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Fatty acid modulation of tumor cell-platelet-vessel wall interaction

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Summary

Prostaglandins and other eicosanoids have been studied extensively in their physical, biochemical, biophysical and pharmacological aspects. However, studies on their role in tumor progression, especially metastases are relatively recent. Following a brief overview of the history of discovery and metabolism of eicosanoids and other fatty acids, we discuss the functions of these fatty acids (with emphasis on prostacyclin, thromboxane A_2, 12-hydroxyeicosatetraenoic acid and 13-hydroxyoctadecadienoic acid) in cell transformation, tumor promotion and particularly in tumor cell metastasis. The relation between these monohydroxy fatty acids and tumor cell metastasis is discussed from three different perspectives, i.e., their effects on tumor cells, on platelets and on endothelial cells. The mechanism of these effects are then addressed at cell adhesion molecule, motility, protease, cell cytoskeleton, protein kinase and eicosanoid receptor levels. Finally, regulation of three key enzymes which generate eicosanoids (phospholipase, prostaglandin endoperoxide synthase and lipoxygenase) is explored.

Prostagladins (PGs), thromboxanes (TXs), leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs) have been studied extensively since their discoveries. The vast biological activities of these eicosanoids and their pharmacological importance are beyond the scope of our review. Here, we will focus on thromboxane A_2 (TXA_2), prostacyclin (PGI_2) and 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE] and their activities which can affect tumor metastatic behavior. We will also discuss the effect of 13(S)-hydroxyoctadecadienoic acid [13(S)-HODE], a metabolite of linoleic acid, on tumor cells. However, a brief introduction on arachidonic acid (AA) and linoleic acid (LA) metabolism is desirable for readers who are not familiar with eicosanoids and other polyunsaturated fatty acids.

Historical overview

Research on prostaglandins and other fatty acids can be traced back about 60 years ago to two independent groups. In 1929, two biochemists George Burr and Mildred Burr at University of Minnesota discovered that fats contain a substance essential for growth and health. One year later, they identified the substance as linoleic acid, now known as one of the essential fatty acids. This 18-carbon fatty acid can be metabolized to hydroxyderivatives or converted to other essential fatty acids, i.e., dihomo-γ-linolenic acid and arachidonic acid. These two fatty acids then can be bioconverted to prostaglandins and other eicosanoids. Eicosanoid is a generic term, introduced by E.J. Corey, for oxygenated metabolites of polyunsaturated 20 carbon fatty acids. Greek eikosi means twenty. In 1930, New York gynecologist Raphael Kurzrok and pharmacologist Charles Leib observed that human semen could cause uterine contractions in some

women but relaxation in others, and strips of human uterine tissue would contract or relax when treated with different semen samples. This work was extended by Maurice Goldblatt in England and Ulf von Euler at the Karolinska Institute in Sweden. They reported that several substances in human seminal fluid influenced not only muscle contraction, but also blood pressure in animals. The name prostaglandin was suggested by von Euler in his paper of 1935. Impeded by World War II, purification and structural characterization did not occur until the 1950s. PGE_1 and $PGF_{1\alpha}$ (metabolites of dihomo-γ-linolenic acid) were, the first eicosanoids crystallized, which led to their structural elucidation in 1962. Soon, biosynthesis of prostaglandins and their analogs was elaborated and a variety of their biological activities was studied. With the discovery by Sir John Vane that aspirin and other nonsteroidal anti-inflammatory agents (NSAID) inhibited the biosynthesis of prostaglandins, significant attention focused on PG synthesis inhibitors for treatment of inflammation. Another major breakthrough in the prostaglandin field was announced in 1973 by Hamberg and Samuelsson at Karolinska as well as by Nugteren and Hazelhof at Unilever in The Netherlands, who isolated the previously postulated, arachidonic acid derived prostaglandin endoperoxides. On the other hand, in 1969, Priscilla Piper and Sir John Vane, then at the Royal College of Surgeons in London, described a short-lived substance formed in guinea-pig lung during anaphylaxis and named it rabbit aorta contracting substance (RCS). RCS appeared to contain a minor amount of PGG_2 and/or PGH_2, and a major unknown component which had a half-life of ~ 30 sec in water. Meanwhile, Svensson, Hamberg and Samuelsson at the Karolinska found a potent platelet aggregating factor resembling the major component of RCS. Later studies defined the structure of this unstable factor, now known as thromboxane A_2. Because they are similar regarding conditions under which they formed and their chemical and biological properties, TXA_2 and the major component of RCS are considered to be identical. In 1976, discovery of a new substance, whose major biological properties are opposite to those of TXA_2, was announced by Salvador Moncada, Ryszard Gryglewski, Stuart Bunting, and Sir John Vane who had moved to Wellcome Laboratories. In a collaborative effort by groups at Wellcome and Upjohn, this substance was identified as the PGI_2 and named prostacyclin by Vane. Prostacyclin is 30 times more active as an inhibitor of platelet aggregation than is PGE_1, and has a half-life of ~ 3 min at 37° C in aqueous solution. Thus, three important arachidonic acid metabolites, i.e., prostaglandins, prostacyclin and thromboxanes were identified. Another important group of metabolites of arachidonic acid, the leukotrienes, were first described in 1930 by Feldberg and Kellaway in England, and named slow-reacting substance (SRS) because of their slow onset of small intestine contraction. In the early 1950s, W.E. Brocklehurst coined the term 'slow reacting substance of anaphylaxis' or SRS-A to describe the reactive material generated during an anaphylactic event. Although SRS and SRS-A are generated by different means, they are considered to be identical and the terms are used interchangeably. During studies on polymorphonuclear leukocytes in 1975, Samuelsson and his colleagues identified one of the polar substances in SRS-A as (5S,12R)-5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid, now called leukotriene B_4. Samuelsson coined the term leukotriene to designate the conjugated triene of arachidonic acid metabolites derived from leukocytes. After the discovery of LTB_4, other LTs soon were identified. Hydroxyeicosatetraenoic acids were first found in plants and later in animals. In 1974, Hamberg and Samuelsson discovered that a human platelet lipoxygenase catalyzes the conversion of AA into 12-S-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12-HPETE) which is rapidly reduced to 12-HETE. Subsequently, they identified 5-HETE from polymorphonuclear leukocytes in 1976 and 15-HETE from neutrophils in 1979. However, until recently, there was little interest in HETEs because they were considered to be biologically inactive.

Arachidonic and linoleic acid metabolism

Arachidonic acid is released from phospholipids

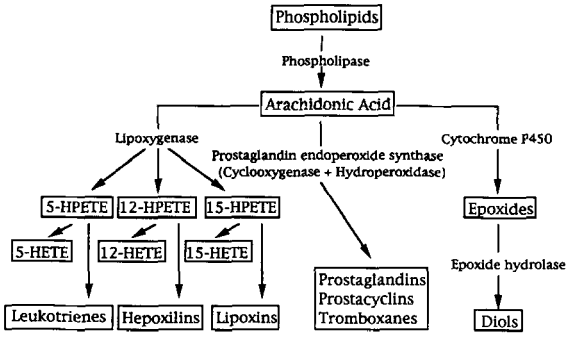

Fig. 1. Metabolism of arachidonic acid by lipoxygenase, prostaglandin endoperoxide synthase and cytochrome P450 monooxygenase. HPETE: hydroperoxyeicosatetraenoic acid; HETE: hydroxyeicosatetraenoic acid.

