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Review Article

A novel approach to anticancer therapies for prostate cancer: lipoxygenase as a new target in the treatment of prostate cancer

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CONTENTS

Abstract

Prostate cancer differs from other urinary tract tumors in that it is hormone-dependent, although angiogenic factors play a significant role in the metastasis of both prostate cancer and tumors of other organs. The metabolism of arachidonic acid (AA) by either a cyclooxygenase (COX) or lipoxygenase (LOX) pathway is considered an important factor in tumor promotion. In this review, we investigated the expression of COX and LOX (5- and 12-LOX) in prostate cancer and the effects of COX and LOX inhibitors. Our findings demonstrated that cell growth and apoptosis of human prostate cancer cells are regulated by LOX pathways. Inhibiting the growth of prostate cancer cells by blocking LOX pathways was associated with induction of apoptosis. Data also support the evidence that a 5-LOX inhibitor is significantly more effective than a 12-LOX inhibitor in preventing cancer cell growth in the prostate, as the 5-LOX pathway is more closely associated with carcinogenesis than the 12-LOX pathway. Downregulation of the AA-metabolizing LOX enzymes therefore provides a novel approach to anticancer therapy.

Introduction

Prostate cancer comprises 32% of all cancers in American men and is on the increase worldwide. Because of increased screening, prostate cancer is frequently diagnosed at a clinically localized stage, making it amenable to therapy. Nevertheless, it remains the second most common cause of cancer death in men. A substantial subset of patients with clinically localized prostate cancer will relapse after local therapy, and up to 20% of all patients still present initially with metastatic disease. These patients generally respond to androgen deprivation therapy, but the vast majority eventually experience disease progression and become refractory to sustained hormonal manipulation. Typically, such patients progress with a rise in their serum prostate-specific antigen (PSA) level, and months later show systemic symptoms such as weight loss, fatigue and complications from metastases, such as bone pain, cord compression or urinary obstruction. Unfortunately, standard therapeutic options at this stage of disease are limited, and while there has been some success with chemotherapy for hormone-refractory prostate cancer (HRPC) patients, the response is generally short-lived (1).

Angiogenic factors play an important role in this organ as in other organs (2), and although various potential angiogenic factors have been identified in prostate cancer, it is still unclear by which process prostate cancer cells become angiogenic. Thus, the challenge is to discover new treatment strategies that target androgen-independent prostate cancer. The identification of molecular targets involved in the tumorigenesis and progression of prostate neoplasms provides opportunities for the development of new agents with greater therapeutic potential and better specificity. Patients with advanced or recurrent disease that has progressed to a hormone-refractory state are suitable candidates for studies testing the efficacy of these new agents. An alternative strategy is to study agents with novel molecular targets prior to local

therapy in patients with clinically localized prostate cancer. This approach provides the advantage of rapid evaluation of both pathological and clinical endpoints. Agents that show promise in this setting can then undergo more traditional testing in a randomized phase II or III adjuvant study. Biological correlations can also be evaluated since tissue is obtained for study when definitive local therapy is given. Moreover, if the agent is effective, it can render the patient more likely to be surgically cured. Finally, this setting facilitates clinical development since fewer patients are required to establish the activity of the agent.

Although few new compounds have progressed to phase III clinical trials, significant strides have been made in understanding the biology of this disease, and numerous functional targets have been identified for exploration. This review describes a novel approach to anticancer therapies for prostate cancer using lipoxygenase inhibitors.

Hormonal manipulation

Androgen deprivation therapy usually provides only temporary control of advanced prostate cancer. It is unclear if this is because hormone-insensitive cells are present in small numbers from the onset, or because they develop after prolonged treatment (3). Although HRPC is associated with amplification and overexpression of androgen receptors (AR) (4), it is likely that alternative signaling pathways are operative for continued growth. These pathways are crucial for the progression from an androgen-dependent to an androgen-independent state and are promising targets for future drug development.

In addition to independent effects on growth and development, many distinct signal transduction pathways transactivate with the AR pathway. For instance, epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I) and keratinocyte growth factor (KGF) all have a role in the expression or transactivation of the AR in HRPC cells (5). Also, interaction of the AR with HER-2/neu (6) and protein kinase A (PKA) signaling (7, 8) has been noted independent of androgen stimulation. Finally, multiple effectors of mitogen-activated protein kinase (MAPK) signaling, such as HER-2/neu (9), EGF (10), IGF-I and Janus kinase signal transducers and activators of transcription (JAK-STAT) (11), have been implicated in AR activation, providing further evidence that other receptors and pathways are closely tied to the AR signaling pathway.

