet known whether this is due to increased expression of the respective enzymes or increased substrate availability (43).

3.3. Receptors

Elucidation of the function of the individual eicosanoids has been hampered by the fact that the same PGs often elicit opposing effects in different cells. This observation led to the search for receptors for the individual PGs, as well as other arachidonate metabolites. As described in detail in several recent reviews (44-46), specific receptors have been shown to exist for the PGs; the action of the PGs depends on the cell type, metabolic activity, location, and state of stimulation by other agonists (47). Three major subtypes of the PGE_2 receptor have so far been identified: EP_1, EP_2 and EP_3 (48). The EP_3 receptor, which belongs to the seven-transmembrane-domain family (49), is currently the most well characterized; the mouse EP_3 receptor has been cloned and shown to inhibit adenylate cyclase via G protein coupling (50). Namba *et al.* (49) have recently shown that there are at least four isoforms of the EP_3 receptor, all produced by alternative splicing, and differing only at their carboxy-terminal tails. This allows for activation of different second messenger systems because of coupling to different G proteins (49). For example, PGE_2 causes down-regulation of EP_{3alpha}, but not EP_{3beta}, by sequestering it away form the cell surface (48).

The EP_2 receptor for PGE_2 has also seven-transmembrane domains; unlike the EP_3 receptor family, it activates adenylate cyclase activity (51). Very recently, at least four isoforms of EP_2 have been identified, which are the result of alternative splicing (52). Activation of the third PGE receptor subtype, EP_1, can cause a rise in intracellular calcium concentration (44).

Receptors have also been identified for thromboxane A_2, prostacyclin, PGF_{2alpha} , and PGD_2; the signaling pathways they activate are slowly being elucidated (44-46). With regards to the lipoxygenase products, several receptors have been identified, eg., specific leukotriene C_4 receptors have been identified that mediate its mitogenic activity in cutaneous psoriatic lesions (53). Binding sites for 12-HETE have also been found in a human squamous cell carcinoma cell line, although the binding protein(s) has not been isolated or characterized (54, 55).

Currently, little is known about whether or how the expression of specific receptors is regulated. There are several reports that suggest that, at least some receptors, can be regulated by exogenous agents. For example, the binding of 12-HETE was found to be inhibited by exposure to ultraviolet-B light exposure (54, 55). Recently Cameron *et al.* (56)

reported that application of phorbol ester to mouse keratinocytes dramatically reduced the number of apparent PGE_2 binding sites, with little change in affinity (56). This dearth of information on modulation of receptor binding points to a clear need to characterize the eicosanoid receptors in normal and pathological states, with regards to distribution, function and regulation.

While the classical PG receptors are cell surface receptors, a class of nuclear proteins may also function as receptors for at least some of the eicosanoids. The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily that includes receptors for the steroid and retinoid hormones. PPARs, which affect the expression of many genes, and particularly those involved in the catabolism and storage of fatty acids, appear to represent another pathway or mechanism through which some eicosanoids exert their biological effects (57-59).

4. HUMAN CANCER AND EXPERIMENTAL ANIMAL MODELS

The strongest evidence that PGs contribute to the development of cancer originates from the numerous studies in experimental animal models, as well as human epidemiologic studies, showing that the incidence of tumorigenesis is reduced in the presence of inhibitors of PG synthesis. Although a number of organ sites have been studied in rodents, the majority of this work has been carried out in skin and colon. The second line of evidence is the elevation in PG levels in many tumors. These models will be discussed below, followed by comments on breast cancer. Potential mechanisms of PG actions will be discussed in the final sections of this review.

4.1. Skin Carcinogenesis

Mouse skin has proven to be one of the best animal model systems for studying the multistage nature of carcinogenesis (60, 61). Skin tumors can be readily induced by the sequential application of a subthreshold dose of carcinogen (initiation stage) followed by repetitive treatment with a noncarcinogenic tumor promoter (promotion stage). In this model, promotion is most often accomplished by using the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), although a variety of agents have been identified as skin tumor promoters, including nonphorbol esters such as benzoyl peroxide, anthralin, dihydroteleocidin B and ethyl phenylpropriolate (61-62). The promotion stage has been further subdivided into additional stages in which the sequential use of incomplete or partial promoters like the ionophore A23187 and mezerein can replace the use of a complete promoter such as TPA (60-61).

