Chapter 6
Eicosanoids in Tumor Progression and Metastasis

Sriram Krishnamoorthy and Kenneth V. Honn

Abstract Eicosanoids and the enzymes responsible for their generation in living systems are involved in the mediation of multiple physiological and pathophysiological responses. These bioactive metabolites are part of complex cascades that initiate and perpetuate several disease processes such as atherosclerosis, arthritis, neurodegenerative conditions, and cancer. The intricate role played by each of these metabolites in the initiation, progression, and metastasis of solid tumors has been a subject of intense research in the scientific community. This review summarizes some of the key aspects of eicosanoids and the associated enzymes, and the pathways they mediate in promoting tumor progression and metastasis.

Keywords Eicosanoids · cyclooxygenase · lipoxygenase · platelets · metastasis

6.1 Introduction

Eicosanoids are 20-carbon lipid molecules derived from the enzymatic breakdown of membrane lipid precursors, chiefly arachidonic acid. Mediators in this family are generally, local and short acting metabolites, that have potent and stereospecific action in a multitude of host physiological and pathological processes like inflammation, asthma, and cancer (Funk, 2001). The three major enzyme families that are actively involved in catalyzing the conversion of arachidonic acid into the bioactive eicosanoids are the cyclooxygenases (COX), the lipoxygenases (LOX), and the epoxygenases (cytochrome p450 enzyme family). Lipid mediators generated by these enzymes are involved in a wide variety of cellular and molecular pathways, including but not limited to apoptosis, cell survival, proliferation, chemotaxis, senescence etc. This review paper will chiefly focus on the molecular mechanisms involved in the regulation of tumor progression and metastasis by these mediators importantly the COX and LOX generated metabolites.

S. Krishnamoorthy
Departments of Pathology, and Chemistry, Wayne State University School of Medicine, Detroit, MI 48202

P.J. Quinn, X. Wang (eds.), *Lipids in Health and Disease*,

6.2 COX and LOX Pathways of Arachidonic Acid Metabolism

Prostaglandins, bioactive metabolites generated from arachidonic acid, were first isolated from seminal fluid in the year 1935 by a Swedish physiologist, Ulf von Euler. This discovery came decades after the analytical synthesis of aspirin, a pain killer, derived from acetyl salicylic acid, the active principle in willow bark. Aspirin and other NSAIDs commonly used in the clinic, are potent inhibitors of the COX enzyme. This enzyme is involved in the conversion of arachidonic acid into prostaglandins (Fig. 6.1A). Cellular phospholipases (PLA_2) cleave membrane phospholipids at the sn-2 position to liberate arachidonic acid which is acted upon by either COX or LOX enzymes. In the COX pathway, the arachidonic acid thus released is presented to the enzyme prostaglandin H synthase (PGHS or COX), which converts it to PGH_2. PGH_2 undergoes further metabolism to downstream products which are formed in a cell type specific fashion, depending on

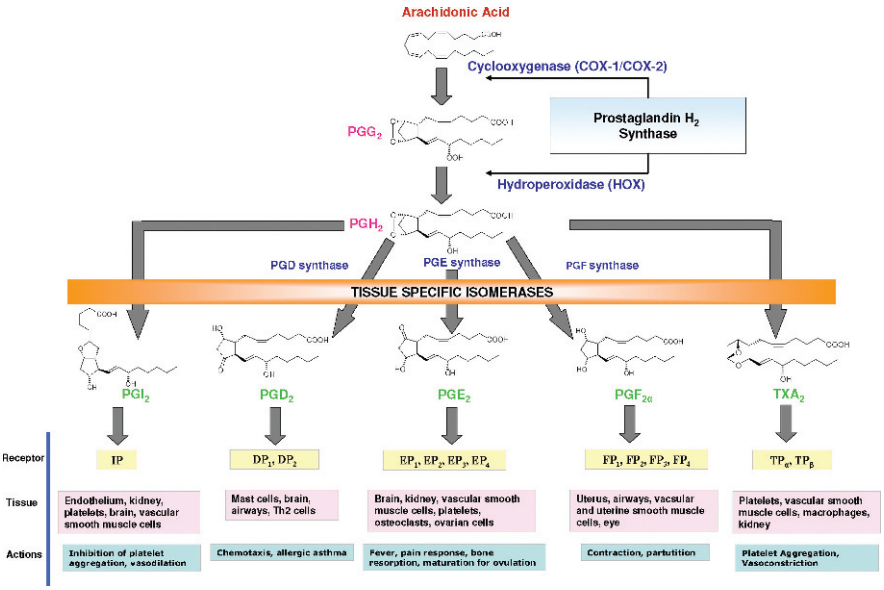

Fig. 6.1 (**A**) Cyclooxygenase pathway of arachidonic acid metabolism. Arachidonic acid generated from cell membrane phospholipids, is acted upon by COX-1 or COX-2 to generate PGG_2 and subsequently PGH_2. Tissue specific isomerases take up PGH_2, and convert it into respective lipid mediators, for example PGE_2 synthase converts PGH_2 to PGE_2. Each of these lipid mediators have specific cell surface receptor(s), which relay signals to the interior of the cell resulting in physiological or pathophysiological consequences. (**B**) Lipoxygenase pathway of arachidonic acid metabolism. In this pathway, arachidonic acid is converted chiefly to the HETEs or the 5-LOX products, leukotrienes, in a cell or tissue specific manner. For example, platelets are abundant in 12-LOX, and produce 12-HETE. Resulting lipid mediators, have several cell type specific actions in both physiological and pathophysiological contexts

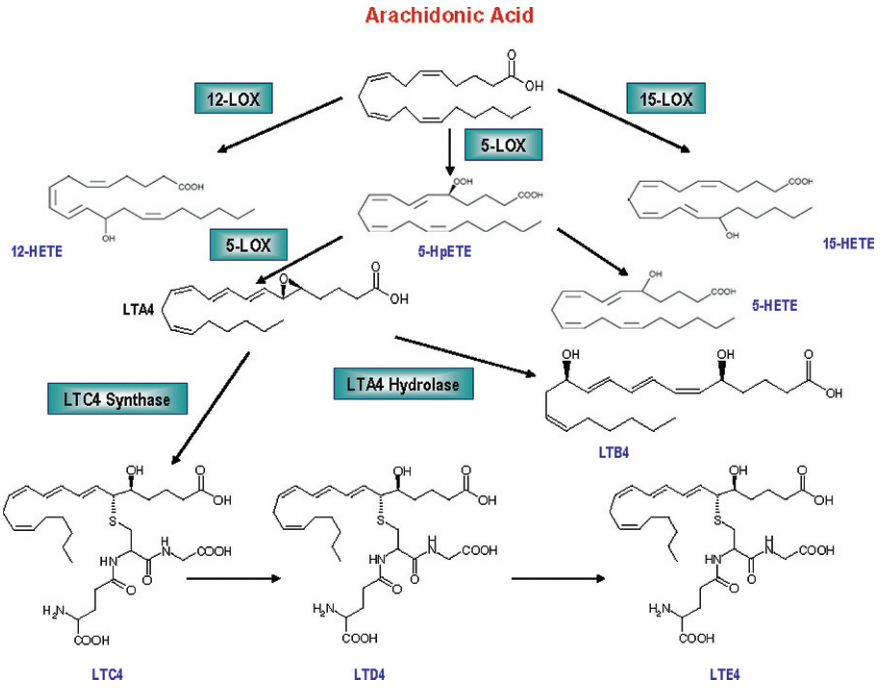

Fig. 6.1 (Continued)

the presence of the enzyme in these cell types. For example, mast cells and brain convert PGH_2 to PGD_2 using the PGD synthase; platelets harbor the enzyme thromboxane synthase and produce thromboxanes from PGH_2; endothelial cells utilize arachidonic acid to form prostacyclin, catalyzed by prostacyclin synthase; uterine cells form $PGF_2\alpha$. Microsomal PGE_2 synthase enzyme is responsible for the formation of PGE_2 from PGH_2.

Conversely, arachidonic acid is also metabolized by various enzymes of the LOX pathway (Fig. 6.1B), to produce bioactive lipid mediators. The chief enzymes of the LOX pathway are the 5-LOX, 12-LOX, and the 15-LOX enzymes, whose names are derived from the position in which molecular oxygen is inserted by the enzyme species into the arachidonic acid backbone. The major bioactive products of the 5-LOX pathway are the leukotrienes (LT). Leukotrienes are formed from arachidonic acid presented to the 5-LOX enzyme by the 5-lipoxygenase-activating protein (FLAP). 5-LOX converts arachidonic acid to LTA4, which can be hyrdrolyzed by LTA4 hydrolase to LTB4. LTA4 can also give rise to glutathione conjugates by the action of LTC4 synthase, giving rise to the cysteinyl leukotrienes, namely LTC4, LTD4, and LTE4. Other enzymes, 12-LOX and 15-LOX give rise to 12-HETE and 15-HETE respectively, upon their action on arachidonic acid.