For these reasons, several novel strategies/targets for the treatment of prostate cancer are under study, including differentiation therapy (vitamin D, peroxisome proliferator-activated receptor γ [PPARγ]), growth factor receptors (EGF, HER-2/neu, platelet-derived growth factor [PDGF]), intracellular pathways, apoptosis (cyclooxgenase type 2 [COX-2], lipoxygenase [LOX]) and others.

Arachidonic acid

It has been reported recently that patients using nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin have a significantly lower risk of colorectal cancer. Consequently, the suppression of carcinogenesis using NSAIDs has been proposed. It has also been reported that the size and number of adenomas were markedly reduced when the NSAID sulindac was given to patients with familial adenomatous polyposis (FAP), which is considered a high-risk group for colorectal cancer (12).

The metabolism of arachidonic acid (AA) by either the COX pathway or the LOX pathway generates eicosanoids, which have been implicated in the pathogenesis of a variety of human diseases including cancer and have a significant role in tumor promotion, progression and metastasis. Arachidonic acid is metabolized to produce a host of proinflammatory substrates, called eicosanoids, through pathways involving LOX, including 5-LOX, 12-LOX and 15-LOX, and COX (COX-1 and COX-2) (13, 14). The LOXs convert arachidonic, linoleic and other polyunsaturated fatty acids to biologically active metabolites that influence cell signaling, structure and metabolism (15). 5-, 12- and 15-LOX are considered the main LOX isoforms catalyzing the biosynthesis of biologically active compounds such as leukotrienes and hydroxyeicosatetraenoic acids (HETEs). One group of LOXs, including 5- and 12-LOX, has a procarcinogenic role and appears to work primarily within the AA pathway. On the other hand, two isozymes of 15-LOX have anticarcinogenic activity and act either on the linoleic or the arachidonic acid pathway. Among the LOX pathways, 5-LOX catalyzes the first step in the oxygenation of AA to produce 5-hydroperoxyeicosatetraenoic acid (5-HPETE), with the subsequent production of 5-HETE and leukotrienes (13, 14). 12-LOX, including platelet and leukocyte 12-LOX, oxygenates AA at position C-12 to produce 12-HPETE and then 12-HETE.

The expression of 5- or 12-LOX mRNA and protein has been detected in various types of cancer cell lines (16). 5- Or 12-HETE promotes several tumorigenic events such as invasion and metastasis (17), increasing the adhesion of tumor cells to the matrix protein fibronectin (18) and microvessel endothelium (19), enhancing cell migration during tumorigenesis (20) and promoting tumor spread (21). Findings have indicated that increased levels of 5- and 12-HETE stimulate the growth of lung and colon tumor cells (22).

We have therefore examined the expression of 5- and 12-LOX in human prostate cancer tissues and prostate cancer cell lines, the effect of LOX inhibitors on human prostate cancer cell lines, and whether or not LOX inhibitors induce apoptosis of prostate cancer cells.

Expression of lipoxygenase in the prostate

The LOX-catalyzed products play important roles in the pathophysiology of inflammation and carcinogenesis

(23). Increased levels of LOX metabolites have been found in patients with various forms of cancer, including lung, breast, colon, prostate and skin cancer (22) and leukemia (24). Studies have shown a marked increase in the activity of 5-LOX in lung tumor cells (25) and colon tumors (22), as well as dramatically increased levels of 5-HETE, sufficient to support the proliferation of prostate cancer cells, with the addition of AA to both hormone-responsive and -unresponsive human prostate cancer cells (26). Additional studies showing reduced DNA synthesis and growth inhibition of prostate cancer cells with specific 5-LOX inhibitors support the involvement of the LOX pathway in prostate cancer. Increased 5-LOX activity and 5-HETE levels are also associated with the development and progression of several tumor types, including prostate cancer. The AA metabolite of 12-LOX, 12-HETE, is one of the major lipid metabolites influencing tumor progression, by stimulating tumor cell proliferation and motility, protecting tumor cells from apoptosis and promoting angiogenesis. Several reports have identified 12-LOX expression in a variety of tumor cells and have indicated the involvement of 12-HETE in modulating cell growth. However, the exact role of 12-LOX in human cancer cells is still unclear.