Investigations into the role of arachidonic acid metabolites in tumor promotion were spurred by the observation that TPA induces cytotoxicity, inflammation and increased vascular permeability (63). A crucial role for inflammation in phorbol ester promotion was suggested by several early studies showing that the anti-inflammatory steroids, dexamethasone and fluocinolone acetonide, were extremely effective in preventing tumor development (32, 64). Although other mediators of inflammation, including vasoactive amines, cytokines and growth factors have been and continue to be investigated, most of the emphasis has been on arachidonic acid and its oxidation products (65-67).

The early observations that steroidal anti-inflammatory agents are potent anti-promoters led to two approaches in assessing the importance of eicosanoids in tumor promotion: first, determination of whether tumor development could be modified through exogenous application of PGs and second, the effect on promotion of inhibitors of various parts of the arachidonic acid cascade, including use of a series of NSAIDs. In one study, skin tumor experiments were carried out in which PGE_2, PGF_{2alpha}, PGD_2 or arachidonic acid were applied either alone or with TPA during tumor promotion. The various PGs were found to be distinct in their modulation of the tumor response and that they depend both on the particular eicosanoid used as well as the dose and time of application (68). Other studies showed that PGE_2 and PGF_{2alpha} enhanced the development of papilloma and squamous cell carcinoma in mouse skin and promoted the growth of basal cell carcinomas in rats initiated with 3-methylcholanthrene (69, 70). Several studies also indicated that neither the PGs nor arachidonic acid were themselves tumor promoters (68, 71, 72).

While these exogenous application studies indicated that PGs could be used to modify tumor yield, understanding their action was confounded by the fact that TPA itself induces considerable PG synthesis. A series of studies have since been carried out using inhibitors of specific enzymes of the arachidonic acid cascade. The PLA_2 inhibitor dibromoacetophenone was shown by several laboratories to have strong inhibitory activity against phorbol ester promotion in several strains of mice (73). The effect of the COX inhibitor, indomethacin, however, is mouse-strain dependent: while inhibition occurs in CD-1 and NMRI mice, enhancement of promotion occurs in SENCAR mice (73). To show that increased PGE_2 synthesis is essential for DNA synthesis and tumor promotion, 'add-back' experiments were carried out in NMRI mice (74). While adding back PGE_2 had little effect on indomethacin reduction of tumor rate or yield, adding back PGF_{2alpha} produced a dose-dependent partial reversal (74, 75). Collectively, these inhibitor studies support the contention that arachidonic acid release and metabolism are essential components of the tumor promotion process.

Another observation supporting a critical role of PGs in the development of skin tumors is that carcinomas from animals no longer being treated with tumor promoters show constitutive overexpression of COX-2, but not COX-1, at the message and protein level (76). This correlates very well with the elevated PGE_2 levels measured in papillomas and especially in carcinomas (76). Although at least several classes of tumor promoters, induce COX-2 (but usually not COX-1) in keratinocytes *in vivo* and *in vitro*, the levels of

expression or levels of PGE_2 synthesis are not as high as seen in tumor promoter-independent tumors (38, 76). The mechanisms involved in the constitutive upregulation of COX-2 expression in skin tumors are currently unknown. Although most skin tumors generated by the multistage protocol carry an activated H-*ras* gene, and activation of the Ras-MAP kinase signaling pathway can induce COX-2, there is not a strong evidence that this drives the overexpression in tumors. This is indicated by the lack of correlation between expression levels and ratios of normal to mutated H-*ras* alleles in several tumor cell lines (38, 76). Several other possibilities exist, including enhanced transforming growth factor alpha production and elevated epidermal growth factor receptor expression in tumors (77). It is expected that these and other studies on the regulation of COX-2 at the level of its promoter region soon will shed light on the mechanism(s) responsible for the elevated COX-2 expression and high PG production.

4.2. Tumors of the Colon

The epithelium of the colon is similar to the epidermis of skin in that it is a self-renewing tissue, i.e., the epithelium in both cases continuously undergoes proliferation, differentiation and sloughing. Over the last decade, the multistage nature of human colon cancer has been defined and has similarities to the mouse skin model with regards to the progressive acquisition of mutations and chromosomal abnormalities. This raises the likelihood that there are similarities in physiological processes as well, including a role for PGs.