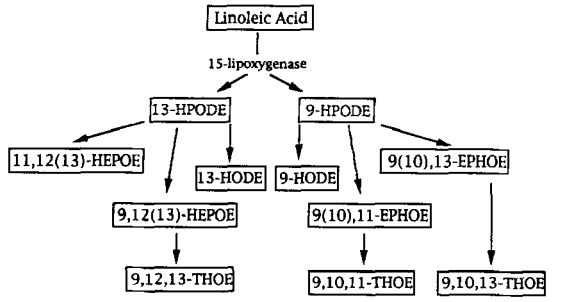

Fig. 2. Metabolism of linoleic acid by lipoxygenase. HPODE: hydroperoxyoctadecadienoic acid; HODE: hydroxyoctadecadienoic acid; HEPOE: hydroepoxyoctadecaenoic acid; THOE: trihydroxyoctadecaenoic acid.

mainly by the action of phospholipase A_2 and is the substrate for lipoxygenases, prostaglandin endoperoxide synthase and cytochrome P-450 monooxygenase (also known as epoxygenase) (Fig. 1). AA is metabolized to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) by 5-lipoxygenase (5-LOX), to 12-HPETE by 12-LOX and to 15-HPETE by 15-LOX. Then, 5-HPETE can be converted to 5-HETE and LTs, 12-HPETE to 12-HETE and hepoxilins (HXs), 15-HPETE to 15-HETE and lipoxins (LXs). AA can also be metabolized to prostaglandins, prostacyclin and thromboxanes by PG endoperoxide synthase, or to epoxides and diols by cytochrome P-450. Linoleic acid is metabolized to 13-hydroperoxyoctadecadienoic acid (13-HPODE) and 9-HPODE by 15-LOX. Subsequently, the HPODEs are converted into hydroxyoctadecadienoic acids (HODEs), hydroepoxyoctadecaenoic acids (HEPOEs) and trihydroxyoctadecaenoic acids (THOEs) (Fig. 2). Many reviews are available on eicosanoid metabolism, and readers interested in details are referred to references 1–4.

5-, 12-, 15-lipoxygenases and prostaglandin endoperoxide synthase are the major enzymes involved in arachidonic acid metabolism. These enzymes have been characterized immunologically and biochemically [2, 5]. Recently, cDNAs have been cloned from human [6] and rat [7] for 5-LOX; from human [8], porcine [9] and bovine [10] for 12-LOX; from human [11] and rabbit [12] for 15-LOX; from human [13], murine [14] and ovine [15] for PG endoperoxide synthase. The lipoxygenases are 662–674 amino acid proteins with a molecular weight ~75 kD, while the PG synthases are ~600 amino acid protein of 65–70 kD. Gene structure of human 5-LOX [16], rabbit 15-LOX [12], human [13] and murine [17] PG synthase have been determined. Human PG synthase have TATA box-like motif and several CCAAT sequences. In contrast to human PG synthase, the murine counterpart does not contain TATA box nor CCAAT box [17]. The promoter of 15-LOX contains TATA box while no CCAAT motif at the canonical position in 15-LOX upstream region was found. Interestingly, the 5-LOX promoter region contains no TATA and CCAAT sequences but multiple GC boxes. This raises the question whether 5-LOX is a housekeeping gene.

Fatty acid effects on tumor cells, platelets and endothelium

Fatty acids and cell transformation

Cell transformation represents the first step of tumorigenesis. In polyoma virus transformed hamster kidney fibroblasts, the production of PGE_2 and $PGF_2\alpha$ was significantly increased [18]. This enhanced PG synthesis was mainly due to the polyoma T antigen as demonstrated by the fact that the biosynthesis of PGs were 2-3 fold higher at the permissive than at the nonpermissive temperature using temperature-sensitive mutant transformed cells [18]. Further studies using polyoma virus

transformed Balb/c 3T3 indicated that polyoma transformation induced cells to lose inhibitory control, to continuously produce PGs without alteration in the PG degradation rate, and to increase the release of AA from cellular lipids [18]. Transformation by another virus of the Papovaviridae family, SV40, gave variable results [19, 20]. Barker *et al.* [21] showed that activation of $p60^{v\text{-}src}$ in chicken embryo fibroblasts increased the release of prostaglandins. They suggested that the effect may be due to alteration in signal transduction upstream of phospholipase A_2 (PLA_2) and PG synthase, and probably involving PLC and protein kinase C (PKC) pathways. Stimulation of PLA_2 also was observed in NIH3T3 transformed by membrane-associated oncogenes src, met and trk as well as by cytoplasmic oncogenes mos and raf, with no effect by nuclear oncogene fos and myc [22]. Han *et al.* [23] reported that PGH synthase (PGHS) was induced in murine fibroblasts transformed by Rous sarcoma virus. Oncogene v-src caused a persistent induction of PG synthase in contrast to the transient expression induced by PDGF. This persistent induction is probably independent of the PKC pathway since down-regulation of PKC inhibited induction of PG synthase by PMA but not by $p60^{v\text{-}src}$ [23]. Recently, a src-inducible PG synthase, termed miPGHSch, was cloned from chicken embryo fibroblasts [24]. It shares 59% homology with sheep PGHS. However, significant differences exist between miPGHSch and sheep PGHS. The most intriguing differences are that miPGHSch is in low abundance in nonproliferating cells and in high abundance in src-transformants, and the miPGHSch mRNA present in resting cells is found to be nonfunctional due to an unspliced intron that separates the signal peptide from the remainder of the protein [24]. Another unique feature is that the 2.3 kb-long, AU-rich 3′ untranslated region of miPGHSch mRNA contains 16 copies of Shaw-Kamen repeats (5′-ATTTA-3′) which confer instability to transcripts. The Shaw-Kamen sequence is found in multiple copies in the 3′ untranslated regions of many mRNAs that encode proteins involved in growth control, including cytokines and transcription factors. The 3′ untranslated region of the sheep 2.8 kb mRNA is not AU-rich, and contains only one copy of the ATTTA sequence.

Fatty acids and tumor promotion

The relationship between eicosanoid synthesis and tumorigenesis has been studied extensively. To various degrees, almost all cancers can form more prostaglandins, especially of the PGE and F series, than the normal tissues from which they are derived. PG synthesis has been studied in a variety of human and animal cancers such as carcinomas of the thyroid, carcinoid and renal phaeochromocytoma, Kaposis sarcoma, ganglioneuroma, neuroblastoma, breast cancer, fibrosarcoma, Moloney sarcoma, Chondrosarcoma, etc. [25]. The effects of PGs on tumor promotion are well documented [26, 27]. Both TPA-type and non-TPA type promoters increase prostacyclin [27] and thromboxane production [28]. In fact, one may expect that TPA stimulates an overall production of PGs and TXs, since it enhances PGH synthase activity. TPA up-regulation of PGHS has been observed in human endothelial cells [29], canine kidney [30] and urothelial [31] cells, rat tracheal epithelial cells [32]. TPA may regulate PGHS at transcription [32] and/or translation [29] levels. It has also been shown that TPA stimulates arachidonic acid release [33]. TPA stimulation may be mediated by protein kinase C [32, 33]. Interestingly, similar stimulation can be achieved by growth factors, cytokines and hormones such as PDGF [34, 35], EGF [36, 37], TNF [38], IL-1 [38–40] and epinephrine [41]. Actually, PGHS is an immediate-early gene induced by IL-1 in human endothelial cells. The inducibility by growth factors, cytokines and hormones plus the alternative splicing control of miPGHSch in resting and src-transformed cells makes the PGHS gene an interesting model to study the regulation of eicosanoid metabolism during cell transformation and tumor progression. In contrast to the work on the relationship between PGHS products and cancer, until recently, little has been done to study the relationship of lipoxygenase products (i.e., HETEs, lipoxins, leukotrienes and hepoxilins) to

tumor initiation, promotion, growth, invasion and metastasis.

