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Bioactive lipid metabolism in platelet "first responder" and cancer biology

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Abstract

Platelets can serve as "first responders" in cancer and metastasis. This is partly due to bioactive lipid metabolism that drives both platelet and cancer biology. The two primary eicosanoid metabolites that maintain platelet rapid response homeostasis are prostacyclin made by endothelial cells that inhibits platelet function, which is counterbalanced by thromboxane produced by platelets during activation, aggregation, and platelet recruitment. Both of these arachidonic acid metabolites are inherently unstable due to their chemical structure. Tumor cells by contrast predominantly make more chemically stable prostaglandin E_2, which is the primary bioactive lipid associated with inflammation and oncogenesis. Pharmacological, clinical, and epidemiologic studies demonstrate that non-steroidal anti-inflammatory drugs (NSAIDs), which target cyclooxygenases, can help prevent cancer. Much of the molecular and biological impact of these drugs is generally accepted in the field. Cyclooxygenases catalyze the rate-limiting production of substrate used by all synthase molecules, including those that produce prostaglandins along with prostacyclin and thromboxane. Additional eicosanoid metabolites include lipoxygenases, leukotrienes, and resolvins that can also influence platelets, inflammation, and carcinogenesis. Our knowledge base and technology are now progressing toward identifying newer molecular and cellular interactions that are leading to revealing additional targets. This review endeavors to summarize new developments in the field.

Keywords Platelets · Cancer · Metastasis · Thromboxane · Prostacyclin · Prostaglandin · Cyclooxygenase · NSAID · COXIB · Aspirin

Abbreviations

15-PGDH	15-Hydroxyprostaglandin Dehydrogenase
AP	Activating Protein
Apc	Adenomatous polyposis coli
AA	Arachidonic Acid
ABC	ATP-Binding Cassette
CCL2	C-C motif Ligand 2
CVD	Cardiovascular disease

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CRC	Colorectal cancer
CI	Confidence interval (95%)
COXIBs	Cyclooxygenase inhibitors
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
DC	Dendritic Cells
DNMT1	DNA methyl transferase
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
ERK	Extracellular signal regulated kinase
FAP	Familial adenomatous polyposis
Gs	G-stimulatory
HK	Hexokinase
HR	Hazard Ratio
HETE	Hydroxyeicosatetraenoic acid
IκB	Inhibitor of nuclear factor of kappa light chain in B-cells
LTB4	Leukotriene B4
LOX	Lipoxygenase
MSI	Microsatellite instability
MDSCs	Myeloid-derived suppressor cells
NK	Natural killer
NSAIDs	Non-steroidal anti-inflammatory drugs
OR	Odds ratio
OATP	Organic ion transporter protein expression
PGT	PG transporter
cPGES/ mPGES	PGE2 synthases, cytosolic/microsomal
PGIS	PGI2 synthase
PI3K	Phosphatidylinositol-3-kinase
PDK1	Phosphoinositide-dependent kinase-1
PLA2	Phospholipase A2
PMNs	Polymorphonuclear cells
PGI2	Prostacyclin
EP	Prostaglandin (prostanoid) E receptors
PGEM	Prostaglandin E metabolite
PGE2	Prostaglandin E2
PGH2	Prostaglandin H2
RCT	Randomized controlled trial
RR	Relative risk
TCR	T-cell receptor
TXS/ TBXAS1	Thromboxane synthase
TxA2	Thromboxane
TLR	Toll-like receptors
TNF	Tumor necrosis factor
VDAC	Voltage-dependent anion channels

1 Platelets as "first responders"

Platelets can be thought of as first responders in many hematogenous and inflammatory diseases [1–4]. Data continue to emerge supporting the notion of platelet bioactive lipid metabolism that is likely central to driving many of these first responder characteristics. The term first responder describes platelet metabolism and biology involved in the hemostasis, wounding, immune, and cancer metastatic processes [1–4]. Typically, platelets may remain overlooked during *in vivo* experimental studies or pathologic observations. This can occur due to their small spherical plate-like morphology and size that is 2.6 to 2.9 μm in diameter. The difficulty in establishing a standardized standard interval range for platelet indices across the different clinical methodologies used has also had an impact on the reported platelet distribution. Despite being visible under high magnification light microscopy, electron microscopic ultrastructural analysis is typically used to identify subcellular structural changes although newer methods can potentially be effective [5–8]. Aggregates and individual activated platelets can be detected by immunohistochemical staining in some cases.