Reports have demonstrated that 12-LOX is overexpressed in tumor tissues, including prostate, breast, colorectal and lung cancer (27). Various cancer cell lines express 12-LOX and produce 12-HETE (28, 29). Moreover, 12-HETE facilitates the invasion and metastasis of tumors by several different ways, including the enhancement of tumor cell motility, proteinase secretion and angiogenesis (30, 31). Although 12-LOX expression has been detected in various epithelial cancer cell lines (colon, prostate, breast and pancreas), no information is available as to whether or not 12-LOX and its metabolites are involved in the regulation of prostate cancer cell survival. Various studies support the involvement of 12-LOX expression and function in tumor progression and/or metastasis (32). Timar *et al.* have demonstrated that metastatic prostate carcinoma cells express 12-LOX at a significantly higher level compared with nonmetastatic counterparts, and that lung colonization *in vivo* may be suppressed by 12-LOX inhibitors (21).

We have demonstrated that 5- and 12-LOX are strongly expressed in prostate cancer. Total RNA isolated from prostate cancer cells was subjected to reverse transcription and nested PCR amplification with primers specific for 5-LOX and platelet-type 12-LOX. The human prostate cancer cell lines DU 145 and PC-3 expressed significant and strong 5- and 12-LOX mRNA bands, while normal prostate cells expressed no 5-LOX mRNA band and a very weak 12-LOX mRNA band (Fig. 1).

Immunohistochemistry was performed in order to examine the expression and localization of 5- and 12-LOX in normal prostate, benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia and prostate cancer tissues. Very weak expression of both immunoreactive 5- and 12-LOX was found in BPH and normal prostate tissues, whereas strong expression in cancer

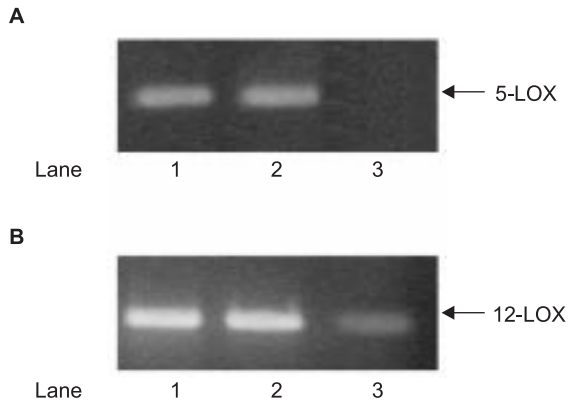

Fig. 1. Nested RT-PCR of 5- and 12-LOX. Human prostate cancer cell lines DU 145 and PC-3 expressed significant and strong 5- and 12-LOX mRNA bands (**A**: 5-LOX; **B**: 12-LOX. Lane 1: DU 145; Lane 2: PC-3), while normal prostate cells expressed no 5-LOX mRNA (**A**; Lane 3) and a very weak 12-LOX mRNA band (**B**; Lane 3).

tissues, including nuclei and cytoplasm (Fig. 2). Thus, 5- and 12-LOX immunostaining of epithelium was significantly more extensive and intense in prostate cancer and prostatic intraepithelial neoplasia tissues than in tissues of BPH and normal prostate. Staining for 5- and 12-LOX was also high in blood vessels and stromal tissues of prostate cancer and prostatic intraepithelial neoplasia, whereas the expression of 5- and 12-LOX in blood vessels and stromal tissues from BPH and normal prostate was low.

Effects of lipoxygenase inhibitors

To investigate the effects of 5- and 12-LOX inhibitors on prostate cancer cell proliferation, we analyzed cell viability *in vitro* using a modified MTT assay. Although the inhibitors (caffeic acid, baicalein, NDGA) had no effect on normal prostate cell proliferation, they reduced cancer cell viability, with half-maximal concentrations for growth inhibition of prostate cancer cell lines (Table I) in the range of 10-80 μM. Furthermore, counting cells at days 1, 2 and 3 clearly showed marked inhibition of cell proliferation using 50 μM of caffeic acid, baicalein or NDGA (Table II). All LOX inhibitors arrested the growth of prostate cancer cells. Although the effect of the nonspecific LOX inhibitor NDGA was the strongest, the effect of the 5-LOX inhibitor caffeic acid was stronger than that of the 12-LOX inhibitor baicalein.