Tumor cell metastasis

Metastasis is a sequential process which requires multiple phenotypic factors expressed by tumor cells and is subjected to distinct stimulus regulation [42]. During the hematogenous phase of metastasis, tumor cells undergo a variety of cell-cell and cell-matrix interactions, some of which are deleterious (i.e., interaction with cells of the immune system) [43] and some of which are favorable to tumor cell survival (i.e., interaction with platelets and attachment to endothelial cells) [44]. Two cell types, i.e., platelets and endothelium with which tumor cells interact during hematogenous metastasis are capable of producing a vast array of lipid mediators by either direct or transcellular metabolism of precursors. Only a few of the many fatty acids, capable of being synthesized by platelets and endothelium, have been examined for their relationship to metastasis. The notable exceptions are PGI_2, TXA_2, 12-HETE and 13-HODE. The remainder of this review will focus on these four fatty acids as well as their synthetic analogs and their relationship to a series of key events in the metastatic cascade. Specially we will discuss tumor cell arrest and adhesion to endothelium, induction of platelet aggregation, induction of endothelial cell retraction, tumor cell motility and spreading on subendothelial matrix, attachment to that matrix and protease dependent extravasation. Tumor cell arrest in the microvasculature is a critical event in hematogenous metastasis [43]. Following arrest and attachment to endothelial cells, platelets rapidly associate with the arrested tumor cell, and then they disappear soon after tumor cell contact with the extracellular matrix is completed [44–46]. While associated with platelets, tumor cells induce endothelial cell retraction [44–47], spread on subendothelial matrix, release proteases and finally migrate through the subendothelial matrix [44, 45].

Platelets are capable of synthesizing PGD_2, PGE_2 and $PGF_{2\alpha}$ but not prostacyclin. The major PGHS product of AA in platelet is TXA_2. Biosynthesis of TXA_2 is associated with intracellular membrane components of the dense tubular system. Platelets also metabolize AA through the lipoxygenase pathway and the major product is 12-HETE. No leukotrienes and other HETEs have been found [48]. The main product of AA in vascular tissue is prostacyclin and endothelial cells are the most active cell type to produce prostacyclin. Synthesis of TXA_2, PGE_2, $PGF_{2\alpha}$ and a small amount of PGD_2 has also been demonstrated in vascular tissue [48]. Endothelial cells also produce a significant amount of 13-HODE from linoleic acid by 15-lipoxygenase [49] and the presence of 5-LOX and leukotriene-like material has been demonstrated as well as 12-LOX activity [4, 50].

In contrast to platelets, vascular endothelial cells elaborate PGI_2 as their major PGHS metabolite which plays a paramount role in maintaining the normal non-thrombogenicity of the vessel wall by preventing platelet adhesion and aggregation. Honn *et al.* [51] first demonstrated that exogenous PGI_2 significantly inhibited B16 amelanotic melanoma (B16a) cell lung colony formation in syngeneic mice, and that inhibitors of PGI_2 synthesis increased the experimental metastases of B16a cells. This experimental finding established, for the first time, that PGI_2, and possibly its synthetic analogs, possess anti-metastatic potential which may have therapeutic significance. Thereafter, a number of experiments conducted in laboratories worldwide have provided confirmative evidence for the anti-metastatic effect of PGI_2 and its analogs including epoprotenol, eptaloprost, iloprost and cicaprost (Table 1 and references therein). So far the anti-metastatic effects have been observed on a wide spectrum of tumor cells from different species and of different histopathological types, including melanoma, fibrosarcoma, reticulum carcinoma, and the carcinomas of the lung, breast, colon, pancreas and the prostate, although a few negative results also have been reported (Table 1).

Based on early experimental observations with exogenous PGI_2, Honn *et al.* [51, 116, 133] proposed that the production of PGI_2 by the vascular endothelium may constitute a natural deterrent

to hematogenous metastasis. Other experiments also revealed that treatment of tumor cells with TXA_2 synthase inhibitors also inhibited their lung-colonizing capacity [133]. This led to the further hypothesis that EC-derived PGI_2 and platelet-elaborated TXA_2 represent two antagonistic biological mediators whose balanced ratio maintains the adhesivity and thrombogenicity of the vessel wall and modulates tumor cell adhesion, that highly metastatic cells disrupte and shift the PGI_2/TXA_2 balance to a proaggregatory condition in favor of thrombosis and metastasis, and that modulation of PGI_2/TXA_2 ratio may be a rational and effective approach to disrupt of the metastatic cascade [53, 134–136]. Many lines of experimental evidence accumulated so far have lent support to the validity of the above proposals. First, it was noted that when mice were perfused with 15-HPETE (hydroperoxy fatty acids in general are potent inhibitors of prostacyclin synthase) 20 min prior to tail vein injection of B16a tumor cells, three to five times more pulmonary metastatic lesions were observed. Increased macroscopic tumors were also found in the liver and spleen. Infusion of 15-HPETE followed by PGI_2 plus theophylline significantly reduced the number of metastases compared to 15-HPETE alone [53; see ref 57 for review]. Second, Menter *et al.* [137] examined tumor cell-platelet-endothelial cell interactions under dynamic conditions involving shear forces. Rat aortic EC were grown on microcarrier beads and coincubated with rat platelets and Walker 256 carcinosarcoma cells in an aggregometry cuvette. These microcarrier grown EC inhibited TCIPA in a dose dependent fashion.

Table 1. Anti-metastatic effects of prostacyclin (PGI_2) and its analogs[#]

Chemical	Effect[§]	Tumor cells	Year reported	Reference
Prostacyclin	+	B16a melanoma	1981	Honn *et al.* [51]
Forskolin*	+	B16-F10	1983	Agarwall & Parks [115]
Prostacyclin	+	B16a, 3LL	1983	Honn [116]
Epoprostenol	+	Lung carcinoma	1984	Tanaka & Fukumoto [117]
Prostacyclin	−	3LL, B16a, colon carcinoma	1984	Karpatkin *et al.* [118]
Prostacyclin	±	Lung carcinoma, B16	1984	Gorelik *et al.* [119]
Prostacyclin	+	B16a	1987	Jeney *et al.* [120]
Prostacyclin	+	Fibrosarcoma	1988	Mahalingam *et al.* [121]
PG12 & Iloprost	+	BL6 melanoma	1988	Constantini *et al.* [122]
PGI2-TEI8153	+	Fibrosarcoma	1988	Niitsu *et al.* [123]
Iloprost	+	3LL	1989	Sava *et al.* [124]
Prostacyclin	+	Pancreatic carcinoma	1989	Kato *et al.* [125]
Prostacyclin	+	Pancreatic carcinoma	1990	Tzanakakis *et al.* [126]
Prostacyclin	+	3LL	1990	Lapis *et al.* [127]
Cicaprost Iloprost Eptaloprost	+	B16BL6 melanoma	1990	Constantini *et al.* [128]
Iloprost Eptaloprost	+	Prostate carcinoma	1990	Schneider *et al.* [129]
Cicaprost Eptaloprost	+	3LL	1990	Giraldi *et al.* [130]
Cicaprost	+	Reticulum carcinoma	1991	Schirner & Schneider [131]
Cicaprost	+	Reticulum carcinoma; 3LL; prostate and mammary carcinoma	1992	Schirner & Schneider [132]

[#] Modified from 71 with permission.

* Not a prostacyclin analog. Included because it also activates adenylate cyclase and thus elevates cAMP.

[§] + Anti-metastatic effect; − no anti-metastatic effect; ± in this case, PGI_2 alone showed little anti-metastatic effect, but combination with heparin resulted in dramatic metastasis-suppressing effect.