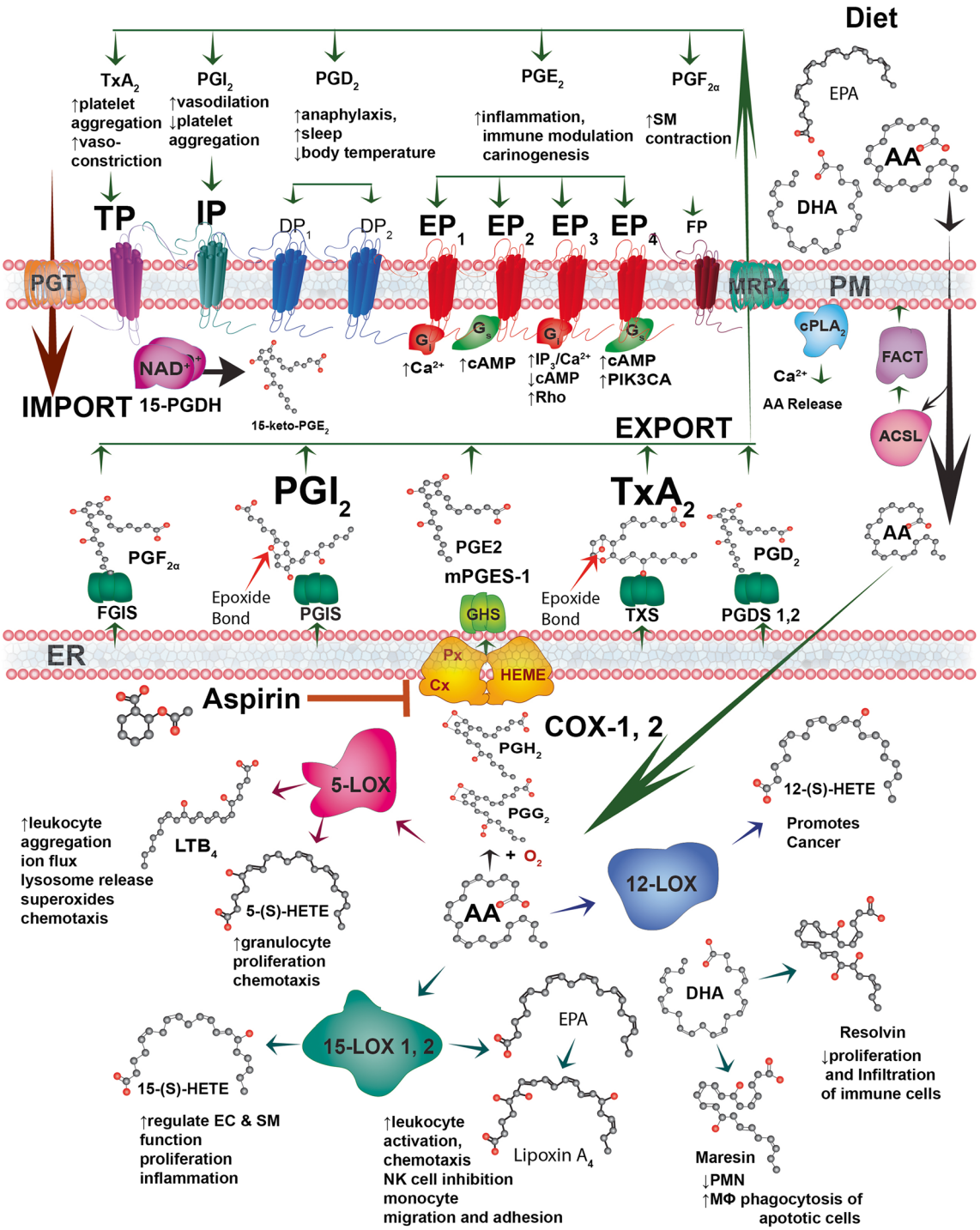
Resting platelets exist as disks that maximize biophysical surface interactions when the plate-face dimension is in contact with vessel walls [9–11]. Their small discoid physical characteristics promote platelet segregation near the outer fluid shear fields of flowing blood [12–19]. These shear characteristics cause platelets distribute at 2–3 times greater numbers at the vessel walls than within the core fluid stream dominated by red blood cells. Platelet

Fig. 1 Bioactive eicosanoid and lipid metabolism. Bioactive lipids are derived from metabolic sources that arise from essential dietary fatty acids. These fatty acids include arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) that are transported into cells. Acyl-CoA is coupled to fatty acids by acyl-coenzyme A synthetases (ACSLs). Fatty acids like AA are then inserted as a storage source into membrane phospholipids by fatty acyltransferases (FACTs). After platelet agonist stimulation, the cytoplasmic form of phospholipase A_2 ($cPLA_2$) catalyzes the release of AA from membrane phospholipids. Once membrane free, AA is enzymatically converted by cyclooxygenases (COXs) to prostaglandin G_2 (PGG_2) followed by prostaglandin H_2 (PGH_2). PGH_2 then serves as a substrate multiple PG synthases. In contrast, DHA and EPA are less effective substrates for COX. PG synthases occur in multiple forms, specifically: PGD_2 synthases (PGDS), PGE_2 synthases (PGES), $PGF_{2\alpha}$ synthase (PGFS), PGI_2 synthase (PGIS), or TxA_2 synthase (TXS). Both PGI_2 and TxA_2 contain epoxide bonds (red arrows) that contain significant chemical bond strain that leads to their rapid hydrolysis and short half-life approaching 30 s. Bioactive lipids are exported outside the cell by multidrug resistance-associated protein 4 (MRP4) and other transport molecules. As PGs accumulate in the extracellular microenvironment, they bind to subtype-specific G-protein-coupled receptors. These receptors include DP1, DP2, EP1–4, FP, IP, and TP. Depending on their function, various receptors interact with subtype-specific G-stimulatory (Gs) or G-inhibitory (Gi) proteins. Downstream signaling molecules stimulate cAMP, Ca^{2+}, inositol phosphates, or IP3/Ca^{2+}, and Rho among others. Metabolic breakdown relies on PG transporter (PGT) followed by inactivation involving NAD + -dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH). Either free AA, DHA, or EPA can be utilized by lipoxygenase (LOX)-5,-12, and -15-1, or -15-2. LOX enzymes generate hydroxyeicosatetraenoic acids (HETEs) or leukotrienes such as LTB_4. LOX-15 also uses EPA to make lipoxins or DHA when coupled with 5-LOX to make resolvins. Additional DHA metabolites are protectins and maresins ▶

distribution patterns and excess numbers near vessel walls can increase the probability of detecting vascular wall breaches or wounds. We and others have described the platelet rapid recognition and response properties associated with extracellular matrix exposure and endothelial retraction elsewhere [1–4]. These rapid response properties are closely linked to the unique stability properties, biochemistry, and metabolism of blood-born bioactive eicosanoids.