6.3 Cyclooxygenases, Metabolites and Tumor Progression

The COX enzymes, chiefly COX-2, have been linked to development and progression of multiple neoplasias. In the early 1980s, it was clinically observed that NSAIDs may inhibit tumor progression in patients with familial adenomatous polyposis (Waddell & Loughry, 1983). This was followed by the finding that administration of NSAIDs regressed intestinal polyps in an autochthonous rat model of intestinal cancer (Pollard et al., 1983). Epidemiological observations revealed that frequent intake of NSAIDs prevented cancers in humans. In a large prospective study, it was reported that people with aspirin intake had a reduced risk of fatal colon cancer (Thun et al., 1991). A few years after these findings, the expression of COX-2 at the message and protein levels was demonstrated to be upregulated in human colorectal adenomas and adenocarcinomas (Eberhart et al., 1994; Kargman et al., 1995) . Subsequent studies elaborated that, chemical inhibition or gene knockout of COX-2 in $Apc^{\Delta 716}$ knockout mice, a model of human familial adenomatous polyposis, led to a dramatic reduction in the number and size of intestinal polyps, which are precursor lesions for colon cancer (Oshima et al., 1996). Among the COX metabolites, prostaglandin E_2 (PGE_2) has been widely implicated in various steps of cancer development and progression such as angiogenesis, cell survival, proliferation, and chronic inflammation (Marnett & DuBois, 2002) (Fig. 6.2). In colorectal carcinomas, PGE_2 levels are maintained in a steady state by the actions of two pathways, i.e., the biosynthetic pathway catalyzed by the PG synthases and the enzymatic breakdown into the 15-keto PGE_2 metabolite by the enzyme 15-hydroxy prostaglandin dehydrogenase (15-PGDH). Loss of 15PGDH expression has been demonstrated in colorectal carcinomas, colorectal cancer cell lines, breast cancer, and lung cancer (Backlund et al., 2005). COX-2 overexpression also has been detected in prostate adenocarcinoma (Gupta et al., 2000; Yoshimura et al., 2000). The expression of COX-2 in tumors, was found to be upregulated by various oncogenes such as Her-2 or *ras* and downregulated by tumor suppressor genes like p53 (Subbaramaiah et al., 1999; Subbaramaiah et al., 2002). Several COX-2 inhibitors such as celecoxib and NS-398, have been demonstrated to induce apoptosis in prostate cancer cell lines (Hsu et al., 2000; Liu et al., 1998).

6.3.1 Prostaglandin E_2

Prostaglandin E_2 (PGE_2) is formed by the action of specific prostaglandin E synthases (PGES) which convert PGH_2 to PGE_2. Three isoforms of PGES exist, ie mPGES-1, mPGES-2, and cPGES. COX-2 and/or mPGES-1 have been found to be upregulated in several epithelial cancers (Muller-Decker & Furstenberger, 2007). The expression of mPGES-1 was found to be upregulated in 80% of non-small cell lung cancers and was localized to neoplastic epithelial cells The same study was able to identify that TNF-α, a pro-inflammatory cytokine, was able

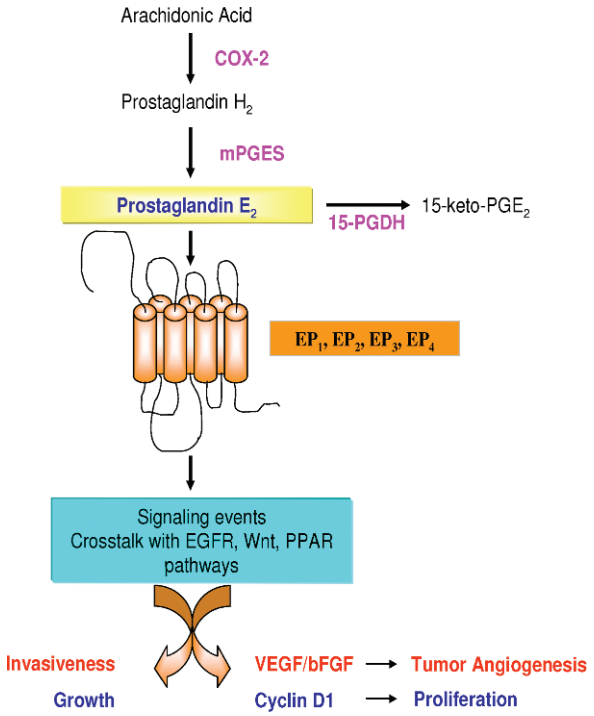

Fig. 6.2 Prostaglandin E_2 – Formation, Degradation, and Role in Cancer progression. PGE_2 is formed by the conversion of arachidonic acid to PGH_2 by COX, which is later converted to the active PGE_2 by mPGES. PGE_2 is metabolised to the inactive keto form by PGDH. PGE_2 mediates its cellular actions by binding to one or more of the specific EP receptors on the cell surface of target cells resulting in the activation of one or more signaling pathways. Studies have suggested that PGE_2 crosstalks with other cell signaling pathways (see text for details), leading to upregulation of target genes of these pathways, which are vital for tumor progression and metastasis. Overexpression of COX-2 and / or mPGES have been reported in various cancers and cancer cell lines, leading to increased production of PGE_2, while downregulation or loss of PGDH in cancers results in poor metabolic inactivation of PGE_2. These events are critical in regulating the availability and actions of PGE_2 in the tumor microenvironmental milieu

to upregulate mPGES-1 in NSCLC cell lines, but failed to do so in a non-tumorigenic bronchial epithelial cell line (Yoshimatsu et al., 2001). Similarly overexpression of mPGES-1 was found in 70% of cases of invasive breast cancer by immunohistochemistry, but was undetectable in normal breast epithelial cells (Mehrotra et al., 2006).

PGE_2 exerts its molecular action by binding to unique cell surface G-protein coupled receptors namely EP1, EP2, EP3, and EP4. EP2 and EP4 signal via intracellular activation of cAMP through the Gαs protein; EP1 signals through Gi raising intracellular calcium concentration; EP3 decreases cAMP formation via Gi. All four receptors have been demonstrated to be associated with tumor

formation and progression, by independent studies (Cha & DuBois, 2007). For example, $Apc^{\Delta 716}$ mice with knockdown of the EP_2 receptor exhibited a decrease in the size and number of polyps. These polyps also showed a decrease in the secretion of the vascular endothelial growth factor (VEGF) linking PGE_2 signaling and angiogenesis in this model (Sonoshita et al., 2001). Growth of xenografts derived from sarcoma 180 and Lewis Lung carcinomas was significantly hampered in EP3 null mice. VEGF production was reduced in tumors lacking the EP3 receptor in this model, thus verifying the findings from the previous study (Amano et al., 2003). EP receptor signaling activated by PGE_2 is an intricate mechanism, in that the pathways emanating from the cell surface get involved in cross-talk with other signaling pathways which are important in tumor progression, especially in colorectal cancer. It has been demonstrated that PGE_2 treatment results in the activation of the Wnt signaling pathway elements, resulting in the translocation of β-catenin to the nucleus, and upregulation of Wnt target genes such as VEGF and cyclin D (Castellone et al., 2005; Shao et al., 2005). These observations lend credence to earlier findings emphasizing the significance of NSAIDs in inhibiting colon cancer progression. PGE_2 induced signaling also activates the peroxisome proliferator activated receptor δ (PPARδ) in colorectal cancer, which signals to activate the Wnt cascade, thus reinforcing the already existing crosstalk via the Wnt pathway. This phenomenon was demonstrated by Wang et al, in $Apc^{\Delta 716}$ mice, where PGE_2 activates PPARδ in a PI3K-Akt dependent fashion. The effect of PGE_2 in this system was abolished when PPARδ was knocked out in these mice (Wang et al., 2004). In addition to these pathways, PGE_2 stimulated signaling via the EP4 receptor, has also been shown to crosstalk with EGFR activated tyrosine kinase pathways through βarrestin 1/c-src signaling complex, resulting in downstream pathophysiological actions such as cancer cell migration and invasion, which is thought to play a crucial role in the metastatic spread of colorectal cancer to liver (Buchanan et al., 2006).

PGE_2 also plays a critical role in the modulation of the tumor angiogenic response. Several studies have clearly demonstrated that PGE_2 upregulates the production of pro-angiogenic factors VEGF and bFGF. In co-culture systems, it was found that COX-2 overexpressing colon cancer cells activated endothelial cell migration and tube formation, by virtue of the secretion of pro-angiogenic factors. These responses were inhibited by treating the cancer cells with aspirin, whereas, treatment of COX-2 negative cells did not affect the endothelial cell responses (Tsujii et al., 1998). It was also demonstrated that PGE_2 upregulates the chemokine receptor CXCR4, via VEGF and bFGF, on microvascular endothelial cells (Salcedo et al., 2003). The molecular mechanism by which PGE_2 upregulates VEGF in human colon carcinoma cells was found to be dependent on the induction of HIF-1α. The expression of VEGF mRNA in these cells, when exposed to PGE_2, was mediated by the transcriptional activator HIF-1α. PGE_2 mediated activation of HIF-1α was found to be signaled via activation of ERK phosphorylation and c-Src kinase activity, which

demonstrates the requirement of multiple signaling pathway in the regulation of angiogenesis by this eicosanoid (Fukuda et al., 2003).

6.3.2 Thromboxane A_2

Thromboxanes are biologically active lipid mediators, first identified in the year 1975, from washed human platelets incubated with arachidonic acid or PGG_2 for 30 s, which led to formation of a highly unstable factor, that induced irreversible platelet aggregation (Hamberg et al., 1975). Thromboxane A_2 (TXA_2), a short-lived lipid mediator, is formed by the action of thromboxane synthase on PGH_2. TXA_2 is extremely labile and breaks down to TXB_2, which is the biologically inactive form. Physiological functions for TXA_2 include platelet activation, aggregation, and thrombosis (Needleman et al., 1976). Human prostate cancer PC-3 cells express functionally active TX synthase enzyme and are able to biosynthesize TXA_2. TX synthase mRNA expression was found to be higher in prostate, renal and breast carcinomas compared to matched normal tissues. Immunohistochemical analysis demonstrated that normal prostate tissues showed weak expression of the enzyme compared to prostate carcinoma. The degree of expression of TX synthase was found to correlate with the severity of prostate carcinoma lesions, with advanced stages and poorly differentiated forms having the highest expression levels. Within the cancer tissue, expression of TX synthase was localized to areas of perineural invasion. The enzyme was found to be involved in motility, but not proliferation or survival, of prostate cancer cells (Nie et al., 2004). Overexpression of thromboxane synthase and/or increased TXB_2 levels were found also in lung cancer tissues, benign and malignant papillary thyroid carcinoma, renal carcinoma, and larynx squamous cell carcinoma (Bryant, 1994; Casey et al., 2004; Chen et al., 2006; Kajita et al., 2005; Pinto et al., 1993). Thromboxane synthase was also found to be over-expressed in several forms of bladder cancers such as transitional cell carcinoma, squamous cell carcinoma, and adenocarcinoma compared to non-tumor tissue. Patients with more than 4-fold increase in TXS expression were found to have a poor prognosis. Treatment of bladder cancer cell lines with TXS inhibitors and TXA_2 receptor antagonists resulted in a decrease in cell survival, migration, and invasion, and the opposite effects were seen with receptor agonists (Moussa et al., 2005). TXA_2 was also shown to mediate endothelial functions such as migration and angiogenesis. Pro-angiogenic factors bFGF and VEGF was demonstrated to enhance TXA_2 synthesis in endothelial cells by 3-5 fold. Inhibition of TXS activity resulted in a decrease in the endothelial cell migration response to VEGF and bFGF. Similar changes were seen with TXA_2 receptor antagonists (Nie et al., 2000a).