In rodent models of colon carcinogenesis COX inhibitors such as indomethacin, piroxicam and sulindac exhibit chemoprotective effects (78-83). Piroxicam is particularly effective, providing up to a 70% reduction in tumor incidence, although only at very high doses (84). Piroxicam is not an irreversible COX inhibitor like aspirin and it has been suggested that it may not be as effective as aspirin in certain cell types (85). Treatment with indomethacin of Sprague-Dawley rats with dimethylhydrazine-induced intestinal tumors reduced the tumor size and incidence by 40% (86). Indomethacin also inhibited the development of colon tumors induced by methyl nitrosourea in CD-Fischer rats (87).

The ability of human colonic mucosa to synthesize PGE_2, PGD_2, PGF_2alpha and thromboxane B_2 has been well demonstrated (87-89). Using different approaches, several laboratories have shown that human colon cancer tissue produces more PGE_2 than the surrounding normal tissue (87). One study measured PGE_2 levels in normal-appearing mucosa and in tumors from patients with adenomatous polyps or colon cancer. The level of PGE_2 in polyps was elevated by about 40% and in colon cancer by almost three fold (90). This increase in PGE_2 with progression from normal to malignant tumor is similar to that described for progression of tumors in the mouse skin model (76). However, when cell lines derived from various tumor types were compared, lines derived from colorectal adenocarcinomas were among the lowest producers of PGE_2 (91). Sine biopsy specimens and tumors contain several cell types, the origin of the PGs has been questioned (4). It has been recently shown that high tumor PGE_2 is due to the very high level of PGE_2 produced by the resident (not peripheral) mononuclear cells (89). Thus, a paracrine mechanism may exist in which the mononuclear cells produce the majority of the PGE_2 while the target or responding cells are the colonic epithelium.

The elevation of PGs in colon tumors suggested that the expression of COX-1 and/or COX-2 is altered in tumors. Elevated levels of COX-2 mRNA and protein have been found in rodent colon tumors induced by treatment with carcinogen (92). In humans, COX-1 can be detected in both normal and tumor colonic tissue, while COX-2, is rarely detectable in normal colonic tissue (93, 94). In a study by Eberhart *et al* (95), COX-1 and COX-2 mRNA levels were measured in normal human colonic mucosa from patients with tumors and in their polyps or carcinomas. COX-2 was increased over normal in 12 of the 14 carcinomas and in half of the adenomas. On the other hand, the levels of COX-1 mRNA were essentially the same in normal and tumor tissues. In another study by Kargman *et al* (94), similar results were reported although the percentage of colon cancers with increased COX-2 expression was lower (19 out of 25 tumors). These and other studies have contributed to the concept that COX-2 expression is responsible for the progressive increase in PGE_2 synthesis in colorectal neoplasia (96).

The importance of elevated COX-2 in the development of colonic tumors has been shown not only through studies using COX inhibitors, but also with a genetic model. Mutant mice carrying a germline mutation in the APC gene were generated that developed mulitple intestinal neoplasms (min) and exhibited a phenotype similar to human familial adenomatous polyposis (97). The adenomas that developed spontaneously in this mouse had a threefold higher level of mRNA and protein expression of COX-2 than normal mucosa. Immunohistochemical localization also indicated the presence of COX-2 in dysplastic foci, suggesting that COX-2 levels may be increased at an early stage in tumor development (98). Recently, the nonselective COX inhibitor, sulindac, was shown to reduce COX-2 expression and to inhibit tumor formation in the min mouse (99). In another min mouse study a COX-2 selective inhibitor was shown to be very effective in reducing the number of polyps (100). An even more convincing demonstration of the contribution of COX-2 to tumor development was the introduction of a knockout mutation of the COX-2 gene into APC knockout mice. These animals exhibited a dramatically reduced number of spontaneous tumors in a gene-dosage dependent manner, strongly suggesting that PGs play a major role in tumor development (100).

Although it is often difficult to extrapolate results from animal experiments to humans, a number of clinical trials and epidemiological studies strongly implicate PGs in the development of human colon cancer. Sulindac substantially reduces the number and size of rectal polyps in

individuals with familial polyposis. Nevertheless, when sulindac administration was discontinued, tumors recurred (101-103). These polyp regression studies indicate that sulindac is inhibiting a process associated with preneoplastic colonic tumor development. Sulindac was shown to reduce PG synthetic capacity in colonic mucosal samples from patients on long-term sulindac therapy (102). However, in another study, with a small number of patients, the COX inhibitor, indomethacin, did not induce regression of polyps (104). This discrepancy has been explained by differences in the pharmacokinetics of sulindac and indomethacin (3). Relatively high concentrations of sulindac metabolites are found in the colon while indomethacin is primarily excreted in the urine, with little amounts reaching the colon (105).