2 Keeping rapid platelet responses local: all about epoxide bond instability

The Honn Laboratory was first to recognize the importance of prostacyclin in metastasis [20]. Prostacyclin is synthesized very rapidly by vascular endothelial cells and is one of the most effective anti-platelet aggregation metabolites of the eicosanoid pathway (Fig. 1). The biological effects of prostacyclin are counterbalanced by thromboxane which is



one of the more potent pro-platelet aggregation metabolites of the eicosanoid pathway and is rapidly synthesized by platelets. Both of these molecules contain epoxide groups in their final bioactive form [21–23]. These exhibit significant ring strain leading to chemical and metabolic instability [21–24], which has been experimentally demonstrated in half-life experiments. The half-life of the bioactive (epoxide-containing) forms is around 30 s *versus* approximately 5 min for the lipid precursor molecule prostaglandin H_2 (PGH_2) [23, 25]. This intrinsic chemical instability helps to regulate the delicate hematologic balance between pro- and anti-platelet aggregation in the blood stream [26–28]. It is nature's elegant way of maintaining rapid responses of platelets to vascular changes that remain localized near the site of vascular lesion recognition and response (Fig. 2). These platelet rapid response characteristics can be subverted during cancer progression and metastasis. At the First International Conference on Prostaglandins and Cancer, we were the first to report on the inhibition of tumor cell-induced platelet aggregation (TCIPA) by prostacyclin, thromboxane A_2, and phosphodiesterase inhibitors [29] and subsequently on the efficacy of prostacyclin on TCIPA and as a deterrent for metastasis [30, 31]. These studies were followed by our report that examined prostacyclin and its synthetic analog carbacyclin and their abilities to inhibit tumor cell-platelet interactions [32]. The extent of cellular interactions during TCIPA was examined ultrastructurally. These studies revealed that tumor cell-platelet interactions began with individual platelets and initiated platelet chain formation in focal association with tumor cell surfaces. By mid-phase aggregation, large homotypic platelet aggregates grew with tumor cells externally positioned at the periphery of emboli. Tumor cell-platelet surface and cytoplasmic interactions became progressively more extensive within growing platelet aggregates. Prostacyclin and carbacyclin showed dose-dependent inhibition of tumor cell platelet interactions. Carbacyclin inhibition of TCIPA lasted longer, but was tenfold less effective than was prostacyclin [32]. Prostacyclin and its analogs significantly decrease blood

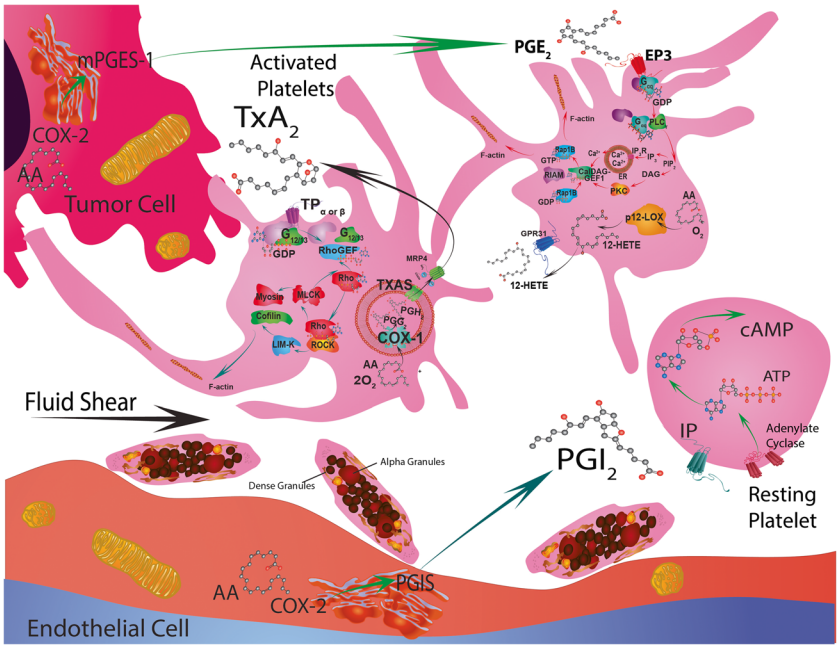


Fig. 2 Platelet first responder bioactive lipid signaling and biology. Platelets circulate near the endothelial cell surface based on their biophysical properties under fluid shear stress. Platelet plasma membranes contain multiple surface receptors that are activated by various agonists or antagonists. These surface receptors can interact with matrix proteins, collagen, other platelets, endothelial cells, immune cells, and tumor cells. Key bioactive receptors include G-protein-coupled receptors. To form eicosanoid metabolites, cyclooxygenase 1 and 2 (COX) couple two oxygens to arachidonic acid to produce PGG_2 and then PGH_2. In turn, PGH_2 is metabolized to various prostaglandins by synthase enzymes. Platelet prostaglandins thromboxane (TX)A_2 synthesized by TXA_2 synthase (TXAS) and PGE_2 synthesized by PGE_2 synthase are the potent pro-aggregatory agents. TXA_2 and PGE_2 stimulate platelet responses through various isoforms of G-protein-coupled TP or EP receptors. TP stimulates $G_{12/13}$ and Rho-GEF followed by Rho-associated kinase (ROCK), LIM domain kinase (LIMK), and cofilin and that interact with actin to initiate shape change, activation, cytoplasmic process generation contraction and alpha- or dense-granule release. Other interactions include binding to myosin light-chain kinase followed by myosin. Also, EP_3 receptors stimulate initiate signal transduction pathways though G_{aq}-calcium release-linked receptors. Another important G_{as}-protein-coupled IP receptor prevents platelet aggregation by binding prostacyclin (PGI_2) followed by stimulating cyclic adenosine monophosphate (cAMP) synthesis *via* adenylate cyclase (AC). Another abundant eicosanoid is 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE] through platelet-type lipoxygenase (p12-LOX) enzymatic activity. Once released, 12-(S)HETE is thought to activate orphan receptor GPR31

pressure [33] including some of the more recently described PGI2 mimetics [34]. Some of these effects may also involve endothelium-derived hyperpolarizing factor [35] which may be partly distinct from the direct effects of nitric oxide and prostacyclin [36].