TXA_2 mediates its cellular functions by binding to its receptors -TPα and TPβ. These are GPCRs which couple to Gq, G11, and G12/13 and elevate intracellular calcium levels, by signaling via PLC dependent inositol phosphate

generation, leading to vasoconstriction and platelet aggregation (Breyer et al., 2001). TPα and TPβ are alternatively spliced variants that differ in amino acid sequences distal to Arg-328 at the C-terminal end of the receptor. mRNAs of both the receptors are expressed widely in organs such as lungs, kidneys, liver, uterus, heart, etc. (Miggin & Kinsella, 1998). Overexpression of the TP receptor has been identified in human breast cancer specimens compared to normal breast tissues. TP overexpression was seen in aggressive tumors and linked with poor prognosis. The same study also found that TXAS expression was significantly low in high grade tumors and in patients with a poor prognosis (Watkins et al., 2005). Recent studies from our group have clearly demonstrated that prostate carcinoma express functionally active TP receptors. Signaling via these receptors were found to regulate prostate cancer cell motility and migration in a Rho dependent fashion and subsequent reorganization of the cytoskeleton (Nie et al., 2008).

6.3.3 Prostacyclin

Prostacyclin was discovered by Sir John Vane's group in the year 1976 as a substance generated by arterial vessel walls mediating relaxation of mesenteric and celiac arteries and inhibiting platelet aggregation (Bunting et al., 1976). The structure of prostacyclin was deduced in the same year (Whittaker et al., 1976). Prostacyclin or PGI_2 is an eicosanoid lipid mediator synthesized chiefly in endothelial cells, by the action of prostacyclin synthase on PGH_2. The major physiological action of PGI_2 is to oppose the functions of thromboxanes, inhibit platelet aggregation, and promote vasodilation. Together, prostacyclins and thromboxanes play a pivotal role in maintaining cardiovascular homeostasis (Wu & Liou, 2005). Prostacyclin mediates its cellular actions by binding to the cell surface PGI_2 receptor, named IP, a seven transmembrane GPCR (Narumiya et al., 1999). Knockout models of the IP receptor in mice have demonstrated the occurrence of thrombosis, reperfusion injury, intimal hyperplasia, and restenosis (Cheng et al., 2002; Murata et al., 1997; Xiao et al., 2001). Prostacyclin, in concert with thromboxanes play a major role in regulating metastasis of cancers *(see below in section on role of eicosanoids in platelet-tumor interactions)*.

6.4 Lipoxygenases and Tumor Progression

Lipoxygenases (LOX) constitute a family of lipid peroxidizing enzymes, which are distributed widely in the plant and animal kingdoms. These enzymes preferentially metabolize substrates that are polyunsaturated fatty acids containing a series of *cis* double bonds, which are the essential fatty acids for human beings (Kuhn & Thiele, 1999). Lipoxygenases are dioxygenases in nature and

catalyze the stereospecific insertion of molecular oxygen into polyunsaturated fatty acids (PUFA). The primary products of the lipoxygenase reaction are the hydroperoxy fatty acids. Based on the currently used nomenclature, lipoxygenases are classified with respect to their positional specificity of arachidonic acid oxygenation. In mammalian cells, there are three major types of lipoxygenases – 5-LOX, 12-LOX, and 15-LOX. The major end product of LOX mediated enzymatic breakdown of arachidonic acid is hydroxyeicosa 5, 8, 10, 14-tetraenoic acid (HETE). 12-LOX introduces molecular oxygen at carbon 12 and forms 12-HpETE which is converted to 12-HETE nonenzymatically. Similarly 5-LOX and 15-LOX catalyze the conversion of arachidonic acid into 5-HETE and 15-HETE respectively (Marks & Furstenberger, 1999). In case of the 5-LOX enzyme, 5-HETE is further metabolized in the presence of the 5-Lipoxygenase Activating Protein (FLAP), to the epoxide intermediate, leukotriene A4 (LTA4). LTA4 could be subsequently converted enzymatically by LTA4 hydrolase to leukotriene B4 (LTB4). LTA4 can also undergo enzymatic conjugation to glutathione, generating the cysteinyl leukotrienes LTC4, LTD4, and LTE4. Each LOX is predominantly expressed in a specific tissue. For example, 5-LOX is abundantly expressed in polymorphonuclear leukocytes (Funk & FitzGerald, 1991; Funk et al., 1989). Similarly, 15-LOX represents one of the major proteins besides hemoglobin in reticulocytes during anemia (Fleming et al., 1989; Rapoport et al., 1979; Turk et al., 1982) whereas platelets constitutively express 12-LOX (Chen & Funk, 1993).

6.4.1 5-LOX and metabolites

5-LOX enzymatic activity was first described in 1976 by Borgeat et al in rabbit polymorphonuclear leukocytes (Borgeat et al., 1976). 5-LOX catalyzes the conversion of arachidonic acid to 5-HETE or LTA4, which generates LTB4 and the cysteinyl leukotrienes. Majority of the 5-LOX metabolites such as LTB4 and the cysteinyl leukotrienes play crucial roles in the inflammatory processes of the host. LTB4 is a potent neutrophil chemotactic factor and also stimulates transendothelial migration of these cells. Cys-LTs are chiefly involved in mounting the allergic inflammatory response. Specific receptors for LTB4 and Cys-LTs have been identified. Two receptors for LTB4 have been cloned – BLT1 and BLT2. The Cys-LTs mediate their physiological actions by binding to the Cys-LT1 and Cys-LT2 receptors (Funk, 2001).

Several reports have suggested an association between 5-LOX, 5-(S)HETE formation and carcinogenesis. The expression of 5-LOX has been documented in several cancers including prostate, colon, lung, breast, pancreas, bone, brain, and mesothelium (Romano & Claria, 2003). Inhibition of 5-LOX enzymatic activity by specific chemical inhibitors blocked the stimulatory effect of arachidonic acid on the growth of prostate cancer cells. Conversely, addition of 5-HETE but not leukotrienes promoted the growth of prostate cancer cells,

suggesting the involvement of this metabolite in modulating growth stimulatory effects in prostate cancer (Ghosh & Myers, 1997). Similarly, 5-LOX and 5-HETE were found to mediate growth of lung cancer cells, and inhibition of this pathway led to growth arrest and apoptosis of these cells (Avis et al., 1996). In the case of pancreatic cancer and mesothelioma, low or undetectable levels of 5-LOX were observed in normal cells and tissues, compared to the tumor cells, which had high expression and activity of this enzyme (Hennig et al., 2002; Romano et al., 2001). Specific inhibition of 5-LOX led to apoptosis in prostate cancer cells and blocked proliferation in human leukemia cell lines (Ghosh & Myers, 1998; Tsukada et al., 1986). Overexpression of LTB4 receptor was also identified in human pancreatic cancer tissues by immunohistochemistry (Hennig et al., 2002). Upregulation of LTD4 receptor, Cys-LT1 has been detected in colorectal adenocarcinoma (Ohd et al., 2003). In a human malignant mesothelioma model, it was reported that 5-LOX and LTA4 but not LTB4, upregulated the expression and secretion of the proangiogenic factor VEGF. Selective inhibition of VEGF, a prosurvival factor for mesothelioma cells, brought about by 5-LOX antisense or inhibitors resulted in apoptotic cell death, suggesting the involvement of 5-LOX and its metabolites in promoting tumor cell survival (Romano et al., 2001).

6.4.2 12-LOX and 12(S)-HETE

The conversion of arachidonic acid to 12S-hydroxy-5,8,10,14 eicosatetraenoic acid 12(S)-HETE was first demonstrated in human and bovine platelets (Yoshimoto & Takahashi, 2002). The three predominant forms of 12-LOX are platelet-type 12-LOX, epidermis-type 12-LOX, and leukocyte-type 12-LOX (Limor et al., 2001; Siebert et al., 2001; Yamamoto et al., 1997). These are distinct enzymes by sequence, catalytic properties, and function. 12-LOX and its product 12-HETE have been shown to be involved in a variety of cancers. The mRNA of 12-LOX has been detected in erythroleukemia, colon carcinoma, epidermoid carcinoma A431 cells, human glioma, prostate, and breast cancer cells. Additionally, this enzyme also has been detected in smooth muscle cells (Kim et al., 1995), keratinocytes (Krieg et al., 1995), and endothelial cells (Funk et al., 1992). The product of 12-LOX in amelanotic melanoma cells has been found to be the S enantiomer (12(S)-HETE) by GC-MS spectral analysis (Liu et al., 1994). Production of endogenous 12S-HETE has been documented in human colon carcinoma, rat Walker carcinosarcoma, mouse melanoma and lung carcinoma (Chen et al., 1994). In human prostate carcinoma, the level of platelet-type 12-LOX expression was correlated with the tumor stage and grade (Gao et al., 1995; Timar et al., 2000). Platelet-type 12-LOX mRNA was shown to be increased in prostate cancer tissues, and the expression correlated with clinical stage of the disease (Timar et al., 2000). Studies using inhibitors of platelet-type 12-LOX have shown that the blocking 12-LOX activity results in

the arrest of cell cycle progression and induction of apoptosis in prostate cancer cell lines (Pidgeon et al., 2002). These studies have demonstrated that the 12-LOX pathway plays an important role in regulating prostate cancer progression and apoptosis. Urinary levels of 12(S)-HETE, the metabolite produced by 12-LOX, in prostate cancer patients are significantly elevated when compared to normal individuals and removal of the prostate gland results in a significant decrease in the urinary concentration of this eicosanoid (Nithipatikom et al., 2006) In contrast, other HETEs (i.e. 5-and 15-HETE), although detected remain unchanged following radical prostatectomy (Nithipatikom et al., 2006). Approximately 38% of the 138 prostate cancer patients studied exhibited an elevated expression of 12-LOX at the mRNA level in prostate tumor tissues compared to matched normal tissues. This elevated 12-LOX mRNA expression was found to have a positive correlation with advanced stage and poor differentiation of prostate cancer (Gao et al., 1995). 12-LOX and 12-HETE have been shown to be important determinants of tumor cell survival and apoptosis (Honn et al., 1996).