The mechanism by which LOX inhibitors prevent growth in prostate cancer cells requires clarification. To address this problem, we examined whether or not apoptosis is involved in growth suppression in these cancer cells. To determine whether or not cell death induced by LOX inhibitors was via apoptosis, we evaluated the chromatin morphology of human prostate cancer cell

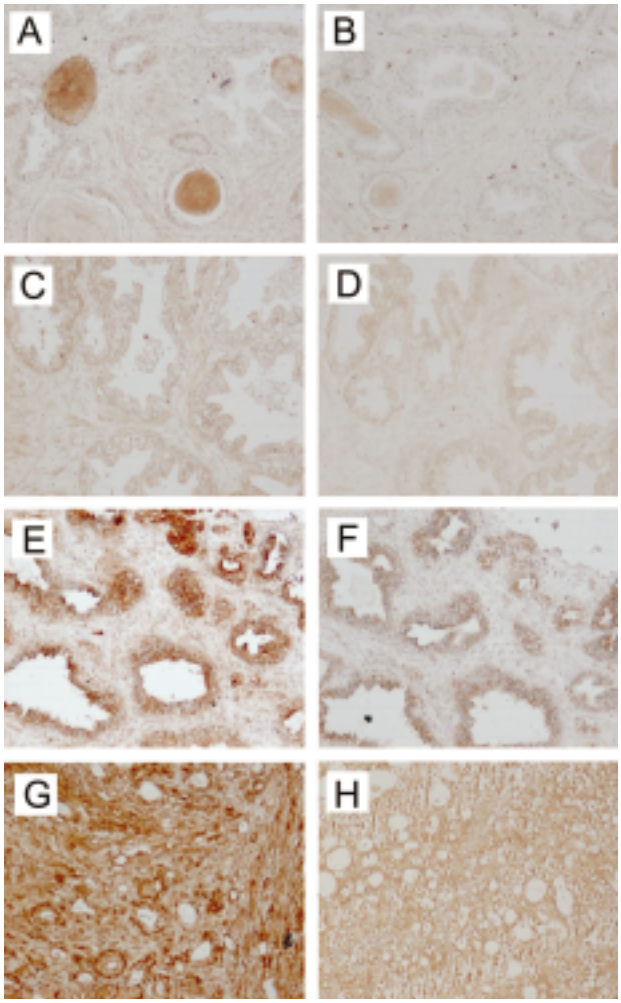

Fig. 2. Representative immunostaining for 5- and 12-LOX in prostate cancer. Very weak expression of immunoreactive 5- and 12-LOX was found in normal prostate tissues (**A**: 5-LOX; **B**: 12-LOX) and benign prostatic hyperplasia tissues (**C**: 5-LOX; **D**: 12-LOX). In contrast, we found significant expression of immunoreactive 5- and 12-LOX in cancer tissues, including nuclei and cytoplasm, in the prostatic intraepithelial cancer group (**E**: 5-LOX; **F**: 12-LOX) and all prostate cancer groups (**G**: 5-LOX; **H**: 12-LOX).

lines using Hoechst 33258 staining. Cells treated with the 5-LOX inhibitor caffeic acid and the nonspecific LOX inhibitor NDGA showed significant chromatin condensation, cellular shrinkage, small membrane-bound bodies (apoptotic bodies) and cytoplasmic condensation, changes which are typical characteristics of apoptosis. However, cells incubated with the 12-LOX inhibitor baicalein revealed only slight apoptotic changes (Fig. 3).

Expression of lipoxygenase in other cancers

LOX inhibitors also significantly inhibit the growth of other cancers such as renal cell carcinoma and bladder tumor cell lines in a concentration- and time-dependent manner. The effect of the nonspecific LOX inhibitor was the strongest, and the effect of the 5-LOX inhibitor was stronger than the 12-LOX inhibitor. Thus, it appears that blocking the 5-LOX pathway may be more effective than blocking the 12-LOX pathway for inhibiting the growth of urological cancer cells. We also examined whether or not apoptosis is involved in growth suppression in these cancer cells. At a concentration of 50 μM the LOX inhibitors induced apoptosis in urological cancer cells, providing support for the involvement of apoptosis in the cancer cell growth-inhibitory effects of 5- and 12-LOX inhibitors. These results indicate that LOX inhibitors, particularly 5-LOX inhibitors, may lead to both inhibition of the proliferation and metastasis of cancer and inhibition of cancer carcinogenesis. The data also provide evidence that cell growth and apoptosis of cancer cells are regulated by LOX pathways. These findings further suggest that 5-LOX inhibition is significantly more effective than 12-LOX inhibition in preventing cancer cell growth, due to the fact that the 5- LOX pathway is more closely associated with carcinogenesis than the 12-LOX pathway.