3 Inhibition of cyclooxygenase (COX)-1/2

The use of cyclooxygenase-2 (COX-2) inhibitors (COXIBs) to prevent cancer development has strong experimental support. COX-2 inhibition in the tumor and surrounding microenvironment [37, 38] is thought to occur specifically through acetylation of the active site serine side chain (Ser516) and inhibition of prostaglandin biosynthesis at the level of prostaglandin H_2 (PGH_2) substrate generation [39]. Since PGH_2 is the rate-limiting substrate required for all prostaglandin production, its inhibition impacts all PG bioactivities whether they are pro-inflammatory, immunomodulatory, prooncogenic, or otherwise bioactive. Similar chemical acetylation of cyclooxygenase-1 (COX-1) active site serines to limit PGH_2 substrate availability for thromboxane production by thromboxane synthase in platelets [40]. This has a more narrow scope of activity that remains a largely unexplored mechanism of limiting carcinogenesis and metastasis [40]. There are also non-enzymatic transacetylation reactions with the *N*-terminal amino groups of proteins as well as side-chain amino, hydroxyl, and sulfhydryl groups [41], suggesting that other cyclooxygenase-independent effects of aspirin also exist [41, 42].

Prostaglandin synthesis from membrane-derived AA by cyclooxygenase (COX)-1 and COX-2 enzymatic activity can decrease systemic arterial pressure and increase pulmonary arterial pressure in mouse models, and this observation was further supported in KO mouse studies [43]. Mouse models have also been particularly useful in revealing the role of prostaglandin genesis in cancer progression and metastasis. In one mouse model, direct evidence was provided in mice with a truncation mutation in adenomatous polyposis coli at amino acid 716 ($Apc^{\Delta 716}$) which predisposes them to adenoma formation in the small intestine [44]. In another mouse model strain, genetic knock-out of COX-2 or pharmaceutical blockage approaches both led to polyp reduction. In this case, both COX-1 null/$Apc^{Min/+}$ and COX-2 null/$Apc^{Min/+}$ mice had decreased numbers of intestinal polyps [45]. Genetically engineered and carcinogen-induced animal models consistently show the importance of the COX1/2 pathways in a variety of organ systems [46]. These findings prompted a celecoxib clinical trial in patients with familial adenomatous polyposis (FAP) that has resulted in significantly reduced adenomas [47]. These COX-2-specific inhibitor-related outcomes led the FDA to approve celecoxib for use in FAP patients as an adjunct to surgery. Subsequently, individuals who were previously diagnosed with adenomas also showed reductions in adenoma recurrence in similar COXIB trials, particularly in those patients with advanced adenomas, the recurrence was reduced following treatment with celecoxib. One proposed mechanism of action suggests that this effect results from decreased inflammation and lower levels of pro-inflammatory cytokines downstream of prostaglandin E_2 (PGE_2)-mediated signal transduction. Although this may help to explain why most COX-1/2 inhibitors prevent cancer in a number of organ sites, the role of platelets in these processes remains to be fully explored [48–50].

As metabolically produced bioactive lipids, PGs are derived from arachidonic acid (AA), which is mobilized from membrane phospholipids. Any given prostaglandin pathway subtype activation varies depending on the cell type, tissue involved, and the expression of surface receptors present on target cells. Pro-inflammatory stimuli mobilize bioactive lipid genesis by catalyzing the release of AA from membrane phospholipids *via* phospholipase A_2 [51–54]. AA released from the phospholipid layer is converted into bioactive lipids by a series of enzymatic reactions. AA is first converted to prostaglandin H_2(PGH_2) with the incorporation of two oxygen molecules by the COX enzymes. This substrate generated by COX enzymes is used by a number of enzymes downstream of COXs that generate a variety of PGs, each with specific mode of action and a different biological function. COX enzymes catalyze the rate-limiting reactions within this pathway and thereby serve as targets in limiting all PG production.

By targeting the rate-limiting COX activity, both PGE_2 pro-inflammatory effects, and those of other critical PGs that influence hemostasis would be attenuated. One key PG that regulates homeostasis is prostacyclin (PGI_2). Prostacyclin is continuously produced in nucleated vascular endothelial cells by PGI_2 synthase (PGIS; PTGIS) downstream of COX-2. Vascular endothelial cell PGIS synthesizes PGI_2, which is transported to the bloodstream. PGI_2 has a very short half-life (seconds in solution) and acts locally on blood vessels by inducing vasodilation and inhibiting platelet aggregation.

Because endothelial cells are nucleated and contain all of the gene expression machinery, PGIS is constantly turned over and replaced. NSAIDs and COXIBs are typically competitive inhibitors that occupy the catalytic site of COX enzymes. Pro-inflammatory and pro-oncogenic stimuli stimulate COX-2 synthesis and enzymatic activation.

COX-1 by contrast is a constitutively synthesized housekeeping gene that is elevated primarily in smooth muscle cells and platelets. Antiplatelet activity is counterbalanced by COX-1 that is linked to thromboxane (TxA_2) production by TBXAS1 (TXS) in circulation. In circulating platelets, COX-1 synthesizes PGH_2 which is then converted to TxA_2 by TXS [55]. Aspirin irreversibly acetylates COX-1 at Ser 530 [55] eliminating PGH_2 biosynthesis and inhibiting platelet function

[56]. This inhibition can have an impact on both inflammatory and carcinogenic processes.

New platelets must be produced by the megakaryocytes in bone marrow to reconstitute platelet function in the circulation. Once produced and exported into circulation, TxA_2 activates platelet functions, including aggregation, adhesion, additional platelet recruitment, and vessel contraction. As a whole, COXIBs and NSAIDs can effectively inhibit inflammation due to PGE_2 inhibition. In the case of platelet-endothelial cell homeostasis, balance between COX-1/TxA_2 production and COX-2/PGI_2 is important. Using COX-2-selective COXIBs would inhibit endothelial cell PGI_2 synthesis and shift the balance toward platelet TxA_2 production leading to cardiovascular thrombosis in certain high-risk individuals. However, acetylation of COX-1 by low-dose aspirin eliminates both downstream TxA_2 production by platelets, along with PGI_2 and PGE_2 while reducing the risk of cardiovascular thrombosis. As an unwanted side effect, aspirin cause severe gastropathy in susceptible individuals, which some studies show can be limited by combining with phosphatidylcholine [57]. The use of these drugs depends on their application in the appropriate clinical context.