12-LOX has been found to be an important marker for cancer progression within the melanoma system, and therefore could be a useful biomarker and therapeutic target for melanoma chemoprevention (Winer et al., 2002). It also has been found that 12(S)-HETE is involved in the proliferation of pancreatic carcinoma cells (Ding et al., 2001) and inhibition of the 12-LOX has been shown to cause apoptosis of these cells (Tong et al., 2002). 12-LOX and 12(S)-HETE were also found to enhance proliferation and survival of gastric cancer cell lines (Wong et al., 2001).

Overexpression of the platelet-type 12-LOX in human prostate cancer PC-3 cells stimulated growth by enhanced tumor angiogenesis (Nie et al., 1998) and 12(S)-HETE, the sole and stable end product of arachidonic acid metabolism by the platelet-type 12-LOX, has been shown to protect tumor cells from apoptosis and induce invasion, motility, and angiogenesis (Gao & Honn, 1995; Honn et al., 1994a; Nie et al., 2000c) as well as surface expression of $\alpha v\beta 3$ integrin (Tang et al., 1993b). Promotion of such divergent biological functions by 12(S)-HETE is indicative of a complex signaling mechanism leading to metastasis and survival of tumor cells. Attempts at understanding the 12(S)-HETE signaling mechanisms revealed several interesting features involving G-proteins. While specific 12(S)-HETE receptor(s) is yet to be identified, both high and low affinity binding sites have been identified on B16a murine melanoma cells (Liu et al., 1995), keratinocytes (Arenberger et al., 1993), and A431 cells (Szekeres et al., 2000a). We have recently elucidated some of the signaling events down stream of the putative 12(S)-HETE receptor (Szekeres et al., 2000a; Szekeres et al., 2000b). 12(S)-HETE stimulates phosphorylation of PLCγ1, which in turn is responsible for the activation of PKCα. PKCα plays a significant, but not exclusive role in ERK1/2 activation. Further, we have demonstrated the 12(S)-HETE induced activation of Src family kinases, and the subsequent phosphorylation of adapter proteins Shc and Grb2, which lead to

activation of ERK1/2 *via* Ras. Our work also suggested that protein tyrosine phosphatases are involved in 12(S)-HETE signaling in A431 cells. Additionally, 12(S)-HETE also was shown to activate PI3 kinase which is rate limiting for ERK1/2 activation (Szekeres et al., 2000b). PI3 kinase mediated activation of ERK by 12(S)-HETE involves PKCζ. Thus, 12(S)-HETE activates (through its putative receptor) PKCα *via* PLCγ1 and stimulates PKCζ *via* inositide kinase. Both PKC isoforms contribute to phosphorylation of the Raf/MEK/ERK cascade. The natural convergence point for this vast array of mitogenic signaling mechanisms is the extremely variable transcriptional machinery.

One possible integrator of these 12(S)-HETE signaling mechanisms in tumor cells is the pleiotropic transcription factor NF-κB, which plays an important role in the control of cell proliferation and apoptosis. Using PC-3 prostate cancer cells, we have shown that either overexpression of the platelet-type 12-LOX or exogenously added 12(S)-HETE activates NF-κB (Kandouz et al., 2003). NFκB is normally sequestered in the cytosol where it is bound to IκBα proteins (Ghosh et al., 1998). 12(S)-HETE induced the degradation of IκBα, which resulted in the nuclear translocation of NF-κB and enhanced transcriptional activity. Among the activators and regulators of NF-κB complex are several members of the MEK kinase family (MEKK1, 2, and 3 and NIK), TAK1, PKCζ, and S6 kinase (Baumann et al., 2000; Lee et al., 1998; Nakano et al., 1998; Nemoto et al., 1998; Pearson et al., 2001). Given the role of 12(S)-HETE in the activation of the MAP kinase signaling pathway to induce Raf, PLC, PKCζ, as well as PI3 kinase (Szekeres et al., 2000a; Szekeres et al., 2000b) and the role of MAP kinase cascade in the activation of NF-κB, it is highly likely that the 12(S)-HETE activation of NF-κB proceeds *via* the MAP kinase cascade. A recent report on the activation of NF-κB by MEKK1 in Raf mediated cell transformation suggests the potential for a 12(S)-HETE induced Raf participation in the signaling pathway (Baumann et al., 2000).

The effect of 12-LOX on tumor growth in vivo, has been positively correlated with its ability to increase tumor angiogenesis (Nie & Honn, 2002). Studies have revealed that prostate cancer cells expressing high levels of platelet-type 12-LOX are more angiogenic than those expressing none or low levels of 12-LOX. The same study also revealed that the increased angiogenicity of the 12-LOX overexpressing cells is directly related to the ability of the metabolite 12(S)-HETE to stimulate endothelial cell migration (Nie et al., 1998). It was identified by two separate groups that 12-LOX induced stimulation of angiogenesis involved the upregulation of the pro-angiogenic factor VEGF (McCabe et al., 2006; Nie et al., 2006). The 12-LOX inhibitor BMD 122 (Nbenzyl-N-hydroxy-5-phenyl-pentanamide) was found to reduce endothelial cell proliferation stimulated by basic fibroblast growth factor (bFGF) or by vascular endothelial growth factor (VEGF). This inhibition could be partly restored by the addition of 12(S)-HETE. The same inhibitor also blocked *in vitro* blood vessel formation by rat vascular endothelial cells. These findings taken together have proved that arachidonic acid metabolism in endothelial cells through the 12-LOX pathway plays a critical role in angiogenesis (Nie et al., 2000b).

Overexpression of 12-LOX in breast cancer cells has resulted in enhanced tumor angiogenesis and growth in a fat pad animal model (Connolly & Rose, 1998).

Many studies have demonstrated that 12(S)-HETE is a proangiogenic agent. 12(S)HETE has a variety of effects on endothelial cells. This metabolite has been shown to upregulate the surface expression of $\alpha v\beta 3$ integrin in rat aorta endothelial cells and murine pulmonary vascular endothelial cells (Tang et al., 1993a, b, 1994, 1995a). 12(S)HETE has also been found to act as a mitogen for microvascular endothelial cells (Tang et al., 1995b). Taken together, the above findings clearly illustrate a definitive role for 12-LOX in tumor angiogenesis.

6.4.3 15-Lipoxygenase and Metabolites

Two isoforms of 15-LOX exist – 15-LOX-1 and 15-LOX-2. 15-LOX-1 was originally described in rabbit reticulocytes in the year 1975 as an enzyme that oxidizes phospholipids in intact mitochondria and cell membrane (Schewe et al., 1975). In 1988, the human ortholog of 15-LOX-1 was purified from eosinophilic leukocytes (Sigal et al., 1988). In 1997, a second isoform of 15-LOX, namely 15-LOX-2 was identified from human hair roots. This enzyme has only a low degree of sequence similiarity with 15-LOX-1, and was found to be expressed in prostate, skin, lung, and cornea (Brash et al., 1997). The 15-LOX-1 isoform is capable of utilizing both arachidonic acid and linoleic acid as substrates. It inserts molecular oxygen at C-15 of arachidonic acid to generate 15-HPETE, and at the C-13 of linoleic acid to produce 13-HODE (Kuhn et al., 2002). Conversely, 15-LOX-2 does not act efficiently on linoleic acid, and converts arachidonic acid to 15(S)HETE. This enzyme shares the highest homology with the mouse 8-LOX enzyme (Tang et al., 2007).

The role of 15-LOX-1 in carcinogenesis is controversial, and both pro and anti-tumor properties have been assigned to this enzyme by various researchers. Ikawa et al, identified stronger immunostaining for 15-LOX-1 in tumors compared to normal tissue, in 18 of 21 matched pairs of colorectal tumor samples and adjacent normal tissues (Ikawa et al., 1999). In contrast, a study by Shureiqi et al., reported weak immunohistochemical scores for 15-LOX-1 and lower levels of 13HODE in colorectal cancer when compared with normal tissue. It was concluded that 15-LOX-1 expression and 13-HODE generation may have tumor suppressor properties in colorectal cancer, by suppressing cell proliferation and promoting apoptosis (Shureiqi et al., 1999). Later, it was identified that COX inhibitors upregulated 15-LOX-1 expression in colon cancer cells, and this upregulation may be related to triggering apoptotic pathways in these cells (Shureiqi et al., 2000). Similarly, 15-LOX-1 expression and 13-HODE production was found to be downregulated in esophageal cancer cells, and inhibition of 15-LOX-1 activity was found to dampen the cytotoxic effects of NSAIDs in

these cells, which was rescued by exogenous addition of 13(S)-HODE, suggesting the tumor suppressor roles for these molecules (Shureiqi et al., 2001). Spindle et al, have identified that the levels of 13(S)-HODE, measured by enzyme immunoassay, is elevated in human prostate carcinoma. In another study, 15LOX-1 was found to be coexpressed with a mutant p53 isoform, in 48 prostatectomy specimens with a high degree of statistical significance ($p < 0.001$), and enzyme expression correlated with Gleason staging of prostate cancer (Kelavkar et al., 2000). It was later identified that 15-LOX-1 is overexpressed in PC-3 prostate cancer cell lines and prostate adenocarcinoma. Lower levels of 15LOX-1 were identified in normal prostate tissues compared to 15-LOX-2, whereas 15-LOX-1 overexpression and concomitant 15-LOX-2 downregulation was observed in cancerous prostate tissues suggesting the involvement of 15-LOX-1 in prostate cancer (Kelavkar et al., 2006). On the contrary, 15-LOX-1 expression and activity was found to exert anti-tumor properties in human pancreatic cancer. Immunohistochemical analysis demonstrated that pancreatic cancer cell lines show a weak expression of this enzyme, and poor staining was seen in tumor samples compared to normal. Restitution of 15-LOX-1 activity in these cancer cells resulted in decreased cell growth suggesting a tumor suppressor role for this enzyme in pancreatic cancer (Hennig et al., 2007).