The ability of the microcarrier grown EC to inhibit TCIPA was dependent upon their endogenous production of PGI_2. This conclusion was based on the following results: 1) an increased number of microcarrier grown EC caused a prolongation of aggregation lag time; 2) an increased number of microcarrier grown EC caused a proportional increase in PGI_2 synthesis measured as the metabolite, 6-keto-PGF1α; 3) an increased number of microcarrier grown EC resulted in an inverse correlation with TXA_2 production by platelets; 4) indomethacin pretreatment of microcarrier grown EC caused decreased PGI_2 production which resulted in a decreased ability of these EC to inhibit TCIPA and tumor cell adhesion to EC; 5) the dose dependency for inhibition of TCIPA and tumor cell adhesion to EC by endogenous PGI_2 was the same as that observed for exogenous PGI_2. Third, a Rous sarcoma virus transformed EC line (RCEt1) was derived from primary rat cerebral EC [53]. A comparison was made of the adhesion of rat Walker (W256) carcinosarcoma cells to monolayers of RCEt1 and to monolayers of normal rat EC (RAEC) in the presence and absence of platelets. The transformed EC demonstrated a normal morphology and elaborated factor VIII, von Willebrand factor and angiotensin converting enzyme. However, these cells lacked the ability to produce PGI_2 in response to external stimuli. As shown in Fig. 3A, when W256 carcinosarcoma cells alone or together with washed rat platelets were added to monolayers of RCEt1 cells, there was no change in terms of PGI_2 production by these monolayers. In contrast, when W256 cells or W256 cells plus washed platelets were added to normal rat EC a significant increase in PGI_2 production was observed (Fig. 3A). Not unexpectedly, both W256 cells alone and W256 cells plus platelets adhered to the PGI_2 deficient RCEt1 monolayer to a greater extent than to a normal rat EC monolayer (Fig. 3B). Fourth, clinical studies have suggested that PGI_2 synthesis, the PGI_2/TXA_2 ratio, and/or PGI_2 biological $T_{1/2}$ are altered in many cancer patients. For example, a decreased plasma or urinary PGI_2/TxA_2 ratio has been observed in patients with breast cancer, metastatic ovarian cancer, carcinoma of the head and neck, renal cell carcinoma, gestational choriocarcinoma, and also in many animal tumors [57, 138–140]. In comparing the urinary excretion of PGI_2 and TXA_2 metabolites in 19 women with gestational choriocarcinoma and 20 healthy age-matched women, Aitokallio-Tallberg *et al.* [140] observed that PGI_2/TXA_2 ratio was decreased in cancer patients as compared to the controls. The decrease in PGI_2/TXA_2 may be due to a lower synthesis of PGI_2 or a higher synthesis of TXA_2, or even a decreased bioactivity of PGI_2 (i.e., the ability to inhibit tumor cell induced platelet aggregation and the duration of this inhibitory effect) which was observed in some cancer patients [141]. Furthermore, it was recently shown that metastatic cells had decreased NO synthase activity [142] whose enzymatic product is nitric oxide, a potent anti-aggregatory agent, the activity of which is potentiated by PGI_2. Fifth, exogenous PGI_2 or its analogs and TXA_2 synthase inhibitors or TXA_2 receptor antagonists were shown to inhibit tumor cell induced platelet aggregation (TCIPA), tumor cell adhesion to endothelium, and cancer metastasis [143–146]. Some calcium channel blockers could potentiate the antimetastatic effect of PGI_2 [147]. Finally, many other agents which mechanistically mimic the action of PGI_2 (i.e., increasing intracellular cAMP levels), including foskolin, nafazatrom, and phosphodies-

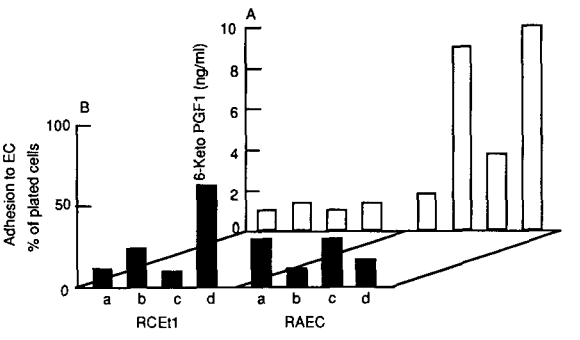

Fig. 3. Endogenous PGI_2 derived from EC inhibits tumor cell adhesion. Depicted is the relationship between the PGI_2 production by RCEt1 (a Rous sarcoma virus transformed EC line), RAEC (rat aortic EC) and platelet enhanced tumor cell adhesion to EC. a, W256 cell (2.5×10^4) alone; b, W256 cells plus washed rat platelets (3×10^8); c, W256 cells plus 0.1% platelet poor plasma (PPP); d, W256 cells plus platelets (3×10^8) and 0.1% PPP. Taken from ref 57 with permission.

terase inhibitors also demonstrated antimetastatic effects [143, 146; see ref 57 and 71 for reviews].

Multiple mechanisms can be involved in the wide-spectrum *in vivo* anti-metastatic effects of PGI_2. PGI_2 (and thromboxane synthetase inhibitors) have been observed to inhibit tumor cell proliferation and DNA synthesis by a cAMP-dependent mechanism [133]. Constantini *et al.* [122, 128] have shown that the metastasis-suppressing effects of PGI_2 or its analogs may be mediated by its potentiation of host immunocompetence. More recent experimental results of ours indicate that inhibition of TCIPA, tumor cell adhesion to endothelium and subendothelium matrix and tumor cell-induced platelet-enhanced endothelial cell retraction by PGI_2 and its analogs, may be the most important and revelant mechanisms [53, 67, 136, 145; see ref 57, 58 and 71 for reviews]. Other mechanisms, such as synergism with nitric oxide (NO, which inhibits TCIPA) may also be involved [142]. At the molecular level, PGI_2 activates adenylate cyclase through membrane-bound G-protein (Gs). Elevated intracellular cAMP activates protein kinase A, which subsequently phosphorylates myosin light chain kinase. Phosphorylated myosin light chain kinase loses its ability to catalyze the phosphorylation of myosin light chain, therefore, cytoskeleton-driven cell shape change is inhibited [see ref 71 for detailed discussions]. This may explain the inhibition by PGI_2 (or its analogs such as cicaprost) of tumor cell-induced endothelial cell retraction. Also, direct phosphorylation of vimentin by protein kinase A leads to the disruption of the vimentin filaments [148]. Increased intracellular cAMP may block the functional activation of aIIbβ3 integrin receptor [149]. Concerted action of these two effects resulting from PGI_2 activation of the protein kinase A explain the decreased TCIPA and tumor cell adhesion to endothelial cells as described above.

Arachidonic acid metabolite regulation of TCIPA

Recently, we demonstrated that a lipoxygenase metabolite of AA, i.e. 12-(S)-HETE, enhances tumor cell induced platelet aggregation [58, 59]. The role of 12-HETE and TXA_2 in TCIPA was examined in a homologous system [60]. Rat Walker 256 carcinosarcoma cells and rat platelets were used in the *in vitro* assay. Tumor cells induced the aggregation of rat platelets via a thrombin-dependent mechanism with concomitant production of 12-HETE and TXA_2. The extent of TCIPA was dependent on the concentration of tumor cells used to induce aggregation and the biosynthesis of platelet PGH synthase (PGHS) and lipoxygenase (LOX) products, i.e. TXA_2 and 12-HETE, were correlated with tumor cell concentration. PGHS and lipoxygenase inhibitors were used to treat platelets before aggregation assays. PGHS inhibitors, but not LOX inhibitors, blocked TCIPA at a low concentration of agonist (8.8×10^4 tumor cells). At a high concentration of agonist (5×10^5 tumor cells), neither PGHS nor LOX inhibitors alone affected platelet aggregation, however, the combination of both inhibitors resulted in subsequent inhibition of TCIPA regardless of agonist concentration. These results suggest that both the platelet PGH synthase and lipoxygenase products are important for tumor cell induced platelet aggregation. TXA_2 and 12-HETE employ different mechanisms during TCIPA but they may act in synergism.