Numerous epidemiologic studies looked for an association between chronic aspirin ingestion and reduced incidence of colon cancer. In one of the earliest reports Kane *et al* (106) found that in a case-control study (n=715) regular aspirin intake was associated with a nearly 50% reduction in incidence of colon cancer. Rosenberg *et al* (107) reported similar findings in an even larger study (n=1326). One of the more recent studies, by Thun *et al* (108), examined mortality from colon cancer. They reported that regular aspirin ingestion was associated with a 50% reduction in colon cancer deaths over a six year follow-up period. There are some caveats in drawing strong conclusions from this study, however. There was little association between the use of acetaminophen and fatal colon cancer even though acetaminophen is a moderate inhibitor of PG synthesis. It was suggested that possibly aspirin induced bleeding, leading to earlier diagnosis and improved survival (108). Greenberg *et al* (109) showed that the protective effect of aspirin was not influenced by the number of prior adenomas and was the same among men and women. Suh *et al* (110) further showed a frequency-of-use effect, i.e., consumption of aspirin several times a day was more protective than less frequent use. Giovannucio *et al* (111) reported that use of aspirin $\geq$ 2 times per week resulted in a reduced risk of total and advanced (metastatic and fatal) colorectal cancers in male health professionals. The issue of dose was also addressed by Gann *et al* (112) who observed that regular use of aspirin at low doses, that recommended for prevention of myocardial infarction, did not reduce the incidence of colon cancer during a five year randomized treatment trial. In disagreement with the above reports, a small study (n=181) by Paganini-Hill *et al* (113) found that there was a 50% increased risk of colon cancer among daily aspirin users. Overall, however, the large majority of these epidemiologic studies and clinical trials show that regular use of aspirin is associated with significant reduction in risk of development of colorectal cancer. This protective effect was originally thought to be limited to the gastrointestinal tract in that little association of aspirin and reduced risk of development of cancers of lung, breast, endometrium, ovary, testis, bladder, lymph nodes, hematopoietic cells or melanocytes were reported by Rosenberg *et al* (107). However, a more recent study found a significant inverse association with cancers of all sites (114).

4.3. Tumors of the Breast

The role of PGs has also been evaluated, in both rodents and humans, in tissues and tumors other than those from the skin and colon, notably the breast. Many of these studies are contradictory, however. For example, although, in carcinogen-induced rat mammary tumors, the PGE_2 content was elevated (115) and could be reduced by indomethacin, indomethacin was reported to have either no effect (116), an inhibitory effect only with diets high in linoleate (117), or to promote tumor proliferation, resulting in increased tumor size (118). The level of PGs in human breast tissue and tumors has also been determined by several laboratories (119-121). As with the rat mammary carcinogenesis model, some contradictions have been noted which have been attributed, at least in part, to the lack of standardization of methodologies (121). The majority of the PGE_2 appears to be synthesized by non-epithelial cells, particularly fibroblasts (122). It has also been suggested that the elevated PG production could be used as a marker of high metastatic potential for breast cancer (119, 123). COX expression levels have been reported for two human breast cancer cell lines, estrogen-dependent MCF-7 and estrogen-independent and aggressive, MDA-MB-231, cells. MCF-7 cells had high COX-1 but barely detectable COX-2 expression; the reverse was seen in the MDA-MB-231 cells. The high PG synthesis by the MDA-MB-231 cells was suggested to be causatively associated with their highly invasive and metastatic phenotype (124). However, another study found no significant correlation between estrogen receptor status, tumor size, lymph node involvement and length of the post-treatment disease-free state, suggesting that PG levels are not of prognostic value (125).

Strong support for a role of PGs in human breast cancer comes from a recent epidemiology study. This case-control study compared 511 cases of breast cancer with 1534 control subjects and found that the relative risk of breast cancer was reduced by the use of NSAIDs (126). A greater risk reduction was seen with more frequent use (daily for $\geq$ five years). It is likely that this study will provide impetus for further investigation into the regulation of COX expression in breast tissue and the function of PGs in breast tumor development.