The second isoform of 15-LOX, namely 15-LOX-2 is considered to be a tumor suppressor, based on studies conducted in various cancers. The expression of 15-LOX-2 is restricted primarily to the prostate and also to the skin, lung, and cornea (Brash et al., 1997). The expression and activity of this enzyme is highly diminished in high grade prostatic intraepithelial neoplasia (HGPIN) and prostate cancer (Shappell et al., 1999; 2001b). Downregulation of 15LOX-2 was also observed in benign and neoplastic sebaceous glands, esophageal cancer and lung cancer (Gonzalez et al., 2004; Shappell et al., 2001a; Xu et al., 2003). The exact molecular mechanisms leading to the loss of 15-LOX-2 expression in prostate cancer cells is still under investigation and many hypotheses have been tested. Studies conducted in normal human prostate epithelial cells (NHP) have revealed that 15-LOX-2 has multiple alternatively spliced isoforms. In these cells 15-LOX-2 functions as a negative regulator of cell cycle progression. The expression of 15-LOX-2 in the NHP cells is regulated by Sp1 and is repressed by Sp3 (Tang et al., 2007).

6.5 Role of Eicosanoids in Platelet-Tumor Interactions

The involvement of platelets in assisting hematogenous spread of metastatic tumor cells and the interactions between platelets, cancer cells, and the blood vessel wall were proposed decades ago. This was confirmed in experimental model systems of thrombocytopenia which showed inhibition of metastasis (Gasic et al., 1973; Kimoto et al., 1993). Honn et al, proposed the first hypothesis on the involvement of bioactive lipid mediators, specifically TXA_2

and PGI_2, produced by the blood vessel wall, platelets, and tumor cells to play a complex and intricate role in the metastatic spread of cancer cells via the blood stream (Fig. 6.3). It was hypothesized that an intricate balance exists between TXA_2 and PGI_2 in mediating cancer cell metastasis in patients, and any pathophysiological disruption in this equilibrium favoring dominance of TXA_2 over PGI_2, would promote metastasis of tumor cells from the primary site (Honn & Meyer, 1981). In agreement with this hypothesis, it was demonstrated that PGI_2 is a potent antimetastatic agent. In this study, experimental metastasis of B16 melanoma cells to the lungs was dramatically inhibited by PGI_2, a response, which was not reproduced by other lipid mediators like PGE_2, which is a vasodilator but not a platelet anti-aggregating factor. PGD_2 which also functions similar to PGI_2 in preventing platelet aggregation, was able to mount this antimetastatic response, but was less potent compared to the latter. Intracellular actions of PGI_2 are mediated by the signaling molecule cAMP. Thus, combination of PGI_2 treatment with a phosphodiesterase inhibitor, which prolongs the half-life of intracellular cAMP, potentiated the actions of PGI_2 in inhibiting platelet aggregation and reducing tumor metastasis (Honn et al., 1981). In a subsequent study, it was revealed that PGI_2 blocks platelet tumor cell aggregation promoted by cathepsin B or calpains, whose actions are similar to cathepsin B (Honn et al., 1982). Yet another study demonstrated that PGI_2 is effective in inhibiting both tumor cell

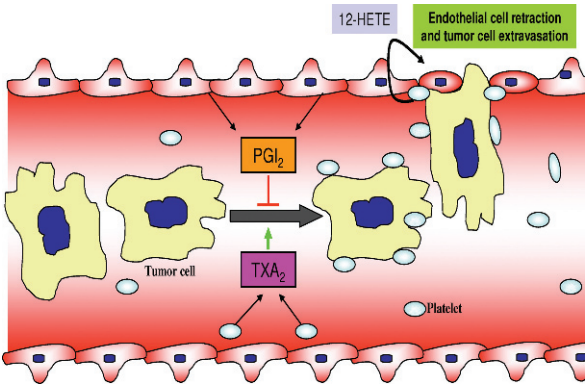

Fig. 6.3 Eicosanoids and Tumor-platelet interactions in metastasis and the role of 12-HETE in tumor cell extravasation. Studies have clearly shown that hematogenous route of metastasis spread of cancer cells, involves interactions with platelets. Tumor-platelet interactions and subsequent aggregation is critically controlled by a delicate balance between the level of endothelium derived PGI_2 and platelet or tumor derived TXA_2. Elevated TXA_2 levels in the circulation can tip the balance towards platelet aggregation and tumor metastasis to distant organs, whereas increases in PGI_2 levels can block this interaction preventing spread of cancer cells. Shown in this illustration is a schematic of a blood vessel, with metastatic tumor cells interacting with platelets. Interactions of tumor cells with platelets and endothelial cells have been demonstrated to induce 12(S)-HETE production, which leads to retraction of endothelial cell layers enabling metastatic tumor cells to extravasate and set up secondary colonies of metastasis

induced platelet aggregation (TCIPA) and platelet facilitated tumor cell adhesion in vitro. In this study, PGI_2 was 100-fold more potent that PGE_1 or PGD_2, and 1000-fold more potent than its non-enzymatic metabolite 6-keto $PGF_2\alpha$. Interestingly, PGE_2 did not inhibit TCIPA but blocked TCIPA induced by PGI_2. It was also demonstrated that platelets promoted the adhesion of W256 cells to the culture dish, and PGI_2 was able to block this platelet induced tumor cell adhesion, suggesting that PGI_2 has antimetastatic actions in vivo (Menter et al., 1984). In agreement with these findings, it was demonstrated that patients with bone tumors had extremely low plasma levels of 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, whereas plasma concentrations of TXA_2 were in the normal range. Patients with malignant bone tumors were also found to have deficiency of plasma factors repsonsible for stabilization of PGI_2. Arterial tissues from patients with malignant disease were found to generate very low concentrations of PGI_2 compared to normal individuals (reviewed by Mehta, P.) (Mehta, 1984). Inhibition of TX synthase activity and treatment with a TXA_2 receptor antagonist were found to inhibit metastasis of B16 melanoma cells injected into the tail vein of mice (Honn, 1983). Later, it was reported that W256 cells can produce TXA_2 and 12-HETE concomitant to inducing platelet aggregation. The role of these eicosanoids in promoting TCIPA was verified by using COX and LOX inhibitors, combination treatment of which led to ablation of platelet aggregation, in repsonse to tumor cells (Honn et al., 1987). Subsequent studies revealed that 12-HETE treatment led to retraction of CD3 endothelial cell monolayers, as assessed by quantitative binding assays and by phase contrast microscopy. Platelet enhanced endothelial cell retraction was blocked by treating either tumor cells or platelets with lipoxygenase inhibitors as well as by PGI_2. It was also found that 12-HETE biosynthesis was enhanced by the addition of platelets in the presence of tumor cell endothelial cell interactions. It was concluded that tumor cell – endothelial cell – platelet interactions lead to enhanced 12-HETE biosynthesis which can lead to endothelial cell retraction facilitating tumor cell extravasation and metastasis (Honn et al., 1994b).

6.6 Conclusion

Eicosanoids are bioactive lipid mediators which have clearly defined roles in mediating tumor progression and metastasis. Both arms of the arachidonic acid metabolism pathway ie the COX and the LOX pathways, have been demonstrated to be pivotal in promoting the malignant phenotype. Some of these metabolites have also been demonstrated to have an anti-tumor effect. Exploiting these pathways will help in better understanding the intricacies of the metastatic cascade and to develop newer therapeutic agents aimed at blocking the actions of these metabolites. Inclusion of specific and safe inhibitors of these pathways along with the usual regimen of anti-tumor drugs, can be beneficial in better control of tumor progression and metastasis.

Acknowledgments Work described in this review contributed by the authors is supported by NIH grant R01CA114051 (K.V. Honn) and National Institutes of Health Grant CA-29997 (K.V. Honn).

References

Amano H, Hayashi I, Endo H, Kitasato H, Yamashina S, Maruyama T, Kobayashi M, Satoh K, Narita M, Sugimoto Y, Murata T, Yoshimura H, Narumiya S, Majima M. Host prostaglandin E(2)-EP3 signaling regulates tumor-associated angiogenesis and tumor growth. J Exp Med. 197 (2003) 221–232.

Arenberger P, Kemeny L, Ruzicka T. Characterization of high-affinity 12(S)-hydroxyeicosatetraenoic acid (12(S)-HETE) binding sites on normal human keratinocytes. Epithelial Cell Biol. 2 (1993) 1–6.

Avis IM, Jett M, Boyle T, Vos MD, Moody T, Treston AM, Martinez A, Mulshine JL. Growth control of lung cancer by interruption of 5-lipoxygenase-mediated growth factor signaling. J Clin Invest. 97 (1996) 806–813.

Backlund MG, Mann JR, Holla VR, Buchanan FG, Tai HH, Musiek ES, Milne GL, Katkuri S, DuBois RN. 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. J Biol Chem. 280 (2005) 3217–3223.

Baumann B, Weber CK, Troppmair J, Whiteside S, Israel A, Rapp UR, Wirth T. Raf induces NF-kB by membrane shuttle kinase MEKK1, a signaling pathway critical for transformation. Proc.Natl.Acad.Sci.U.S.A. 97 (2000) 4615–4620.

Borgeat P, Hamberg M, Samuelsson B. Transformation of arachidonic acid and homo-gammalinolenic acid by rabbit polymorphonuclear leukocytes. Monohydroxy acids from novel lipoxygenases. J Biol Chem. 251 (1976) 7816–7820.