Cyclooxygenase type 2

The cyclooxygenase enzymes COX-1 and COX-2 are responsible for the production of prostaglandins. Initially, cell membrane phospholipid is converted to AA by phospholipase A_2 (PLA_2), followed by transformation of AA to prostaglandin H_2 (PGH_2) through PGG_2 via the COX enzymes. The activation of COX-1 is regularly maintained

Table I: Effects of LOX inhibitors (caffeic acid, baicalein and NDGA) on the viability of human prostate cancer cells, as measured by the MTT assay (expressed as % of control cultures at 48 h).

		Concentration			
Compound	Cell line	10 μM	20 μM	40 μM	80 μM
Caffeic acid	PC-3	112.5%	96.7%	78.8%	45.3%
	DU 145	80.7%	69.2%	22.2%	8.1%
Baicalein	PC-3	117.8%	100.2%	103.8%	76.5%
	DU 145	102.4%	99.1%	85.3%	63.5%
NDGA	PC-3	113.0%	101.7%	51.1%	18.5%
	DU 145	67.7%	42.3%	9.9%	5.2%

Table II: Effects of LOX inhibitors (50 μM) on growth of human prostate cancer cells (expressed as % of control cultures at 0, 24, 48 and 72 h).

		Time			
Compound	Cell line	0 h	24 h	48 h	72 h
Caffeic acid	PC-3		72.1%	28.6%	21.1%
	DU 145		52.3%	44.7%	20.8%
Baicalein	PC-3		83.9%	70.1%	65.3%
	DU 145		76.1%	73.8%	71.9%
NDGA	PC-3		27.2%	8.6%	7.5%
	DU 145		23.9%	7.9%	6.6%

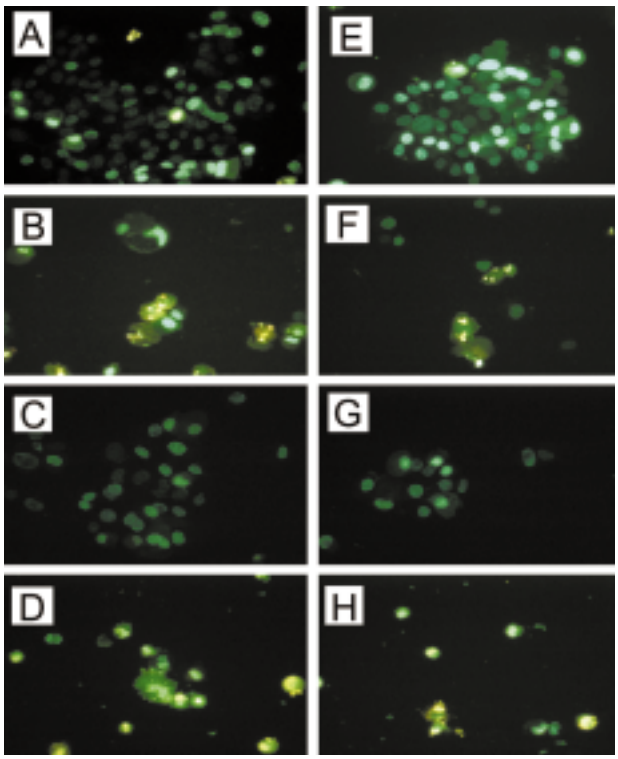

Fig. 3. Effects of LOX inhibitor on induction of apoptosis in human prostate cancer cells. Prostate cancer cells (DU 145: **A, B, C, D**; PC-3**: E, F, G, H**) treated with the 5-LOX inhibitor caffeic acid (**B, F**) and the nonspecific LOX inhibitor NDGA (**D, H**) showed chromatin condensation, cellular shrinkage, small membrane-bound bodies (apoptotic bodies) and cytoplasmic condensation. Cells incubated with the 12-LOX inhibitor baicalein showed only slight apoptotic changes (**C, G**). In contrast, untreated cells (**A, E**) maintained normal chromatin condensation.