Adhesion to endothelium

Tumor cell interaction with endothelial cells of the vessel wall is a critical step in tumor cell metastasis. 12(S)-HETE increased the adhesion of murine B16a cells to endothelium derived from pulmonary microvessels. All other monohydroxy and dihydroxy fatty acids were ineffective. LTB_4 induced a modest stimulation but LTC_4, LTD_4 as well as LXA_4 and LXB_4 were ineffective [61]. The 12(S)-HETE enhanced adhesion of B16a cells was inhibited by pretreatment with 13(S)-HODE but not by 13(R)-, 9(S)-HODE or 13-OXO-ODE [61b]. 12(S)-HETE stimulated adhesion was also observed in Lewis lung carcinoma cells (3LL) [62] and Walker carcinosarcoma cells (W256) [150]. 12(S)-HETE, but not 12(R)-HETE, enhanced 3LL cell adhesion to endothelial cells, subendothelial matrix and fibronectin, but not type IV collagen [62].

Similar enhancement of adhesion can be obtained by treatment of tumor cells with phorbol ester PMA. Interestingly, the PMA effect appears to be mediated by lipoxygenase metabolites [possibly by 12(S)-HETE], as it can be reversed by lipoxygenase inhibitors but not PGHS inhibitors. These results suggest that 12(S)-HETE can be a physiological mediator during cell processes such as adhesion. 12(S)-HETE stimulated adhesion of both 3LL and W256 cells to endothelium can be blocked by 13(S)-HODE [62, 150].

Tumor cell induced endothelial cell retraction

The induction of EC retraction is crucial for tumor cell interaction with subendothelial matrix and subsequent extravasation. Large vessel ECs demonstrated reversible retraction in response to 12(S)-HETE, but not the stereoisomer 12(R)-HETE or other lipoxygenase metabolites i.e. 5(S)-HETE and 15(S)-HETE. The induction of retraction by 12(S)-HETE was both dose and time dependent with no cytotoxic effect on EC. Scanning electron microscopy confirmed the 12(S)-HETE induced EC retraction and revealed collapsed filopodia on the cell surface, appearance of spaces between EC and underlying subendothelial matrix, and large gaps between adjacent EC [63]. Similarly, 12(S)-HETE also induces the retraction of pulmonary microvascular EC [65 and unpublished observation]. The EC retraction was observed 15 min after 12(S)-HETE treatment, and reached a peak level between 1–2 h. The monolayer reformed by 24 h. Silver staining and 'gap-FRAP' experiments suggest that 12(S)-HETE altered the normally apposed cell junctions and impaired gap junction-mediated cell-cell communication. It appeared that the 12(S)-HETE effect was mediated by cytoskeletal alteration. The first observed alteration in EC cytoskeleton following 12(S)-HETE stimulation is vimentin bundling, followed by the rearrangement and disruption of vinculin-containing adhesion plaques and/or simultaneous redistribution of α-actinin, and disruption of spectrin. These changes are accompanied by progressive microfilament dissolution. During the same time interval, α-actinin is mobilized to the cell periphery at cell 'ruffles'. The distribution of myosin along the stress fibers also appeared to be disrupted in some cells. However, 12(S)-HETE showed little or no effects on the actin binding proteins filamin and tropomyosin, or on microtubules.

Spreading

Tumor cell adhesion to matrix protein such as fibronectin can be described by two distinct steps, i.e. the initial tumor cell attachment to matrix and spreading on that matrix. Treatment of B16a cells with 12(S)-HETE shortens their spreading time from 50 min (control) to 15 min [64], suggesting that 12(S)-HETE has a significant effect on the spreading process. However, 12(S)-HETE may have multiple effects on tumor cells and it may accelerate other processes during the tumor cell-endothelium interaction other than spreading.

Source of 12(S)-HETE and 13(S)-HODE

Platelets are a rich source of 12(S)-HETE and they synthesize 12(S)-HETE in response to tumor cells as an agonist [66], therefore, platelets may be the primary contributors of 12(S)-HETE to facilitate tumor cell induced endothelial cell retraction. When murine platelets and 3LL carcinoma cells were simultaneously added to murine pulmonary microvessel EC monolayers, a platelet dose dependent increase in EC retraction was observed over the retraction induced by 3LL cells alone or platelets alone [57]. Pretreatment of platelets with the LOX inhibitor NDGA, but not the PGHS inhibitor indomethacin, abolished their ability to enhance tumor cell induced EC retraction. These results suggest that platelet derived 12(S)-HETE is the agent responsible for the acceleration of tumor cell induced EC retraction. When platelets were pretreated with PGI_2 or its analogues Cicaprost and Iloprost, the ability of these treated platelets to enhance tumor cell induced EC retraction was abolished as well [67]. Yet, the ability of tumor cells to induce EC retraction was not significantly

affected. Since 12(S)-HETE is also produced by a number of tumor cells such as 3LL [68] and Walker 256 carcinosarcoma cells [150], pretreatment of tumor cells with NDGA, reduced their ability to induce EC retraction while indomethacin was ineffective. Treatment of 3LL tumor cells with PGI_2, Iloprost or Cicaprost also inhibits their ability to induce EC retraction. These results indicate that platelet enhanced, tumor cell induced EC retraction can be either blocked by decreasing the level of 12(S)-HETE with LOX inhibitors or counterbalanced by prostacyclin. Similarly, if the EC monolayer was treated with PGI_2 or its analogues prior to the addition of tumor cells, the EC monolayer no longer retracts in response to tumor cell induction [57]. Collectively, 1) 12(S)-HETE is a stimulant for tumor cell induced EC retraction; 2) 12(S)-HETE can be derived from both platelets and tumor cells, therefore treatment of tumor cells and/or platelets with LOX inhibitors can inhibit tumor cell induced EC retraction; 3) platelets, tumor cells and endothelial cells can be the three targets for PGI_2 inhibitory action.

Previously, we discussed the inhibitory effect of 13(S)-HODE on 12(S)-HETE stimulated tumor cell adhesion. 13(S)-HODE may also have an inhibitory effect on the subsequent processes following adhesion such as tumor cell induced EC retraction and metastasis. Endothelial cells (EC) produce a significant amount of 13-HODE which acts as a chemorepellant to maintain the vessel wall thromboresistance. The 13-HODE production is decreased by thrombin, calcium ionophore, and trypsin stimulation [49] as well as by synthetic peptide fMLP and cytokines such as IL-1, TNF treatment [69]. When 13-HODE synthesis is increased in the resting EC by adding exogenous dbcAMP or by growing the cells in the presence of a phosphodiesterase inhibitor (dipyridamole), there is a corresponding dose-related decrease in the ability of tumor cells to adhere to the EC surface. In contrast, when 13-HODE is decreased, tumor cell adhesion to EC increases [69]. It has been shown that 13-HODE, produced either by tumor cells or endothelial cells, can inhibit tumor cell adhesion to EC and pulmonary metastasis. Secondary tumor cell metastasis is blocked more effectively by increasing vessel wall 13-HODE level via diet than by increasing tumor cell 13-HODE [70]. However, exogenous 13(S)-HODE reduces lung colony formation by high metastatic B16a cells as well as 12(S)-HETE stimulated lung colony formation by low metastatic B16a cells [61].