4 Thromboxane synthase inhibition

As mentioned above, TXA_2 is synthesized by TXAS as a prominent pathway in platelet biology and lies downstream of platelet COX-1. Moreover, thromboxane biosynthesis in activated human platelets does not involve COX-2 [58]. More selective TXAS inhibitors and platelet TXAS have been studied since the 1970s [25, 59, 60]. In particular, Upjohn Company chemists produced a synthetic prostaglandin analog 9,11-azoprosta-5,13-dienoic acid inhibitor. This nitrogen-substituted azo analog structurally resembled PGH_2 and was shown to be particularly potent at inhibiting oxygen-based endoperoxide containing PGH_2 as well as ADP, epinephrine, and collagen-induced platelet aggregation [25] and as previously mentioned TCIPA [29]. In a follow-up study, platelet cyclooxygenase (TXA_2 production) 12-lipoxygenase (12-HETE production) enzyme inhibitors each alone were unable to inhibit TCIPA but when combined inhibited TCIPA even at higher concentrations of tumor cells [61]. In other studies, a novel thromboxane modulator BM-567 (II/II) inhibited platelet function [62] along with TCIPA and TXA_2 release [63]. Similarly, 1-alkyl (*N*-alkyl)-imidazole derivatives such as OKY-046 (Ozagrel) are TXAS inhibitors, especially in human platelets that have been studied since the early 1980s [64, 65] and have been shown to inhibit platelet function, TCIPA [66, 67] and hepatic metastasis [68, 69]. Likewise, R-68070 (Ridogrel) is a combination of TXAS inhibitor-TxA2 receptor antagonist [70, 71] that prevents platelet aggregation. Also, various natural compounds can inhibit platelet TXAS function and aggregation [72].

Finally, platelet surface glycoprotein alpha IIb beta 3 (GPIIb/IIIa) plays an important role in platelet aggregation and surface expression. This platelet integrin serves in the adhesion of tumor cells to platelets and may promote tumor metastasis. Inhibiting this platelet GPIIb/IIIa-mediated interaction with heparin (modified heparins), peptides, or blocking antibodies could prevent TCIPA [73, 74].

5 Prostaglandin E_2-related mechanisms

PGE_2, the most common PG, is present at high levels in a variety of cancers [75] and serves as a primary driver of carcinogenesis. Activated platelets also release PGE_2 contributing to vascular modulation and weakened immune responses [76]. PGE_2 regulates tumor cell's pro-survival and anti-apoptotic pathways by acting on the four E Prostanoid receptors highlighted in later sections. Decreasing PGE_2 levels in microenvironment of tumors by various mechanisms can reduce its pro-tumorigenic effects. Anti-inflammatory drugs such as steroids and NSAIDS have cancer-preventive functions as they inhibit PGE levels. The production of PGE_2 is dependent on PGE_2 synthases that are now gaining importance as more viable target enzymes downstream of COXs without triggering major side effects.

6 Synthesis, transport, and catabolism of prostaglandins

A more targeted approach to reduce PGE_2 levels is to directly inhibit PGE_2 synthase. This has the added benefit of reducing the toxicity associated with COX inhibition since it does not eliminate PGs that control platelet hemostasis [50, 77, 78]. The first PGE_2 synthase identified was microsomal PGE_2 synthase-1 (mPGES-1, PTGES-1). Subsequently, two additional isoforms of PGE_2 synthases were discovered, cytosolic PGES (cPGES) and mPGES-2 (PTGES-2). The expression of mPGES-1 generally remains low in most normal tissues while cPGES and mPGES-2, by comparison, remain at steady-state expression levels. Stimuli that can be pro-inflammatory or oncogenic in nature can induce the expression of mPGES-1 [79]. The protein structure of these enzymes contains three transmembrane subunits and a glutathione active center [80]. Both COX-2 and mPGES-1 in particular are overexpressed in cancers compared to normal tissue. One of the mechanisms by which mPGES-1 expression could be upregulated is *via* tumor necrosis factor alpha, a cytokine involved in cancer progression [81]. Several ongoing efforts are focused on developing inhibitors that can specifically target mPGES-1 isoform without targeting the COX enzymes and avoiding the

cardiovascular and gastrointestinal side effects of NSAIDs and COXIBs [82, 83]. PGs could also be modulated by eliminating their transport or increasing their catabolism. PGE_2 transport and accumulation out of the cell in the tumor microenvironment correlates with oncogenic activity. ATP-binding cassette (ABC) transporter ABCC/multidrug resistance protein-4 (MRP4) is responsible for energy-dependent transport of PGs like PGE_2. This transport can also be inhibited by using drugs such as indomethacin and celecoxib [84–86]. Similarly, increased expression of organic anion transporter proteins (OATP2A1, OATP3A1, and OATP4A1) can transport PGs and PGE_2 into the extracellular microenvironment to influence oncogenesis [87] and these proteins can be inhibited by NSAIDs to improve cancer outcomes. The PGs that are transported into the cell by the PG transporter (PGT) are then inactivated by NAD+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH). This converts PGs into 13,14-dihydro-15-keto-PGs, a stable metabolite excreted in the urine, resulting from the dehydrogenation of PGs at carbon 15. In the case of PGE_2, this forms prostaglandin E metabolite (PGEM). It has been shown that aspirin intake reduced incidence of colorectal cancers that were associated with high 15-PGDH expression, but had no influence on low 15-PGDH levels in normal colon mucosa [88] thereby serving as a biomarker of benefit from aspirin chemopreventive use.

7 Prostaglandin receptor antagonists

Eicosanoid receptors are typically G-protein-coupled receptors designated by their PG ligand molecular subclass. This is typically a letter-based identification-based nomenclature. In the case of platelets, surface receptors also facilitate TC-platelet cross talk [89, 90]. Identifying critical receptor-ligand interactions that mediate TC-platelet activation could serve as effective therapeutic targets.