Brash AR, Boeglin WE, Chang MS. Discovery of a second 15S-lipoxygenase in humans. Proc Natl Acad Sci U S A. 94 (1997) 6148–6152.

Breyer RM, Bagdassarian CK, Myers SA, Breyer MD. Prostanoid receptors: subtypes and signaling. Annu Rev Pharmacol Toxicol. 41 (2001) 661–690.

Bryant J. Correlation between metastatic patterns in renal cell carcinoma and tissue distribution of thromboxane synthetase. Acta Oncol. 33 (1994) 708–709.

Buchanan FG, Gorden DL, Matta P, Shi Q, Matrisian LM, DuBois RN. Role of beta-arrestin 1 in the metastatic progression of colorectal cancer. Proc Natl Acad Sci U S A. 103 (2006) 1492–1497.

Bunting S, Gryglewski R, Moncada S, Vane JR. Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac ateries and inhibits platelet aggregation. Prostaglandins. 12 (1976) 897–913.

Casey MB, Zhang S, Jin L, Kajita S, Lloyd RV. Expression of cyclooxygenase-2 and thromboxane synthase in non-neoplastic and neoplastic thyroid lesions. Endocr Pathol. 15 (2004) 107–116.

Castellone MD, Teramoto H, Williams BO, Druey KM, Gutkind JS. Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. Science. 310 (2005) 1504–1510.

Cha YI, DuBois RN. NSAIDs and cancer prevention: targets downstream of COX-2. Annu Rev Med. 58 (2007) 239–252.

Chen GG, Lee TW, Yip JH, Xu H, Lee IK, Mok TS, Warner TD, Yim AP. Increased thromboxane B(2) levels are associated with lipid peroxidation and Bcl-2 expression in human lung carcinoma. Cancer Lett. 234 (2006) 193–198.

Chen X-S, Funk CD. Structure-function properties of human platelet 12-lipoxygenase: Chimeric enzyme and in vitro mutagenesis studies. FASEB J. 7 (1993) 694–701.

Chen YQ, Duniec ZM, Liu B, Hagmann W, Gao X, Shimoji K, Marnett LJ, Johnson CR, Honn KV. Endogenous 12(S)-HETE production by tumor cells and its role in metastasis. Cancer Res. 54 (1994) 1574–1579.

Cheng Y, Austin SC, Rocca B, Koller BH, Coffman TM, Grosser T, Lawson JA, FitzGerald GA. Role of prostacyclin in the cardiovascular response to thromboxane A2. Science. 296 (2002) 539–541.

Connolly JM, Rose DP. Enhanced angiogenesis and growth of 12-lipoxygenase gene-transfected MCF-7 human breast cancer cells in athymic nude mice. Cancer Lett. 132 (1998) 107–112.

Ding XZ, Tong WG, Adrian TE. 12-lipoxygenase metabolite 12(S)-HETE stimulates human pancreatic cancer cell proliferation via protein tyrosine phosphorylation and ERK activation. Int J Cancer. 94 (2001) 630–636.

Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 107 (1994) 1183–1188.

Fleming I, Thiel BA, Chester J, O'Prey J, Janetzki S, Aitken A, Anton IA, Rapoport SM, Harrison PR. The complete sequence of the rabbit erythroid cell-specific 15-lipoxygenase mRNA: Comparison of the predicted amino acid sequence of the erythrocyte lipoxygenase with other lipoxygenases. Gene. 79 (1989) 181–188.

Fukuda R, Kelly B, Semenza GL. Vascular endothelial growth factor gene expression in colon cancer cells exposed to prostaglandin E2 is mediated by hypoxia-inducible factor 1. Cancer Res. 63 (2003) 2330–2334.

Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 294 (2001) 1871–1875.

Funk CD, FitzGerald GA. Eicosanoid forming enzyme mRNA in human tissues. J.Biol. Chem. 266 (1991) 12508–12513.

Funk CD, Funk LB, FitzGerald GA, Samuelsson B. Characterization of human 12-lipoxygenase genes. Proc Natl Acad Sci U S A. 89 (1992) 3962–3966.

Funk CD, Hoshiko S, Matsumoto T, Rådmark O, Samuelsson B. Characterization of the human 5lipoxygenase gene. Proc.Natl.Acad.Sci.USA. 86 (1989) 2587–2591.

Gao X, Grignon DJ, Chbihi T, Zacharek A, Chen YQ, Sakr W, Porter AT, Crissman JD, Pontes JE, Powell IJ, Honn KV. Elevated 12-lipoxygenase mRNA expression correlated with advanced stage and poor differentiation of human prostate cancer. Urology. 46 (1995) 227–237.

Gao X, Honn KV. Biological properties of 12(S)-HETE in cancer metastasis. Adv.Prostaglandin Thromboxane Leukot.Res. 23 (1995) 439–444.

Gasic GJ, Gasic TB, Galanti N, Johnson T, Murphy S. Platelet-tumor-cell interactions in mice. The role of platelets in the spread of malignant disease. Int J Cancer. 11 (1973) 704–718.

Ghosh J, Myers CE. Arachidonic acid stimulates prostate cancer cell growth: critical role of 5lipoxygenase. Biochem Biophys Res Commun. 235 (1997) 418–423.

Ghosh J, Myers CE. Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells. Proc Natl Acad Sci U S A. 95 (1998) 13182–13187.

Ghosh S, May MJ, Kopp EB. NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. Annu.Rev.Immunol. 16 (1998) 225–260.

Gonzalez AL, Roberts RL, Massion PP, Olson SJ, Shyr Y, Shappell SB. 15-Lipoxygenase-2 expression in benign and neoplastic lung: an immunohistochemical study and correlation with tumor grade and proliferation. Hum Pathol. 35 (2004) 840–849.

Gupta S, Srivastava M, Ahmad N, Bostwick DG, Mukhtar H. Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. Prostate. 42 (2000) 73–78.

Hamberg M, Svensson J, Samuelsson B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci U S A. 72 (1975) 2994–2998.

Hennig R, Ding XZ, Tong WG, Schneider MB, Standop J, Friess H, Buchler MW, Pour PM, Adrian TE. 5-Lipoxygenase and leukotriene B(4) receptor are expressed in human pancreatic cancers but not in pancreatic ducts in normal tissue. Am J Pathol. 161 (2002) 421–428.

Hennig R, Kehl T, Noor S, Ding XZ, Rao SM, Bergmann F, Furstenberger G, Buchler MW, Friess H, Krieg P, Adrian TE. 15-lipoxygenase-1 production is lost in pancreatic cancer and overexpression of the gene inhibits tumor cell growth. Neoplasia. 9 (2007) 917–926.

Honn KV. Inhibition of tumor cell metastasis by modulation of the vascular prostacyclin/thromboxane A2 system. Clin Exp Metastasis. 1 (1983) 103–114.

Honn KV, Aref A, Chen YQ, Cher ML, Crissman JD, Forman JD, Gao X, Grignon D, Hussain M, Porter AT, Pontes EJ, Powell II, Redman B, Sakr W, Severson R, Tang DG, Wood DP, Jr. Prostate Cancer -Old Problems and New Approaches. (Part II. Diagnostic and Prognostic Markers, Pathology and Biological Aspects). Pathol Oncol Res. 2 (1996) 191–211.

Honn KV, Cavanaugh P, Evens C, Taylor JD, Sloane BF. Tumor cell-platelet aggregation: induced by cathepsin B-like proteinase and inhibited by prostacyclin. Science. 217 (1982) 540–542.

Honn KV, Cicone B, Skoff A. Prostacyclin: a potent antimetastatic agent. Science. 212 (1981) 1270–1272.

Honn KV, Meyer J. Thromboxanes and prostacyclin: positive and negative modulators of tumor growth. Biochem Biophys Res Commun. 102 (1981) 1122–1129.

Honn KV, Steinert BW, Moin K, Onoda JM, Taylor JD, Sloane BF. The role of platelet cyclooxygenase and lipoxygenase pathways in tumor cell induced platelet aggregation. Biochem Biophys Res Commun. 145 (1987) 384–389.

Honn KV, Tang DG, Gao X, Butovich IA, Liu B, Timar J, Hagmann W. 12-lipoxygenases and 12(S)HETE: role in cancer metastasis. Cancer Metastasis Rev. 13 (1994a) 365–396.

Honn KV, Tang DG, Grossi IM, Renaud C, Duniec ZM, Johnson CR, Diglio CA. Enhanced endothelial cell retraction mediated by 12(S)-HETE: a proposed mechanism for the role of platelets in tumor cell metastasis. Exp Cell Res. 210 (1994b) 1–9.

Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. J Biol Chem. 275 (2000) 11397–11403.

Ikawa H, Kamitani H, Calvo BF, Foley JF, Eling TE. Expression of 15-lipoxygenase-1 in human colorectal cancer. Cancer Res. 59 (1999) 360–366.

Kajita S, Ruebel KH, Casey MB, Nakamura N, Lloyd RV. Role of COX-2, thromboxane A2 synthase, and prostaglandin I2 synthase in papillary thyroid carcinoma growth. Mod Pathol. 18 (2005) 221–227.

Kandouz M, Nie D, Pidgeon GP, Krishnamoorthy S, Maddipati KR, Honn KV. Platelet-type 12lipoxygenase activates NF-kappaB in prostate cancer cells. Prostaglandins Other Lipid Mediat. 71 (2003) 189–204.

Kargman SL, O'Neill GP, Vickers PJ, Evans JF, Mancini JA, Jothy S. Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. Cancer Res. 55 (1995) 2556–2559.

Kelavkar U, Lin Y, Landsittel D, Chandran U, Dhir R. The yin and yang of 15-lipoxygenase-1 and delta-desaturases: dietary omega-6 linoleic acid metabolic pathway in prostate. J Carcinog. 5 (2006) 9–13.

Kelavkar UP, Cohen C, Kamitani H, Eling TE, Badr KF. Concordant induction of 15-lipoxygenase-1 and mutant p53 expression in human prostate adenocarcinoma: correlation with Gleason staging. Carcinogenesis. 21 (2000) 1777–1787.