Mechanism of action

Effects on cell adhesion receptors

Platelet adhesion to vascular endothelial cells and subendothelial matrix, and interaction with circulating cells, e.g. leukocytes, tumor cells, depend on surface adhesion molecules. So far, members of three major families of adhesion molecules, i.e., integrins, immunoglobulins (Igs) and selectins have been localized to platelets [71]. In addition, platelets also express some leucine-rich glycoproteins (LRG) and some other minor groups of proteins that may serve certain adhesional functions. No cadherins have been identified on platelets [71]. Integrins are heterodimeric (an α subunit non-covalently associated with a β subunit) surface glycoproteins which are involved in both cell-cell and cell-matrix interactions. Platelets express at least five integrin receptors, i.e. $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha IIb\beta 3$ and $\alpha v\beta 3$, and the $\alpha IIb\beta 3$ which is the most abundant adhesion receptor and plays a key role in platelet aggregation [71]. On unstimulated platelets, $\alpha IIb\beta 3$ is randomly dispersed on the surface and is capable of recognizing only immobilized fibrinogen. Platelet stimulation and aggregation rapidly causes the $\alpha IIb\beta 3$ complex to become associated with cytoskeleton [72] and to develop binding affinities for several soluble adhesive glycoproteins including fibrinogen, fibronectin, vitronectin, von Willebrand factor and thrombospondin [73]. Like platelets, tumor cells express a variety of adhesion molecules. Among them, integrin $\alpha IIb\beta 3$ is one of the most interesting receptors and best studied by us on tumor cells. The $\alpha IIb\beta 3$ was considered to be expressed only in platelets and cells of megakaryoblastic potential. We first observed that an $\alpha IIb\beta 3$-like protein was expressed in solid tumor cell lines by immunocytochemistry [74]. Later, mRNA en-

coding for αIIb and β3 were detected by Northern blot analysis in several human and murine tumor cells [75]. Recently, αIIb and β3 mRNA were amplified from B16a cells by reverse transcription-polymerase chain reaction (RT-PCR) using specific primers. Partial DNA sequencing revealed that B16a αIIb shares 80% and 90% homology with human and rat αIIb, respectively, and only 26% homology with human αv [76]. In addition, RT-PCR combined with sequencing was employed to screen the expression of αIIb on a wide spectrum of tumor cells (about 20 lines) from different species and of different histological and pathological types. Preliminary results indicate that αIIb is widely expressed among the tumor cells [77].

Integrin αIIbβ3 complex or individual subunits were detected by immunoprecipitation with complex specific antibody or by Western blotting with subunit specific antibodies [76]. Tumor cells organize the αIIbβ3 receptor to focal adhesions, cell filapodia, or to the cell surface [74, 78]. Intracellularly, this integrin receptor colocalizes with the intermediate filament vimentin [59]. Treatment of tumor cells with anti-αIIbβ3 antibodies inhibited tumor cell-induced platelet aggregation [59, 74, 78, 79], tumor cell adhesion to matrix proteins or to endothelium [62, 79] and experimental metastasis [79, 80]. As we know, 12-HETE and TXA_2 are synthesized by activated platelets in response to tumor cells [66]. Pretreatment of tumor cells with anti-αIIbβ3 antibody inhibited the generation of platelet TXA_2 and 12-HETE [59], indicating integrin-integrin interaction transduces a signal for eicosanoid production. Cell surface immunoreactivity of B16a cells for αIIbβ3 can be enhanced by pretreatment with exogenous 12-(S)-HETE in a dose dependent manner while other lipoxygenase metabolites are ineffective. B16a cells possess a large intracellular pool of αIIbβ3, from which the receptor complex translocates to the cell surface following 12-(S)-HETE pretreatment. This pretreatment of tumor cells enhances their adhesion to and spreading on fibronectin, which is mediated exclusively by αIIbβ3 receptors [64, 78]. In contrast, 13(S)-HODE [62], prostacyclin or its analogues [81] and lipoxygenase inhibitors [60] down-regulated αIIbβ3 cell surface expression. However, the enhanced αIIbβ3 expressed was not inhibited by a PGHS inhibitor (aspirin), a thromboxane synthase inhibitor (CGS-14854) or stimulated by a TXA_2 mimic (pinane-TXA_2) [60]. 12-(S)-HETE also facilitates the redistribution of αIIbβ3 in the plasma membrane with localization at the focal adhesion plaques. Disruption of intermediate filaments and/or microfilaments prevents the 12-(S)-HETE induced increase in plasma membrane αIIbβ3, and 12(S)-HETE enhanced tumor cell adhesion to fibronectin. The microtubule-disrupting agent, colchicine, is ineffective in both respects. These results suggest that 12-(S)-HETE regulates the surface expression and function of αIIbβ3 integrin in B16a cells in a cytoskeleton-dependent manner [78]. Disruption of tumor cell cytoskeleton also prevented tumor cell-platelet interaction [59]. In addition, 12(S)-HETE not only can regulate integrin αIIbβ3 at the post-translational level as discussed above, but also may have an effect on the transcription of αIIbβ3. Four subpopulations of B16a cells, possessing different abilities to induce platelet aggregation and to form lung colonies, were isolated by centrifugal elutriation. A gradial increase in mRNAs and cell surface immunoreactivity of the αIIbβ3 receptor, but not in their gene copies, was observed from the low to the high metastatic subpopulations. The ability of tumor cells to adhere to fibronectin and subendothelial matrix increased in parallel. In the high metastatic cells, the αIIbβ3 receptors were localized to focal adhesion plaques. Incubation of the high metastatic cells with αIIbβ3 specific antibodies reduced their matrix adhesion, TCIPA and lung colonization abilities. The data indicate that enhanced metastatic potential of B16a subpopulations may be mediated by an increased expression of αIIbβ3 receptors and that expression of these receptors may be regulated at the transcriptional level. Interestingly, when comparing the level of endogenous 12(S)-HETE and 13(S)-HODE biosynteshis, a 3-fold greater amount of 12(S)-HETE was found in the high metastatic subpopulation than that in the low metastatic subpopulation while the amount of 13(S)-HODE is similar in both subpopulations [82, and unpublished observations]. However, this correlation may be casual rather than causal. Fur-

ther studies are needed to elucidate the mechanism. Based on the above observations, we hypothesized that tumor cell αIIbβ3 serves as a multifunctional receptor mediating tumor cell-platelet-endothelium interactions. Indeed, a nearly linear correlation (R = 0.918–0.978) can be observed in B16a cells between the αIIbβ3 expression and cell adhesion to matrix, or TCIPA or lung colonization [79]. In the previous section we discussed that 12(S)-HETE induces large vessel and microvessel endothelial cell retraction. It is intriguing that the biological response to 12(S)-HETE treatment is significantly different in EC from tumor cells, i.e., 12(S)-HETE induces EC retraction whereas it induces tumor cell spreading. Vitronectin receptor (αvβ3) and fibronecting receptor (α5β1) are two major integrins in endothelial cells. These two receptors were localized to both cell-matrix (focal adhesions) and cell-cell (cell borders) contact sites in confluent EC monolayer. Both receptors were functional in that specific antibodies inhibited EC adhesion to intact subendothelial matrix while only αvβ3 antibody disrupted the monolayer integrity. 12(S)-HETE induced a significant redistribution of the αvβ3-containing focal adhesions, leading to a decrease in αvβ3-containing plaques, whereas 12(S)-HETE did not demonstrate any significant effect on the distribution pattern and the surface expression of α5β1 [83]. Therefore, 12(S)-HETE induced EC retraction may be mediated by redistribution of αvβ3 integrins.