5. MECHANISMS OF PROSTAGLANDIN ACTION

One of the primary approaches to the understanding of the function of PGs in any tissue has been through the use of inhibitors of COX activity. The NSAIDs that have most commonly been used in *in vitro* and *in vivo* studies include drugs such as aspirin, indomethacin, sulindac, ibuprofen and piroxicam, all of which inhibit PG synthesis albeit by different mechanisms. Aspirin inhibits the COX but not peroxidase activities of both COX-1 and COX-2 by acetylating a particular serine and thus blocking the channel that leads to the active site. This results in irreversible inhibition of PG synthesis although a concurrent increase in 15(R)-HETE occurs, particularly with COX-2 (127,128). Indomethacin forms a tight, slowly dissociable complex with COX that

induces an inhibitory conformational change (129). Ibuprofen and piroxicam, on the other hand, compete with arachidonic acid for the active site (130, 131). 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 (132).

The recent demonstration that COX-2 is the inducible isoform and that its high expression occurs at sites of inflammation has spurred the development of isoform specific inhibitors. An additional impetus is the observation that inhibition of PG production, via inhibition of both COX-1 and COX-2, can lead to gastrointestinal lesions and nephrotoxicity. Thus, it was viewed as desirable to reduce the inflammatory effects of COX-2 while retaining the cytoprotective effects of COX-1 (133). As a result, a variety of COX-2 specific inhibitors have recently become available for testing One of the first inhibitors, NS-398, has an IC_{50} of 3.8×10^{-6} M *in vitro* for COX-2 and exerts no effect on COX-1 at 10^{-4} M (134). *In vivo*, NS-398 blocked COX-2 expression in inflammatory cells induced by exogenous stimuli and completely inhibited PG synthesis from these cells whereas it did not not affect PG production from COX-1 in the stomach (133). NS-398 and another COX-2 selective inhibitor, DuP697, cause conformational changes in COX-2 that lead to irreversible loss of activity (135). CGP28238 and L-745,337 also exhibit a dramatic COX-2 selectivity when tested on several cell types and in *in vivo* models of inflammation (136, 137). Together, these pharmaceutical studies strongly support the hypothesis that COX-1 is utilized for cytoprotection and that COX-2 primarily plays a pro-inflammatory role. However, the recent generation of knockout mice for COX-1 and COX-2 suggest that this view may be too simplistic. Contrary to the prediction that COX-1 knockouts would have spontaneous gastrointestinal ulcerations, this pathology was not found. Furthermore, these mice were less sensitive than normal mice to the ulcerating effect of indomethacin (138). The COX-2 knockout mice also produced unexpected results. In these animals topical TPA treatment caused edema to the same extent as in wild type mice (139). In keeping with high PG levels, COX-2 knockouts, however, did show a reduced endotoxin-induced hepatocellular cytotoxicity (140). It is clear from these animal models that, to a large extent, the tissue specific function of COX-1 and COX-2 remains undefined.

5.1. Cell Proliferation

Prostaglandins have many biological effects on different tissues and thus act as endogenous biological modifiers. As mentioned above, specific receptors have been identified for each of the major PGs, as well as for prostacyclin and thromboxane (47), which are coupled to different signal transduction pathways (47, 53). The rapid metabolic breakdown in the circulation suggests that PGs are not classical hormones but act as autocrine or paracrine factors within a given tissue (141). In spite of extensive research on the effects of specific eicosanoids, little is known about their mechanism(s) of action at the cellular and molecular level. For most tissues, it is also not clear whether the effect on a given tissue of a particular PG is the same for normal and malignant cells.

For some, but not all cells, a link has been established between synthesis of PGs and control of cell growth. In Balb/c 3T3 cells, epidermal growth factor-dependent proliferation is inhibited by the COX inhibitor, indomethacin (142). In these cells, PGF_{2alpha} is not a mitogen by itself but acts a permissive factor that allows the mitogenic action of a growth factor. PGF_{2alpha} stimulates proliferation of osteoblast, MC3T3-E1, cells by increasing the number of high affinity binding sites for insulin-like growth factor-1 (143). Thromboxane A_2 has also been shown to stimulate mammary epithelial cell growth (144).