Thromboxane A_2 (TP) receptors play a key role in the early and fast platelet activation events. As previously mentioned, TxA_2 is produced locally during platelet aggregate formation [23, 25] but is rapidly hydrolyzed in solution to inactive TxB2 in ~ 30 s by epoxide ring opening. TxA_2 interactions with TP receptors are among the most rapid proaggregatory platelet agonists [31, 32, 91–93]. TxA_2 pathway activation is a major stimulus and amplification trigger of heterotypic aggregate formation with tumor cells [31, 32, 91–93]. This supports the role of the TxA2 pathway in the rapid responses that mediate TC-platelet heterotypic interactions. TPs can signal through different G protein families of receptors, however, TP mediated platelet activation signals through $G_{12/13}$ and Rho-GEF followed by its downstream Rho-associated kinase (ROCK), leading to the activation of LIM domain kinase (LIMK) regulating actin reorganization [94]. Additional downstream signaling from the TP-G_{13} interactions include those with myosin light chain kinase leading to platelet cytoskeletal changes. TP receptor antagonists include terutroban, daltroban, picotamide, sulotroban, CAY10535, Ifetroban, SQ 29,548, BM 567, or pinane.

E prostanoid (EP) receptors mediate the pro-tumorigenic effects of PGE_2, which can include direct effects on precancerous and cancer cells. There are four different EP1–4 receptors that respond through G-stimulatory (Gs) or G-inhibitory (Gi) protein coupling and activating the signaling cascade involving second messengers such as cAMP, Ca2+, or inositol phosphates. EP1 receptor signals by regulating Ca2+ flux, EP2 and EP4 receptors increase cAMP levels by coupling to Gs, whereas EP3 receptor has three known isoforms generated by alternative splicing that regulate cAMP levels by being bound to Gi or Gs. EP3 isoforms have been reported to function differently from each other and can also increase IP3/Ca2+ and activate Rho [95]. Platelets also express EP2–4 receptors with the exception of EP1 [76]. EP receptors (1–4) show variable sensitivities and upon PGE_2 binding activate different downstream signaling pathways, thereby PGE_2-mediated responses could be activating or inhibiting and vary according to the cell type, state, and stage of maturation [96].

8 Prostaglandin influences on immune response modulation

NSAIDs and COXIBs impact on cancer prevention and reducing mortality result not from a single enzyme inhibition but from a combination of pathway interference leading to a complex interaction within the tumor microenvironment. For example, PGE_2 helps to influence the local immunosuppressive tumor microenvironment along with systemic responses. When the influence of PGs on the immune system is considered along with the direct effects on tumor cells and platelets, this sheds more light on the potential depth of bioactive lipid-driven mechanisms. In the case of PGE_2, it recruits myeloid-derived suppressor cells (MDSCs) into the tumors by CXCR2 signaling [97, 98]. MDSC infiltration plays a critical role in cancer progression as these cells once within the tumor can inhibit CD8+ T-cell mediated cytotoxicity [97, 99, 100]. PGE_2, as a mediator of both inflammation and cancer, also suppresses dendritic cell differentiation, and this, combined with inducing MDSC function, promotes tumorigenesis. In other mouse models, the use of COX-2 inhibitors modulated MDSC functions and blocked tumor growth [101], inhibited PGE_2 synthesis, and delayed tumor progression [102]. More selective EP2 receptor antagonists were shown to prevent the differentiation of MDSCs and tumor progression [103, 104]. PGE_2 can also directly affect CD8 + T cells by suppressing their proliferation, cytotoxicity, and interferon (INF)-γ release. Furthermore, PGE_2 causes the acquisition of

DNMT3A-dependent tolerogenic functions in human MDSC as an immunological hallmark of cancer [105].

The effects of PGE_2 are based on the developmental state of the cell. PGE_2 is inhibitory toward immature B cells, induces apoptosis in immature thymocytes, and promotes regulatory T cell development by enriching selective immune cell populations [96, 106]. Elevated circulatory PGE_2 levels can also affect T cell signaling responses [107]. Activation of EP2 and EP4 signaling pathways by PGE_2 was shown to result in increase in PD-1-mediated immune tolerance in tumor microenvironment. Similarly, PGE_2 produced by COX2/mPGES1 pathway was shown to increase PD-L1 in tumor-infiltrating myeloid cells mediating tumor immune evasion [108, 109].

In the case of natural killer (NK) cells, PGE_2 can impact their crosstalk with cytotoxic CD8 + T cells and other immune suppressive cells. PGE_2 can also directly suppress NK cell function [110, 111]. This suppression of NK cell function was shown to be mediated by EP2 and EP4 receptors, suggesting that NK cell activity can be re-established by specific receptor antagonists. PGE_2 can also modulate the activities of regulatory T cells (T_{reg}) that play a key role in immunosuppression [112, 113]. Furthermore, the infiltration of immunosuppressive T_{reg} cells could be inhibited by using COX-2 inhibitors or EP1, EP2, and EP4 receptor antagonists.

NSAIDs can also modulate dendritic cell (DC) activity and regulate their recruitment to the sites of tissue inflammation. In addition, tumor necrosis factor α (TNFα) alteration was also shown in multiple studies [114, 115]. PGE_2 modulates DC functions along with their differentiation, maturation, and ability to secrete cytokines which could be reduced with aspirin [116]. In the case of macrophages, plasticity is altered by PGE_2 [117]. The conversion of M1 (inflammatory macrophages) to M2 (immunosuppressive macrophages) has been observed in various tumor types [118–120].

9 Lipoxygenases and Monooxygenase activity

Monooxygenases such as cytochrome P450,or lipoxygenase (LOX) can act on AA that has been released from the phospholipid bilayer [121, 122]. Unlike COX which inserts two oxygen molecules into AA, monooxygenases insert a single oxygen into a given lipid. Platelet 12-LOX is a key AA metabolizing monooxygenase pathway utilized by platelets to generate 12-hydroxyeicosatetraenoic acid (12-HETE) [123, 124]. The platelet 12-LOX pathway was also shown to promote metastasis [123, 124] and can stimulate vascular endothelial cell growth factor expression [125]. 12-LOX activity in tumor cells stimulates a wide variety of signaling mechanisms and cellular responses along with the production of autocrine motility factor that elevates invasion [126–131]. In contrast, 15-LOX-1 is an inducible enzyme that synthesizes 15-hydroxyeicosatetraenoic acid (15-HETE), mediates in reducing or resolving inflammation, and its loss has been observed in various cancers.