Effect on cell motility and release of protease

Besides the effects on integrins, 12(S)-HETE also appears to regulate other factors which are important to tumor cell metastasis. Pretreatment of K-1735 murine melanoma cells with 12(S)-HETE at a concentration of 0.1 μM stimulated (2-fold) cell migration. An analysis of cell surface for the expression of the autocrine motility factor receptor (AMF-R) by immunofluorescence revealed an increase in the amount of AMF-R at the leading edge of the treated cells [84]. B16a cells in culture were shown to release both native and latent forms of cathepsin B. Release of native form and total cathepsin B activity from B16a cells was significantly increased by 12(S)-HETE treatment. In contrast, the release of latent cathepsin B activity was not altered. The effect of 12(S)-HETE on release of cathepsin B was time dependent; values returned to control levels after 30–60 min of exposure [85 and unpublished observations]. In addition, incubation with 12(S)-HETE for 15 min resulted in a 40% decrease in the activity of heat stable cysteine proteinase inhibitors (CPIs) in the media of B16a cells [85]. Thus 12(S)-HETE can have opposite effects on the release of cathepsin B and CPIs.

Effect on cell cytoskeleton

The ability of 12(S)-HETE to enhance cell surface expression of a variety of proteins and receptors may be due to its profound effect on cytoskeleton components. In B16a cells, the main cytoskeletal protein F-actin is found principally in cytoplasmic stress fibers or as a homogenous cortical accumulation. Stimulation of tumor cells with 12(S)-HETE induces a rapid and reversible rearrangement of the F-actin skeleton. Within 5 min treatment the cytoplasmic stress fibers disappear and focal F-actin aggregates form under the cell body and in filopodia. Later, the cytoplasmic stress fibers regenerate and the focal cell body aggregates disappear. 12(S)-HETE treatment also induces a reversible, pronounced bundling of the vimentin network which is usually found in the perinuclear region irradiating toward the plasma membrane in untreated control cells. Cytoskeletal rearrangements generally were followed by alteration in actin-binding proteins. Vinculin, an actin-binding protein which localizes principally in cell body-adhesion plaques, is redistributed to the peripheral plasma membrane after 12(S)-HETE treatment. The cytoskeletal alteration appears to be driven by protein phosphorylation. Myosin light chain (MLC) and vimentin are the main phosphorylated cytoskeletal proteins besides the myosin heavy chain (MHC) and a 90 kD protein. 12(S)-HETE induces a rapid (within 5 min) hyperphosphorylation of these proteins [86].

Effect on protein kinase C

It is known that 12(S)-HETE treatment enhances tumor cell adhesion (see previous section). Liu *et al.* [87] showed that 12-HETE enhanced adhesion of W256 cell was blocked by protein kinase inhibitors, staurosporine, calphostin C and 1-(5-isoquinoline-sulfonyl)-2-methylpiperazine (also known as H7). Depleting PKC from W256 cells abolished their ability to respond to 12(S)-HETE. Treatment of W256 cells with 12(S)-HETE induced a 100% increase in membrane-associated PKC activity whereas 13(S)-HODE inhibited the effect of 12(S)-HETE on PKC translocation. The stimulatory effect of 12(S)-HETE and inhibitory effect of 13(S)-HODE on PKC translocation were also observed in B16a cells [87]. Only PKCα isozyme has been found in B16a cells [87]. Thus, it appears that 12(S)-HETE and 13(S)-HODE can up- and down-regulate PKCα activity, respectively. It is still an open question whether these two monohydroxy fatty acids have similar effects on the other PKC isozymes. Besides the direct activation of PKC by 12(S)-HETE, evidence demonstrates that PKC is involved in 12(S)-HETE enhanced tumor cell adhesion [87], spreading [64], cytoskeletal rearrangement [88], EC retraction [88], integrin redistribution [83] and experimental metastasis [82, 89]. At present, the involvement of other protein kinases is still elusive.

Eicosanoid receptors

It is known that the effect of many eicosanoids is mediated through receptors. For example, PGE_2 is able to operate through two different receptors; a stimulatory receptor coupled to Gs and involved in activation of adenylate cyclase (AC), and an inhibitory receptor coupled to Gi and involved in inhibition of vasopressin-stimulated AC. Although it is still unclear for HETEs, evidence suggests that all prostanoids and leukotrienes operate through G protein-linked receptors [90]. Receptors for PGA, PGE, PGF, PGD, PGI_2, TXA^2/PGH^2 and LTs have been described [1, 2, 91–94] and different subclasses of PGI_2 and TXA_2/PGH_2 receptors have been found [95, 96]. No conclusive data shows the presence of HETE receptors, however, a binding site for 12(S)-HETE has been reported in human epidermal cells [97–99]. G-protein coupled eicosanoid receptors play important roles in cell signal transduction upon ligand binding. For instance, in platelets, TXA_2 binding can activate phospholipase C (PLC). Activation of PLC produces two intracellular second messengers, inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases Ca^{++} from the platelet dense tubular system, raising the cytosolic free Ca^{++} concentration. Elevated Ca^{++} level in turn activates PLA_2 localized on both the membrane of the dense tubular system and plasma membrane. PLA_2 releases AA from membrane phospholipid such as phosphatidylcholine. Arachidonate is then metabolized to PGHS products such as TXA_2, and LOX products such as 12(S)-HETE. TXA_2 diffuses out of the cell, binds again to its receptors, activates G'p (a pertusis toxin-resistant G protein) and causes further platelet activation [100]. 12(S)-HETE, as discussed earlier, can exert a variety of biological effects. Increased Ca^{++} also binds to calmodulin and Ca^{++}/calmodulin complex activates myosin light chain kinase (MLCK), which subsequently phosphorylates myosin light chain (MLC) and initiates contraction and cell shape change. DAG, together with Ca^{++}, activates PKC, triggering granule secretion and the activation of integrin αIIbβ3.

Phospholipases

Based on the broad activities of eicosanoids and their roles in cell transformation, tumor progression and metastasis, understanding the regulation of phospholipases, PG synthases and lipoxygenases has a critical importance. Here, we will not discuss the enzyme activity regulated by factors such as ions, inhibitors, suicide substrates, etc. These aspects have been reviewed previously [1, 5, 101, 102]. Instead, we focus on more recent work and put emphasis on their genetic regulation and regulation by growth factors and cytokines. Phospholipase A_2 constitute a diverse family of enzymes that hydrolyze the sn-2 fatty acyl ester bond of

phosphoglycerides liberating free acids and lysophospholipids [103]. cDNAs of extracellular secreted PLA_2s from pancreas and snake venoms have been cloned and sequenced, however little is known about the molecular structure and properties of non-pancreatic mammalian PLA_2s. Extracellular PLA_2s are abundant in pancreatic secretions, but are also present in plasma, lymph and pulmonary alveolar secretions. Intracellular PLA_2s are found in all cells where they are either located in the cytosolic compartment associated with the plasma membrane or stored within secretory granules. PLA_2s have been separated into two distinct structural classes [104]; Group I, exemplified by bovine pancreas PLA_2, and Group II, exemplified by C. atrox venom PLA_2. The control and regulation of enzymatic activity of the granule-associated and cytosolic PLA_2s are distinct. PLA_2s sequestered in granules are inactive and are activated upon secretion into the extracellular space. However, the activity of cytosolic PLA_2 is regulated by various factors such as Ca^{2+}, PKC and PLA_2 activating protein (PLAP). It is accepted that upon stimulation of cells (e.g., platelets) with agonists, the initial phospholipid response of the cell is inositol phospholipid hydrolysis by phospholipase C followed by activation of phospholipase A_2. The common assumption is that PLC generates IP_3 and DAG upon stimulation and then IP_3 activates PLA_2 by elevation of intracellular Ca^{2+} concentration while DAG stimulates PLA_2 by activation of PKC [105]. Recently, the PLAP sequence structure has been elucidated and other studies indicate that it may play a pivotal role in control of the activity of PLA_2s in nucleated cells [106].