Normal murine epidermal cell proliferation does not appear to depend on PGs. This concept is based on the inability of PGE_2 or PGF_{2alpha} or their more stable, 15(S)-15-methyl, derivatives to stimulate DNA synthesis and the lack of inhibition of normal proliferation by the PG synthetase inhibitor, indomethacin (145-147). In human keratinocyte cultures, however, PGs do appear to be required for normal cell proliferation. A correlation was observed between PGE_2 production and cell proliferation and this proliferation was inhibited by indomethacin in a manner that could be overcome by addition of PGE_2 but not PGF_{2alpha} (148). In rat skin, topical administration of PGE_1, PGE_2 and PGF_{2alpha} produced marked increases in DNA, RNA and protein synthesis, further suggesting that PGs are regulatory factors in epidermal cells (149). Unlike in normal mouse skin, *in vivo* topical application of TPA (and other irritants) results in a proliferation that is dependent on specific PGs. Indomethacin was shown to inhibit TPA-induced epidermal hyperproliferation and this inhibition could be overcome by topical application of PGE_2 but not PGF_{2alpha} (145, 146, 150). In addition, although not mitogenic by itself, PGE_2 is co-mitogenic when applied with TPA (151).

With regards to colon, two transformed human colon cancer cell lines were recently evaluated. The *in vivo* growth and *in vitro* colony formation of one cell line, HCA-7, which constitutively expresses high levels of COX-2 protein was inhibited by the selective COX-2 inhibitor, SC-58125. The other cell line, HCT-116, however, does not express COX-2 protein and is not growth inhibited by SC-58125. This study suggests that SC-58125 is acting in a specific manner rather than via nonspecific cytostatic mechanisms and that PGs contribute to the growth of some, but not all, colon cancers (152). It was also noted that the responding HCA-7 cells are more differentiated than the HCT-116 cells, which may be an important determinant (152). The response of two human colon adenocarcinoma cell lines, SW111 and HT-29, to the proliferative or apoptotic effects of a series of eicosanoids was compared. While HT-29 cells showed increased proliferation in response to PGE_2 and leukotriene B_4, SW111 cells also responded to PGI_2 and PGF_{2alpha}, although neither distribution of cells in the cell cycle nor rate of spontaneous apoptosis was altered (153). On the other hand, the NSAID,

sulindac, reduced the proliferation rate of HT-29 cells and increased the rate of apoptosis (154). Other NSAIDs such as aspirin, indomethacin and piroxicam also reduced cell cycling and all except aspirin induced apoptosis in these cell lines (155). In addition, another study suggests that COX-2 participates in the proliferation of colon cancer cells. Several gastrointestinal cancer cell lines that highly expressed COX-2 were inhibited by both COX-2 selective and nonselective inhibitors whereas those cell lines with low COX-2 expression showed minimal response (156).

The growth stimulating effects of PGs in many cases appear to be linked to other biological modifiers, particularly the polyamines. Elevated polyamines are associated with an increase in DNA synthesis and result from induced ornithine decarboxylase (ODC) activity. Tumor promoters, both exogenous and endogenous (bile salts) induce the synthesis of ODC in a PGE_2-dependent process, based on inhibition by indomethacin and restoration by exogenous PGE_2 (157). In this respect, the colonic epithelium responds in a manner similar to murine epidermis, a tissue in which tumor promoter-induction of ODC is also PGE_2 dependent (71). In the skin multistage carcinogenesis model, ODC induction is required for tumor development; inhibitors of arachidonic acid metabolism inhibit both ODC induction and tumor development (158). Therefore, it seems that a clear link exists between PGE_2, enhanced proliferation and tumor development.

In addition to effects on proliferation, PGs are associated with enhanced invasive and metastatic potentials of tumor cells. Recently, several studies have reported that increasing the expression of COX-2 was associated with alterations in cell behavior. When rat intestinal epithelial cells were transfected with a COX-2 expression vector, they showed enhanced cell adhesion due to upregulated E-cadherin expression; this was reversed by addition of sulindac sulfide (159). When human colon cancer cell line, Caco-2, was transfected with a COX-2 vector, they exhibited an enhanced invasive phenotype that was associated with an elevated expression of membrane metalloproteinase. Sulindac sulfoxide reversed both the increased invasiveness and PG synthesis (156).