Kim JA, Gu JL, Natarajan R, Berliner JA, Nadler JL. A leukocyte type of 12-lipoxygenase is expressed in human vascular and mononuclear cells. Evidence for upregulation by angiotensin II. Arterioscler Thromb Vasc Biol. 15 (1995) 942–948.

Kimoto M, Ando K, Koike S, Matsumoto T, Jibu T, Moriya H, Kanegasaki S. Significance of platelets in an antimetastatic activity of bacterial lipopolysaccharide. Clin Exp Metastasis. 11 (1993) 285–292.

Krieg P, Kinzig A, Ress-Loschke M, Vogel S, Vanlandingham B, Stephan M, Lehmann WD, Marks F, Furstenberger G. 12-Lipoxygenase isoenzymes in mouse skin tumor development. Mol Carcinog. 14 (1995) 118–129.

Kuhn H, Thiele BJ. The diversity of the lipoxygenase family. Many sequence data but little information on biological significance. FEBS Lett. 449 (1999) 7–11.

Kuhn H, Walther M, Kuban RJ. Mammalian arachidonate 15-lipoxygenases structure, function, and biological implications. Prostaglandins Other Lipid Mediat. 68–69 (2002) 263–290.

Lee FS, Peters RT, Dang LC, Maniatis T. MEKK1 activates both IkB kinase a and IkB kinase beta. Proc.Natl.Acad.Sci.U.S.A. 95 (1998) 9319–9324.

Limor R, Weisinger G, Gilad S, Knoll E, Sharon O, Jaffe A, Kohen F, Berger E, Lifschizt-Mercer B, Stern N. A novel form of platelet-type 12-lipoxygenase mRNA in human vascular smooth muscle cells. Hypertension. 38 (2001) 864–871.

Liu B, Khan WA, Hannun YA, Timar J, Taylor JD, Lundy S, Butovich I, Honn KV. 12(S)hydroxyeicosatetraenoic acid and 13(S)-hydroxyoctadecadienoic acid regulation of protein kinase C-a in melanoma cells: role of receptor-mediated hydrolysis of inositol phospholipids. Proc.Natl.Acad.Sci.U.S.A. 92 (1995) 9323–9327.

Liu B, Marnett LJ, Chaudhary A, Ji C, Blair IA, Johnson CR, Diglio CA, Honn KV. Biosynthesis of 12(S)-hydroxyeicosatetraenoic acid by B16 amelanotic melanoma cells is a determinant of their metastatic potential. Lab Invest. 70 (1994) 314–323.

Liu XH, Yao S, Kirschenbaum A, Levine AC. NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates bcl-2 expression in LNCaP cells. Cancer Res. 58 (1998) 4245–4249.

Marks F, Furstenberger G. *Prostaglandins, Leukotrienes and Other Eicosanoids*. Weinheim: Wiley-VCH, 1999, 388p.

Marnett LJ, DuBois RN. COX-2: a target for colon cancer prevention. Annu Rev Pharmacol Toxicol. 42 (2002) 55–80.

McCabe NP, Selman SH, Jankun J. Vascular endothelial growth factor production in human prostate cancer cells is stimulated by overexpression of platelet 12-lipoxygenase. Prostate. 66 (2006) 779–787.

Mehrotra S, Morimiya A, Agarwal B, Konger R, Badve S. Microsomal prostaglandin E_2 synthase-1 in breast cancer: a potential target for therapy. J Pathol. 208 (2006) 356–363.

Mehta P. Potential role of platelets in the pathogenesis of tumor metastasis. Blood. 63 (1984) 55–63.

Menter DG, Onoda JM, Taylor JD, Honn KV. Effects of prostacyclin on tumor cell-induced platelet aggregation. Cancer Res. 44 (1984) 450–456.

Miggin SM, Kinsella BT. Expression and tissue distribution of the mRNAs encoding the human thromboxane A2 receptor (TP) alpha and beta isoforms. Biochim Biophys Acta. 1425 (1998) 543–559.

Moussa O, Yordy JS, Abol-Enein H, Sinha D, Bissada NK, Halushka PV, Ghoneim MA, Watson DK. Prognostic and functional significance of thromboxane synthase gene overexpression in invasive bladder cancer. Cancer Res. 65 (2005) 11581–11587.

Muller-Decker K, Furstenberger G. The cyclooxygenase-2-mediated prostaglandin signaling is causally related to epithelial carcinogenesis. Mol Carcinog. 46 (2007) 705–710.

Murata T, Ushikubi F, Matsuoka T, Hirata M, Yamasaki A, Sugimoto Y, Ichikawa A, Aze Y, Tanaka T, Yoshida N, Ueno A, Oh-ishi S, Narumiya S. Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. Nature. 388 (1997) 678–682.

Nakano H, Shindo M, Sakon S, Nishinaka S, Mihara M, Yagita H, Okumura K. Differential regulation of IkB kinase a and beta by two upstream kinases, NF-kB-inducing kinase and

mitogen-activated protein kinase/ERK kinase kinase-1. Proc.Natl.Acad.Sci.U.S.A. 95 (1998) 3537–3542.
Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. Physiol Rev. 79 (1999) 1193–1226.
Needleman P, Minkes M, Raz A. Thromboxanes: selective biosynthesis and distinct biological properties. Science. 193 (1976) 163–165.
Nemoto S, DiDonato JA, Lin A. Coordinate regulation of IkB kinases by mitogen-activated protein kinase kinase kinase 1 and NF-kB-inducing kinase. Mol.Cell.Biol. 18 (1998) 7336–7343.
Nie D, Che M, Zacharek A, Qiao Y, Li L, Li X, Lamberti M, Tang K, Cai Y, Guo Y, Grignon D, Honn KV. Differential expression of thromboxane synthase in prostate carcinoma: role in tumor cell motility. Am J Pathol. 164 (2004) 429–439.
Nie D, Guo Y, Yang D, Tang Y, Chen Y, Wang MT, Zacharek A, Qiao Y, Che M, Honn KV. Thromboxane A2 receptors in prostate carcinoma: expression and its role in regulating cell motility via small GTPase Rho. Cancer Res. 68 (2008) 115–121.
Nie D, Hillman GG, Geddes T, Tang K, Pierson C, Grignon DJ, Honn KV. Platelet-type 12lipoxygenase in a human prostate carcinoma stimulates angiogenesis and tumor growth. Cancer Res. 58 (1998) 4047–4051.
Nie D, Honn KV. Cyclooxygenase, lipoxygenase and tumor angiogenesis. Cell Mol Life Sci. 59 (2002) 799–807.
Nie D, Krishnamoorthy S, Jin R, Tang K, Chen Y, Qiao Y, Zacharek A, Guo Y, Milanini J, Pages G, Honn KV. Mechanisms regulating tumor angiogenesis by 12-lipoxygenase in prostate cancer cells. J Biol Chem. 281 (2006) 18601–18609.
Nie D, Lamberti M, Zacharek A, Li L, Szekeres K, Tang K, Chen Y, Honn KV. Thromboxane A(2) regulation of endothelial cell migration, angiogenesis, and tumor metastasis. Biochem Biophys Res Commun. 267 (2000a) 245–251.
Nie D, Tang K, Diglio C, Honn KV. Eicosanoid regulation of angiogenesis: role of endothelial arachidonate 12-lipoxygenase. Blood. 95 (2000b) 2304–2311.
Nie D, Tang K, Szekeres K, Li L, Honn KV. Eicosanoid regulation of angiogenesis in human prostate carcinoma and its therapeutic implications. Ann.N.Y.Acad.Sci. 905 (2000c) 165–176.
Nithipatikom K, Isbell MA, See WA, Campbell WB. Elevated 12-and 20-hydroxyeicosatetraenoic acid in urine of patients with prostatic diseases. Cancer Lett. 233 (2006) 219–225.
Ohd JF, Nielsen CK, Campbell J, Landberg G, Lofberg H, Sjolander A. Expression of the leukotriene D4 receptor CysLT1, COX-2, and other cell survival factors in colorectal adenocarcinomas. Gastroenterology. 124 (2003) 57–70.
Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell. 87 (1996) 803–809.
Pearson G, English JM, White MA, Cobb MH. ERK5 and ERK2 cooperate to regulate NF-kB and cell transformation. J.Biol.Chem. 276 (2001) 7927–7931.
Pidgeon GP, Kandouz M, Meram A, Honn KV. Mechanisms controlling cell cycle arrest and induction of apoptosis after 12-lipoxygenase inhibition in prostate cancer cells. Cancer Res. 62 (2002) 2721–2727.
Pinto S, Gori L, Gallo O, Boccuzzi S, Paniccia R, Abbate R. Increased thromboxane A2 production at primary tumor site in metastasizing squamous cell carcinoma of the larynx. Prostaglandins Leukot Essent Fatty Acids. 49 (1993) 527–530.
Pollard M, Luckert PH, Schmidt MA. The suppressive effect of piroxicam on autochthonous intestinal tumors in the rat. Cancer Lett. 21 (1983) 57–61.
Rapoport SM, Schewe T, Wiesner R, Halangk W, Ludwig P, Janicke-Hohne M, Tannert C, Hiebsch C, Klatt D. The lipoxygenase of reticulocytes. Purification, characterization and biological dynamics of the lipoxygenase; its identity with the respiratory inhibitors of the reticulocyte. Eur J Biochem. 96 (1979) 545–561.

Romano M, Catalano A, Nutini M, D'Urbano E, Crescenzi C, Claria J, Libner R, Davi G, Procopio A. 5-lipoxygenase regulates malignant mesothelial cell survival: involvement of vascular endothelial growth factor. FASEB J. 15 (2001) 2326–2336.

Romano M, Claria J. Cyclooxygenase-2 and 5-lipoxygenase converging functions on cell proliferation and tumor angiogenesis: implications for cancer therapy. FASEB J. 17 (2003) 1986–1995.

Salcedo R, Zhang X, Young HA, Michael N, Wasserman K, Ma WH, Martins-Green M, Murphy WJ, Oppenheim JJ. Angiogenic effects of prostaglandin E2 are mediated by up-regulation of CXCR4 on human microvascular endothelial cells. Blood. 102 (2003) 1966–1977.