As another monooxygenase-based effect, aspirin-acetylation of COX converts the cyclooxygenase into a lipoxygenase that catalyzes the formation of 15-HETE and 11-HETE from AA [132]. In contrast, acetylation of COX-1 renders it inactive. A shunting mechanism also exists following the inhibition of COX which shifts AA pathway to the 5-LOX pathway and leukotriene B4 (LTB4) production. Additionally, in several cancers including colon, lung, breast, and head and neck squamous cell carcinoma, 5-LOX overexpression is also reported [133–135]. Thus, monooxygenase activities can play an important role in cancer progression and metastasis.

10 Resolution of inflammation *via* resolvins and maresins

Inflammation is optimized as a host-defense mechanism to protect against infections and harmful stimuli. By definition, acute inflammation generally resolves quickly. In some cases, inability to resolve the acute phase can lead to chronic inflammation. Precancerous lesions are sometimes influenced by strong chronic inflammatory conditions such as hepatitis, gastritis, colitis, and so on that can result in cancer formation. In some instances, timely resolution of the inflammatory process may help to lower the risk of developing cancer.

Evidence suggests the resolution of inflammation is not a passive process but is activated by turning off or blunting pro-inflammatory stimulatory signals. Inflammation is triggered by Toll-like receptor (TLR) signaling and activation of polymorphonuclear cells (PMNs) and macrophages. These activated immune cells migrate to inflammatory sites and release cytokines, including interleukin (IL)-8, IL-1, and IL-6 [136].

During the inflammatory response period, platelets as "first responders" once activated can recruit PMNs or macrophages to the sites of inflammation [56, 137]. This may occur after platelet infiltration and migration into the tissues through cytokine release or leaky blood vessels or neoangiogenesis [1]. Anti-inflammatory cytokines IL-4, IL-5, IL-10, IL-13, TNFα, and transforming growth factor β (TGF-β) take part in the resolution of inflammation [138, 139]. Bioactive lipids derived from AA take part in both initiation and resolution processes of inflammation. When platelets interact with leukocytes, this leads to lipoxin A_4 and B_4 formation that promotes resolution. Bioactive lipid functions are governed by tissue-specific isomerases and the receptor profiles on the cells within the microenvironment. LOX activation, which could be enhanced with aspirin, results in leukotriene and lipoxin

synthesis. Collectively, these eicosanoids trigger autocrine and paracrine mechanisms to dynamically modulate inflammation, immunity, vascular permeability, smooth muscle contraction, cognition, and synaptic plasticity. Acetylated COX-2 can also act on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) resulting in the synthesis of resolvins, maresins, and protectins [138, 140–144]. As the name reflects, resolvins initiate the resolution phase of inflammation and can suppress cytokine generation. Together, these resolvins, protectins, and maresins can clear inflammatory signatures, promote homeostasis, and return tissue to normalcy.

Concisely, the exact mechanisms of action for NSAIDs' and aspirin's cancer-preventive efficacy are likely to involve a variety of eicosanoid metabolites. These will likely include not only TxA_2, PGI_2, and PGE_2 but other bioactive lipids along with a variety of altered proteins. As new molecular mechanisms emerge, the overall benefit of these drugs may involve multiple targets and microenvironmental influences not the least of which will include platelets and other immune factors during multiple stages of carcinogenesis.

11 Effect on glucose metabolism

The adaptive metabolic shift in cancer cells with a higher glycolytic rate is known as the Warburg effect. Upregulation in the expression of glycolytic enzymes and glucose transporters in cancer cells impart the ability to uptake glucose at a higher rate [145]. Targeting the glycolytic enzymes has been considered as a viable option for targeting cancer cells as their metabolism is much higher than that of the normal cells. Aspirin was shown to inhibit purified 6-phosphofructo-1-kinase (PFK), which regulates the glycolytic pathway [146], in a dose-dependent fashion. Aspirin also acetylates and selectively inhibits glucose-6-phosphate dehydrogenase, a member of the pentose phosphate pathway involved in ribonucleotide biosynthesis, potentially leading to reduced nucleic acid biosynthesis and cell proliferation [147]. Aspirin can also acetylate many other glucose metabolic pathway proteins but this acetylation did not inhibit their enzyme activities [147, 148]. Finally, aspirin has also been reported to affect the subcellular localization of hexokinases which control the first committed step in glycolysis.

The mitochondrial membrane potential ($\Delta\psi m$) dictates the conformations of voltage-dependent anion channel (VDAC) resulting in the exchange of ions and metabolites across the mitochondrial membrane. Due to the high energy demand in cancer cells, HK is translocated into mitochondria in association with VDAC. Aspirin-induced closure of VDAC correlates with the elevation of mitochondrial Ca2+, a strong apoptotic signal. Additionally, aspirin dissociated HK-II from mitochondria that cumulatively decreased cell viability. Treatment with aspirin reduced HK (II) amount in the mitochondria due to VDAC inhibition leading to the alteration of the membrane potential and cell death [149].

Platelets in diabetic (type 2) patients are hypersensitive with elevated surface CD41, Cd42b, CD62, and CD63 receptors and more active compared to those of normal individuals [150]. With highly activated platelets, these patients could benefit more from aspirin treatment. Low-dose aspirin in Japanese diabetic patients was shown to reduce cancer incidence in individuals aged < 65 years of age but not ≥ 65 years [151]. However, high-dose aspirin treatment in type 2 diabetic patients enhanced insulin sensitivity, reduced insulin clearance, and improved their blood glucose levels [152]. Data on aspirin use and dosage recommendations by diabetic patients in reducing their cancer risk and platelet inhibition is limited.