5.2. Role in Apoptosis

The biochemical basis for the antineoplastic characteristics of NSAIDs has generally been attributed to their ability to reduce PG levels by inhibiting COX. However, this category of drugs may have other effects that are responsible for, or at least contribute to, their anti-cancer properties. Studies using cultured tumor cell lines have shown that a variety of NSAIDs can induce apoptosis (160-164). Apoptosis, frequently referred to as programmed cell death, is a specific morphological and biochemical form of cell suicide. The apoptotic pathway can be triggered by extracellular agents, pathological processes and also occurs during normal development and tissue remodeling. Apoptosis differs from necrosis in that the apoptotic cell kills itself in manner that does not harm neighboring cells nor elicits an inflammatory response. Activation of apoptosis in tumors has thus been a target for some chemotherapies (165).

Although NSAIDs can both inhibit PG synthesis and induce apoptosis, there is recent evidence that the apoptotic effects is likely not due to COX inhibition. The most striking example comes from studies with sulindac and its metabolites. Sulindac sulfoxide is consumed as a prodrug that has no COX inhibitory activity. It is readily reduced to the sulfide form in the liver and in the colon via bacterial microflora; it is this sulfide form that is responsible for the anti-inflammatory characteristics of sulindac (166, 167). However, sulindac sulfoxide is also oxidized in an irreversible manner in the liver to a sulfone that is excreted in the bile and intestine. The sulfone metabolite is devoid of COX inhibitory or other anti-inflammatory activity (168) although it still retains the ability to inhibit tumor cell growth and induce apoptosis (161). Thompson *et al* (168) were the first to report that the sulfone metabolite has cancer chemoprotective activity. In a chemically induced rat mammary cancer model, sulindac sulfone was found to be comparable to sulindac sulfoxide in reducing tumor incidence and numbers and in inducing apoptosis (168). Sulindac sulfone also inhibits formation of aberrant crypts in a rodent colon carcinogenesis model and recently was shown to inhibit azoxymethane-induced colon carcinogenesis in rats (160, 169). In a recent study, it was also shown that in addition to having no COX-inhibitory activity, the sulfone metabolite does not inhibit lipoxygenases or phospholipase A_2 (170).

The relationship between NSAIDs, PGs and apoptosis was further explored in the HT-29 colon adenocarcinoma cells which expresses COX and synthesizes PGs and in the HT-15 carcinoma cell line which does not exhibit these properties. Addition of PGE_2 to HT-15 cells enhanced proliferation only slightly (25%); addition of sulindac or piroxicam severely reduced proliferation and this could not be reversed by addition of PGs. The PG producing HT-29 cells were also growth inhibited by sulindac and piroxicam but unlike similar experiments described above for indomethacin treatment of keratinocytes, growth could not be restored by addition of PGs. The conclusion drawn is that these NSAIDs reduce proliferation in human colonic cells through a PG-independent mechanism (171).

These and related studies have raised the question of exactly how the NSAIDs exert their anti-proliferative effects. As reviewed by Abramson and Weissmann (172), NSAIDs affect a variety of membrane processes not related to PG synthesis. These include inhibition of superoxide anion generation by NADPH oxidase, phospholipase C and 12-HPETE peroxidase and coupling of mitochondrial oxidative phosphorylation (172). The ability of NSAIDs to have biological activity independent of PGs was clearly demonstrated with the drug, flurbiprofen. As a chiral compound, it can exist in the R and S enantiomeric forms. The S enantiomer was shown to be 500 fold more potent than

the R form in inhibiting COX-1 and COX-2. However, when both forms were tested for anti-proliferative activity in the colon, they were equally potent. Although the R form has not been tested in a carcinogenesis model, it has been suggested that it could a be useful chemopreventive agent that is nonulcerogenic and does not interfere with normal PG production (4).

5.3. Carcinogen Metabolism

Besides metabolizing arachidonic acid, the peroxidase component of PG synthetase can, by a co-oxidation reaction, oxidize a wide range of xenobiotics, including several classes of carcinogens (5, 173, 174). Among these, heterocyclic and aromatic amines and dihydrodiol derivatives of polycyclic hydrocarbons are activated to mutagenic derivatives by PG synthetase (173, 175). These oxidations are inhibited by aspirin by preventing cyclooxygenase-catalyzed generation of the hydroperoxide substrate for the peroxidase. Notable examples of this extrahepatic metabolism include the urinary tract carcinogens benzidine and the nitrofuran derivative (N-[4,5-nitro-2-furyl]-2-thiazole) formamide (FANFT), both of which are activated by renal COX (176). FANFT-induced bladder tumors are prevented by administration of aspirin (177). With regards to the colon, COX can activate the heterocyclic aromatic amine IQ, found in food, to mutagens and the NSAID indomethacin blocks both activation and tumors induced by IQ (178). Thus, inhibiting COX may have a direct preventive effect on colon cancer which is independent of the level of PGE_2.