Prostaglandin synthases

In the previous section, we discussed that PGHS can be stimulated by phorbol ester, growth factors, cytokines and hormones. PMA is capable of stimulating endothelial cells to synthesize eicosanoids and the stimulation pattern is in sharp contrast to that exhibited by histamine, ionophore A23187 and bradykinin. Due to the fact that PMA increases the *de novo* synthesis of PGHS, its stimulation of eicosanoid production lasts more than 12 hrs [29]. However, histamine, ionophore A23187 and bradykinin induce a rapid generation of eicosanoids within minutes, and the eicosanoid production reaches its peak at 30 min and declines [29]. This self-limitation of eicosanoid synthesis has been attributed to autoinactivation of PGHS [29]. PDGF has been shown to stimulate *de novo* synthesis of PGHS [35] but the PDGF effect might be mediated by the synthesis of other proteins rather than directly affecting the PGHS gene [35]. EGF [36, 37], TNF [38], IL-1 [38–40] and epinephrine [41] have also been demonstrated to stimulate the *de novo* synthesis of PGHS, yet the molecular mechanisms are still elusive. Human PGHS gene has been cloned and several CAAT and TATA box-like sequences were found in its 5′ region [13], so far little is known about its transcriptional regulation. Based on the fact that cAMP induces, while corticosteroids suppress the PGHS gene [41, 107], it has been speculated that the cAMP responsive element and/or steroid hormone responsive element may exist in the 5′-flanking region of the gene [13]. Indeed, by analyzing mouse PGHS gene, a sequence with similarity to the negative glucocorticoid regulatory element (nGRE) was found at position -2123 and -2009 [17]. In addition, two SP1 sites and three putative AP-1 sites were identified. These results suggest that PGHS gene may be regulated positively by jun/fos, SP-1 and negatively by glucocorticoid receptor. Unexpectedly, a putative dioxin responsive element is located at position -403 to -385. The dioxin responsive element is known to be responsible for the induction of transcription of the cytochrome P450IA1 and other genes involved in detoxification/activation of polycyclic aromatic hydrocarbons. Besides these possible transcriptional controls, alternative splicing may be another regulatory mechanism for PGHS gene [24].

Lipoxygenases

In the case of lipoxygenases, cDNAs of 5-, 12-, 15-LOX have been obtained from different species [6-15]. EGF has been demonstrated to stimulate LOX activity and therefore the production of

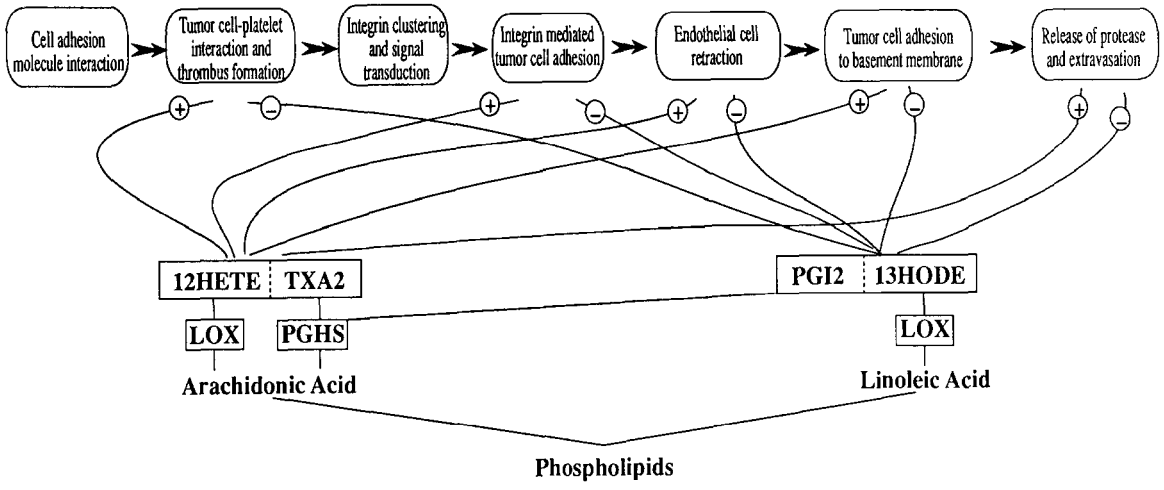

Fig. 4. Schema of the involvement of various metabolites of arachidonic and linoleic acid in tumor cell-platelet-EC interactions, which encompass a series of sequential steps as indicated by arrows. Different metabolites may have either a positive (+) or a negative (−) impact on each distinct step. Generally, 12(S)-HETE and TXA_2 promote tumor cell-platelet-EC interactions while PGI_2 and 13-HODE suppress these processes. For details, refer to relevant discussions in the text.

HETE [108] and HODE [119]. It has been shown that Ca^{2+} activates 5-LOX *in vitro* [110]. Translocation of 5-LOX from cytosol to membrane has also been observed upon activation by ionophore A23187 [110]. The nature of the membrane translocation and its physiological relevance are not yet understood. It is possible that binding of 5-LOX to membrane facilitates the access of the enzyme to substrate (e.g. AA) that is released from phospholipids. Information on gene structure of 5-, 15-LOX but not 12-LOX is available [12, 16], yet little is known about their regulation. Multiple GC boxes were found in the 5′ region, raising the possibility of 5-LOX as a housekeeping gene. However, this is not very likely since LOX activity is highly inducible [110, 111] and its expression demonstrates a tissue specificity [99]. Recent discovery of a 5-lipoxygenase-activating protein (FLAP), which is required for leukotriene synthesis by 5-LOX [112, 113], suggests an alternative mechanism of regulation of 5-LOX. Differentiation of HL-60 cell induced by DMSO is associated with the concurrent induction of both FLAP and 5-LOX, however, LT synthesis depends on FLAP [114].

Altogether, we presented evidence showing the association of eicosanoid metabolism and tumor cell metastasis. Eicosanoids and other monohydroxy fatty acids (i.e., 13(S)-HODE) have profound effects on the behavior of tumor cells, platelets and EC which are reflected by their effects on cell surface receptors, signal transduction pathways and cytoskeletal networks (represented in Fig. 4). Information on the regulation of phospholipases, PG synthases and lipoxygenases is accumulating, which in turn will lead to a better understanding of the regulation of eicosanoids and other fatty acids, and subsequently their role in tumor progression and metastasis.

Conclusions

- Transformation by membrane-associated and cytoplasmic oncogenes enhances PGHS and PLA_2 activity in some cell systems.
- Several tumor promoters, growth factors, cytokines and neurotransmittors can up-regulate PGHS expression whereas glucocorticoid suppress its expression.
- TXA_2 and 12(S)-HETE increase while PGI_2 and 13(S)-HODE decrease tumor cell metastatic potential.
- Tumor cell-platelet-vessel wall interactions are critical for hematogenous phase of metastasis

and these interactions can be positively regulated by TXA_2 and 12(S)-HETE and negatively regulated by PGI_2 and 13(S)-HODE.
- 12(S)-HETE induces PKC translocation to cellular membrane but 13(S)-HODE inhibits PKC translocation.
- 12(S)-HETE increases integrin αIIbβ3 cell surface expression in a cytoskeleton dependent manner.

Unanswered questions

- Whether 12(S)-HETE is a cell signal transduction mediator?
- Can 13(S)-HODE or its stable analogs be used as anti-metastatic agents?
- Are 12(S)-HETE activation and 13(S)-HODE inhibition of PKC isoform specific?
- Are effects of 12(S)-HETE and 13(S)-HODE on PKC direct or indirect?
- Do eicosanoids activate or inhibit other protein kinases?
- How are lipoxygenase, PG synthase and phospholipases regulated at the molecular level?

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