12 Aspirin and NSAIDs for cancer prevention

Aspirin was shown to reduce self-renewal potential and stem cell signaling in pancreatic ductal adenocarcinoma suggesting NSAID use can be effective in targeting cancer stem cell-like properties [153]. Aspirin's function in acting as a cancer preventive could also be attributed to its ability to acetylate and activate the tumor suppressor protein p53, in mutant or wild-type form in colon cancer cell line [154]. In lineage tracing experiments involving β-galactosidase and fluorescent protein expression in stem cells, sulindac was shown to prevent the progression of aberrant crypts in the colon in becoming cancerous lesions.

Activation of AMPK and mTOR inhibition by aspirin adds to the benefits in the use of aspirin as a chemopreventive in CRC. Aspirin's inhibition on mTOR signaling was mediated by targeting the phosphorylation of mTOR pathway effectors S6K1, 4E-BP1, and S6. Additionally, the mechanism of mTOR inhibition was shown to be both dependent and independent of AMPK [155]. Aspirin use can suppress cancer cell migration by inhibiting epithelial to mesenchymal transition and induce antiangiogenic activity by inhibiting vascular endothelial growth factor [156, 157]. Aspirin was also shown to induce the production of TGF-β1 by CRC cell lines which in turn mediated the reduction in the viability and entry into apoptosis phase. Caspase 8 levels were also increased in these cells [109].

13 Cancer prevention by inhibiting eicosanoid metabolism

Meta-analyses of randomized clinical trials provide clear support for NSAID-based reduction of gastrointestinal and other cancer incidence, mortality, and metastasis

[158–169]. The preventive effects of aspirin involve chemical acetylation of cyclooxygenase enzymes 1 or 2 that provide the rate limiting substrate for all prostaglandins. Additional mechanisms are gaining appreciation but there may be some that still needs to be uncovered since aspirin is known to covalently acetylate a variety of different molecules [41, 170–172]. With recent advances in technology, there could be a possibility of gaining new insights into aspirin's mode of action [41, 148, 173, 174]. This article endeavors to describe the mechanisms and supportive evidence that highlights the efficacy of aspirin and other NSAIDs as cancer-preventive drugs.

Aspirin still prevails as one of the most successful drugs ever made, along with serving as an anti-inflammatory agent that can also render effective cancer preventive effects. It is used to prevent cardiovascular disease (CVD) in those who are not at risk for serious bleeding [175, 176]. Several clinical trials and reports show strong evidence that aspirin reduced cancer-associated mortality and prevented CVD with daily use and the beneficial effect improved after 5 years [177]. Remarkably, fatalities from bleeding with aspirin was significantly lower than the control groups. Decreases in cancer incidence [178], particularly for gastrointestinal cancers or cancer-related [179–181] or all-cause mortality, were also shown with aspirin.

Aspirin preventive effects on cancer are based on short-term use that inhibited colorectal adenoma formation. For example, hereditary lynch syndrome patients, or those at high risk of developing CRC [182] or adenomas [183, 184], exhibited a reduction in their adenomas with aspirin.

Celecoxib selectively competes for the active site of COX-2 due stearic constraints compared to COX-1 and was shown to suppress adenoma formation in high-risk patients with familial adenomatous polyposis (FAP) [47] as well as post-polypectomy patients [49, 185]. Unfortunate toxicities that occurred during the trails have eliminated the use of some COX-2-selective inhibitors [50, 77, 78]. Despite these issues, in a phase III trial, NSAID sulindac given in combination with difluoromethylornithine (DFMO) was efficacious in preventing cancer. Collectively, these studies support the notion of aspirin and other NSAID use in preventing cancer.

Even after decades of research, the full explanation of NSAID bioactivity in lowering cancer incidence, progression, and metastasis, mortality, remain incomplete. Furthermore, the biologic complexities and signaling pathways that platelet and cancer-derived bioactive lipids impact they are likely to play different roles depending on when they occur during the cancer continuum.

14 Summary

The rapid response of platelets is an essential part of the first responder behavior. This is primarily due to the eicosanoid metabolites TxA_2 and PGI_2 that maintain hemostatic balance but have very short half-lives in circulation. This is around 30 s due to epoxide ring opening. Both TxA_2 and PGI_2 are mobilized through membrane lipid sources of arachidonate. Arachidonate is converted by COX enzymes to PGH_2, which serves as the substrate for all PG production. PGH_2 is converted *via* COX-1 through TXAS to TxA_2 and exported to then bind platelet TP receptors. TP is a very strong G-protein-coupled receptor that triggers platelet activation and aggregation. This is counterbalanced by the conversion of AA to PGI_2 through COX-2 and then PGIS that binds to IP G-protein-coupled receptor agonist that prevents platelet activation and aggregation. The TxA_2-PGI_2 balance is shifted toward TxA_2 following interactions with tumor cells that activate platelets by a variety of surface receptor interactions and circulating factors. There are a variety of inhibitors that target all of the molecules within the TxA_2 and PGI_2 pathways. These serve as more selective targets than COX molecules and may avoid the cardiovascular and GI toxicity associated with targeting COX and eliminating all PG production. Additional eicosanoid metabolites include lipoxygenases, leukotrienes, and resolvins that can also influence platelets, inflammation, and carcinogenesis which can serve as additive targets along with newer molecular and cellular interactions. Nonetheless, continued study of NSAIDs on cancer strengthens the notion that COX-dependent mechanisms can drive the overall process. The fundamental concept that COX-1/2 play key roles in carcinogenesis provides encouragement that other downstream targets may be equally important, but how can we prioritize these? Although COX-2 inhibitor-targeted therapies seem to work, are the other molecular targets of equal or more value? Will these additional targets provide equal or additive overall benefit, be even more selective and eliminate the toxicity associated with NSAIDs? The risk: benefit associated with targeting platelets in effort to impact cancer progression and metastasis presents challenges considering that platelet count and bleeding risk must be considered. The timing and duration for any platelet-based interventions are also critical to consider. Aspirin seems to be an inexpensive option for now but more targeted approaches may ultimately provide less toxic solutions. In the final analysis, a better understanding of platelets and bioactive lipid biology as a key first responder element in cancer and metastasis will add critical knowledge to our prevention and treatment of cancer.

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