Schewe T, Halangk W, Hiebsch C, Rapoport SM. A lipoxygenase in rabbit reticulocytes which attacks phospholipids and intact mitochondria. FEBS Lett. 60 (1975) 149–152.

Shao J, Jung C, Liu C, Sheng H. Prostaglandin E2 Stimulates the beta-catenin/T cell factor-dependent transcription in colon cancer. J Biol Chem. 280 (2005) 26565–26572.

Shappell SB, Boeglin WE, Olson SJ, Kasper S, Brash AR. 15-lipoxygenase-2 (15-LOX-2) is expressed in benign prostatic epithelium and reduced in prostate adenocarcinoma. Am J Pathol. 155 (1999) 235–245.

Shappell SB, Keeney DS, Zhang J, Page R, Olson SJ, Brash AR. 15-Lipoxygenase-2 expression in benign and neoplastic sebaceous glands and other cutaneous adnexa. J Invest Dermatol. 117 (2001a) 36–43.

Shappell SB, Manning S, Boeglin WE, Guan YF, Roberts RL, Davis L, Olson SJ, Jack GS, Coffey CS, Wheeler TM, Breyer MD, Brash AR. Alterations in lipoxygenase and cyclooxygenase-2 catalytic activity and mRNA expression in prostate carcinoma. Neoplasia. 3 (2001b) 287–303.

Shureiqi I, Chen D, Lotan R, Yang P, Newman RA, Fischer SM, Lippman SM. 15-Lipoxygenase-1 mediates nonsteroidal anti-inflammatory drug-induced apoptosis independently of cyclooxygenase-2 in colon cancer cells. Cancer Res. 60 (2000) 6846–6850.

Shureiqi I, Wojno KJ, Poore JA, Reddy RG, Moussalli MJ, Spindler SA, Greenson JK, Normolle D, Hasan AA, Lawrence TS, Brenner DE. Decreased 13-S-hydroxyoctadecadienoic acid levels and 15lipoxygenase-1 expression in human colon cancers. Carcinogenesis. 20 (1999) 1985–1995.

Shureiqi I, Xu X, Chen D, Lotan R, Morris JS, Fischer SM, Lippman SM. Nonsteroidal antiinflammatory drugs induce apoptosis in esophageal cancer cells by restoring 15-lipoxygenase-1 expression. Cancer Res. 61 (2001) 4879–4884.

Siebert M, Krieg P, Lehmann WD, Marks F, Furstenberger G. Enzymic characterization of epidermis-derived 12-lipoxygenase isoenzymes. Biochem.J. 355 (2001) 97–104.

Sigal E, Grunberger D, Cashman JR, Craik CS, Caughey GH, Nadel JA. Arachidonate 15lipoxygenase from human eosinophil-enriched leukocytes: partial purification and properties. Biochem Biophys Res Commun. 150 (1988) 376–383.

Sonoshita M, Takaku K, Sasaki N, Sugimoto Y, Ushikubi F, Narumiya S, Oshima M, Taketo MM. Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc(-Delta 716) knockout mice. Nat Med. 7 (2001) 1048–1051.

Subbaramaiah K, Altorki N, Chung WJ, Mestre JR, Sampat A, Dannenberg AJ. Inhibition of cyclooxygenase-2 gene expression by p53. J Biol Chem. 274 (1999) 10911–10915.

Subbaramaiah K, Norton L, Gerald W, Dannenberg AJ. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer: evidence for involvement of AP-1 and PEA3. J Biol Chem. 277 (2002) 18649–18657.

Szekeres CK, Tang K, Trikha M, Honn KV. Eicosanoid activation of extracellular signal-regulated kinase1/2 in human epidermoid carcinoma cells. J.Biol.Chem. 275 (2000a) 38831–38841.

Szekeres CK, Trikha M, Nie D, Honn KV. Eicosanoid 12(S)-HETE activates phosphatidylinositol 3kinase. Biochem.Biophys.Res.Commun. 275 (2000b) 690–695.

Tang DG, Bhatia B, Tang S, Schneider-Broussard R. 15-lipoxygenase 2 (15-LOX2) is a functional tumor suppressor that regulates human prostate epithelial cell differentiation, senescence, and growth (size). Prostaglandins Other Lipid Mediat. 82 (2007) 135–146.

Tang DG, Chen YQ, Diglio CA, Honn KV. Protein kinase C-dependent effects of 12(S)-HETE on endothelial cell vitronectin receptor and fibronectin receptor. J Cell Biol. 121 (1993a) 689–704.

Tang DG, Diglio CA, Bazaz R, Honn KV. Transcriptional activation of endothelial cell integrin alpha v by protein kinase C activator 12(S)-HETE. J Cell Sci. 108 (Pt 7) (1995a) 2629–2644.

Tang DG, Diglio CA, Honn KV. Activation of microvascular endothelium by eicosanoid 12(S)hydroxyeicosatetraenoic acid leads to enhanced tumor cell adhesion via up-regulation of surface expression of alpha v beta 3 integrin: a posttranscriptional, protein kinase C-and cytoskeleton-dependent process. Cancer Res. 54 (1994) 1119–1129.

Tang DG, Grossi IM, Chen YQ, Diglio CA, Honn KV. 12(S)-HETE promotes tumor-cell adhesion by increasing surface expression of a V beta 3 integrins on endothelial cells. Int J Cancer. 54 (1993b) 102–111.

Tang DG, Renaud C, Stojakovic S, Diglio CA, Porter A, Honn KV. 12(S)-HETE is a mitogenic factor for microvascular endothelial cells: its potential role in angiogenesis. Biochem Biophys Res Commun. 211 (1995b) 462–468.

Thun MJ, Namboodiri MM, Heath CW, Jr. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med. 325 (1991) 1593–1596.

Timar J, Raso E, Dome B, Li L, Grignon D, Nie D, Honn KV, Hagmann W. Expression, subcellular localization and putative function of platelet-type 12-lipoxygenase in human prostate cancer cell lines of different metastatic potential. Int J Cancer. 87 (2000) 37–43.

Tong WG, Ding XZ, Witt RC, Adrian TE. Lipoxygenase inhibitors attenuate growth of human pancreatic cancer xenografts and induce apoptosis through the mitochondrial pathway. Mol Cancer Ther. 1 (2002) 929–935.

Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell. 93 (1998) 705–716.

Tsukada T, Nakashima K, Shirakawa S. Arachidonate 5-lipoxygenase inhibitors show potent antiproliferative effects on human leukemia cell lines. Biochem Biophys Res Commun. 140 (1986) 832–836.

Turk J, Maas RL, Brash AR, Roberts LJ, II, Oates JA. Arachidonic acid 15-lipoxygenase products from human eosinophils. J.Biol.Chem. 257 (1982) 7068–7076.

Waddell WR, Loughry RW. Sulindac for polyposis of the colon. J Surg Oncol. 24 (1983) 83–87.

Wang D, Wang H, Shi Q, Katkuri S, Walhi W, Desvergne B, Das SK, Dey SK, DuBois RN. Prostaglandin E(2) promotes colorectal adenoma growth via transactivation of the nuclear peroxisome proliferator-activated receptor delta. Cancer Cell. 6 (2004) 285–295.

Watkins G, Douglas-Jones A, Mansel RE, Jiang WG. Expression of thromboxane synthase, TBXAS1 and the thromboxane A2 receptor, TBXA2R, in human breast cancer. Int Semin Surg Oncol. 2 (2005) 23–30.

Whittaker N, Bunting S, Salmon J, Moncada S, Vane JR, Johnson RA, Morton DR, Kinner JH, Gorman RR, McGuire JC, Sun FF. The chemical structure of prostaglandin X (prostacyclin). Prostaglandins. 12 (1976) 915–928.

Winer I, Normolle DP, Shureiqi I, Sondak VK, Johnson T, Su L, Brenner DE. Expression of 12lipoxygenase as a biomarker for melanoma carcinogenesis. Melanoma Res. 12 (2002) 429–434.

Wong BC, Wang WP, Cho CH, Fan XM, Lin MC, Kung HF, Lam SK. 12-Lipoxygenase inhibition induced apoptosis in human gastric cancer cells. Carcinogenesis. 22 (2001) 1349–1354.

Wu KK, Liou JY. Cellular and molecular biology of prostacyclin synthase. Biochem Biophys Res Commun. 338 (2005) 45–52.

Xiao CY, Hara A, Yuhki K, Fujino T, Ma H, Okada Y, Takahata O, Yamada T, Murata T, Narumiya S, Ushikubi F. Roles of prostaglandin I(2) and thromboxane A(2) in cardiac ischemia-reperfusion injury: a study using mice lacking their respective receptors. Circulation. 104 (2001) 2210–2215.

Xu XC, Shappell SB, Liang Z, Song S, Menter D, Subbarayan V, Iyengar S, Tang DG, Lippman SM. Reduced 15S-lipoxygenase-2 expression in esophageal cancer specimens and cells and upregulation in vitro by the cyclooxygenase-2 inhibitor, NS398. Neoplasia. 5 (2003) 121–127.

Yamamoto S, Suzuki H, Ueda N. Arachidonate 12-lipoxygenases. Prog.Lipid Res. 36 (1997) 23–41.

Yoshimatsu K, Altorki NK, Golijanin D, Zhang F, Jakobsson PJ, Dannenberg AJ, Subbaramaiah K. Inducible prostaglandin E synthase is overexpressed in non-small cell lung cancer. Clin Cancer Res. 7 (2001) 2669–2674.

Yoshimoto T, Takahashi Y. Arachidonate 12-lipoxygenases. Prostaglandins Other Lipid Mediat. 68–69 (2002) 245–262.

Yoshimura R, Sano H, Masuda C, Kawamura M, Tsubouchi Y, Chargui J, Yoshimura N, Hla T, Wada S. Expression of cyclooxygenase-2 in prostate carcinoma. Cancer. 89 (2000) 589–596.