Prostaglandins may participate in carcinogenesis via the generation of peroxyl radicals. Peroxyl radicals are amongst the more stable oxy radicals, which also allows for greater diffusion from the site of formation. Peroxyl radicals can, for example, cause epoxidation of the procarcinogen benzo[a]pyrene 7,8-diol to its ultimate carcinogenic form, a diol epoxide. Peroxyl radicals can also epoxidize other carcinogens, such as 3,4-dihydroxy-3,4dihydrobenzo[a]anthracene and aflatoxin B1 to their ultimate carcinogenic forms. Because differences in the relative amounts of DNA adducts generated from peroxyl radicals or cytochrome P450 can be measured, the relative contribution of COX activity can be determined. In mouse skin, peroxyl radicals play a significant role in the metabolism of benzo[a]pyrene 7,8-diol (179). This is likely to be true for a number of carcinogens and in other tissues as well.

Prostaglandins may also contribute more directly to increased DNA damage and mutations via the non-enyzmatic breakdown of PGH to malondialdehyde. Thromboxane sythase also carries out this conversion enzymatically (5). MDA binds to DNA and produces a diversity of base-pair substitutions and frameshift mutations which has led to the suggestion that it may be an important contributor to endogenous mutagenesis (180).

5.4. Immune Modulation

High levels of PGE_2 have been shown to suppress immune surveillance (181-183) and to impair killing of malignant cells (184, 185). These effects appear to be specific to PGE_2 since other eicosanoids have not been shown to have a clear role in the regulation of cellular and humoral immune responses (186). In support of a suppressive effect of PGE_2 is the observation that drugs that inhibit PG synthesis enhance immune responses (184-186). PGE_2 can regulate immune function by acting as a negative feedback inhibitor for such processes as T cell proliferation, lymphokine production and cytotoxicity and for macrophage and natural killer cell cytotoxicity (183, 187). Since the growth of various tumors is associated with immune suppression in animals and humans (181, 188), inhibition of COX may reduce tumorigenesis. This tumor-associated immune suppression may occur as a result of factors such as colony stimulating factor which is released by tumor cells and causes monocytes and macrophages to synthesize PGE_2; this elevated PGE_2 inhibits blastogenesis of T cells and the cytotoxic activity of natural killer cells (188, 189). Examples include the use of indomethacin to reduce the size of tibial bone tumors in maloney sarcoma virus infected mice (190). Indomethacin was also shown to augment the impact of BCG treatment in mice with 3-methylcholanthrene induced fibrosarcomas, which produce large amounts of PGs (191). Conversely, administration of PGE_2 and $PGF_{2\ alpha}$ to syngeneic mice bearing transplanted squamous cell carcinomas enhanced the transplantability of these tumors (192).

Although the growth of some transplanted tumors is reduced by COX inhibitors, when given soon after transplantation, the inhibitory effect is lost over time. At this point, macrophages have been shown to lose their ability to synthesize PGE_2 and their immunosuppressive activity (193). A second phase of immunosuppression ensues that results not from PG production but from enhanced production of bone-marrow derived monocyte-like suppressor cells (193, 194). Thus, while COX inhibitors may be good agents to inhibit initial tumor development, do not seem to be effective chemotherapeutic agents against existing large tumor burdens.

6. SUMMARY

The study of involvement of PGs in the pathogenesis and progression of cancer is currently a lively field of research. The evidence weighs heavily in favor of such a role in many types of cancer. This is based on the observation of high PG levels and elevated expression of synthetic enzymes in various types of tumors. Such a role for PGs is also supported by the numerous animal investigations and human epidemiological studies showing that NSAIDs prevent development of cancer. However, the recent demonstration that these drugs cause apoptosis, and that related anti-cancer drugs that also cause apoptosis do not inhibit PG synthesis, suggest that the story is far from clear. Hopefully, the recently developed knockout and transgenic mouse models will provide the definitive information that is

needed. A clearer demonstration that PG synthesis contributes to the development and/or growth of several types of malignancies offers the opportunity for improved approaches to the prevention of cancer.

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