in tissues such as blood platelets, the stomach and the kidney and is involved in the production of thromboxane A_2 (TxA_2) from platelets, PGI_2 from vascular endothelial and gastric mucosal cells, and PGE_2 from the kidney. This isozyme regulates physiological functions such as the maintenance of platelet aggregation, gastric secretion, diuresis, blood pressure and blood flow. On the other hand, COX-2 is rarely expressed under normal conditions, but rather is expressed in macrophages, fibroblasts, synovial cells, endothelial cells and neurons in response to stimulation by cytokines (IL-1, tissue factor [TF], etc.), carcinogenesis promotors (TPA and others), endotoxins and hormones. This isozyme is associated with inflammation, fever, cancer, vascularization, anaphylactic shock, childbirth, bone metabolism and apoptosis, especially inflammation. The primary difference between COX-1 and COX-2 is that the latter is induced by cytokines, growth factors and carcinogenesis and is the only isozyme that can use AA released in cells by PLA_2. Stomach ulcers, a serious side effect of NSAIDs, occur due to suppression through COX-1 inhibition of the production of PGs with gastric mucosal protective effects. The antiinflammatory effect of NSAIDs, on the other hand, is the result of suppression through COX-2 inhibition of the production of PGs involved in inflammation. Recently, selective COX-2 inhibitors have been developed and shown to suppress cancer cell proliferation with minimal side effects such as stomach ulcers and/or renal damage.

Gupta *et al*. reported strong expression of COX-2 in prostate cancer using immunohistochemical staining and RT-PCR (33). However, Kirschenbaum *et al*. reported strong expression of both COX-1 and COX-2 in prostate cancer compared with the normal prostate using immunohistochemical staining (34). We examined the expression of COX-1 and COX-2 with RT-PCR and immunohistochemical staining in surgical prostate specimens. We did not detect significant expression of COX-1 in normal prostate, BPH, prostatic intraepithelial cancer or prostate cancer tissue, whereas strong expression of COX-2 was seen in prostate cancer tissue. When we grouped the samples by epithelial, blood vessel and stromal tissue, stronger expression of COX-2 was detected in low-differentiation cancer than in high-differentiation cancer of the epithelium (35).

The degree of vascularization in tumors is a factor in the induction of COX-2 expression. Apoptosis is induced in tumor cells following exposure to inhibitors of vascularization, resulting in suppression of tumor growth. Angiogenesis factors such as vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β) are induced by COX-2 expression. Liu *et al.* inoculated human prostate cancer PC-3 cells into nude mice and suppressed VEGF production using the COX-2 inhibitor NS-398 (3 mg/kg i.p. x 3) over a 9-week observation period (36). This resulted in the induction of apoptosis.

Bcl-2 is a key factor suppressing apoptosis associated with the activation of caspase. When Hsu *et al*. applied the selective COX-2 inhibitor celecoxib to two human prostate cancer cells (PC-3, LNCaP), apoptosis was induced due to downregulation of Bcl-2 (37). Celecoxib-induced apoptosis of human prostate cancer cells was also reported to be associated with downregulation of NF-κB, as well as the activation of PPARγ, apoptosis-activating factor-1 (APAF-1) and caspase 3 (38). However, it was recently reported that, among the COX-2 inhibitors, only celecoxib induces apoptosis of cancer cells. We could not verify the induction of apoptosis after exposure to 10-80 μM celecoxib, although we did find slight suppression of bacterial growth on cancer cells using 8 different COX-2 inhibitors and human prostate cancer cell lines (PC-3, DU 145) and human renal cell carcinoma cells. Additionally, no difference was observed between selective and nonselective COX-2 inhibitors (39). Furthermore, no suppression of tumor growth was seen in nude mice inoculated with PC-3 and DU 145 tumor xenografts fed a daily diet supplemented with 10 mg/kg/day of etodolac (data not shown).

Conclusions

Enhanced expression of COX-2 and LOX in gastric and urological tumors has been demonstrated, as well as their involvement in the initiation and promotion of tumors. While it may be possible to use COX-2 and LOX inhibitors as chemopreventive agents, it may be difficult to use these compounds at clinically effective doses to suppress cancer. The clinical application of NSAIDs therefore requires further research and investigation.

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