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The thromboxane synthase and receptor signaling pathway in cancer: an emerging paradigm in cancer progression and metastasis

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Abstract

Thromboxane A_2 (TXA_2) is a biologically active metabolite of arachidonic acid formed by the action of the terminal synthase, thromboxane A_2 synthase (TXA_2S), on prostaglandin endoperoxide (PGH_2). TXA_2 is responsible for multiple biological processes through its cell surface receptor, the T-prostanoid (TP) receptor. Thromboxane A_2 synthase and TP are the two necessary components for the functioning of this potent bioactive lipid. Thromboxane A_2 is widely implicated in a range of cardiovascular diseases, owing to its acute and chronic effects in promoting platelet aggregation, vasoconstriction, and proliferation. In recent years, additional functional roles for both TXA_2S and TP in cancer progression have been indicated. Increased cyclooxygenase (COX)-2 expression has been described in a variety of human cancers, which has focused attention on TXA_2 as a downstream metabolite of the COX-2-derived PGH_2. Several studies suggest potential involvement of TXA_2S and TP in tumor progression, especially tumor

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cell proliferation, migration, and invasion that are key steps in cancer progression. In addition, the regulation of neovascularization by TP has been identified as a potent source of control during oncogenesis. There have been several recent reviews of TXA_2S and TP but thus far none have discussed its role in cancer progression and metastasis in depth. This review will focus on some of the more recent findings and advances with a significant emphasis on understanding the functional role of TXA_2S and TP in cancer progression and metastasis.

Keywords

Thromboxane synthase; Thromboxane receptor; Cyclooxygenase; Cancer progression; Metastasis; Angiogenesis; Cell migration; Apoptosis

1 Introduction

Thromboxane A_2 (TXA_2) was one of the first prostaglandins to be identified from washed platelets (in 1975) and has been widely implicated in a range of cardiovascular diseases, owing to its acute and chronic effects in promoting platelet aggregation, vasoconstriction, and proliferation [1–3]. TXA_2 is a biologically active metabolite of arachidonic acid (AA) formed by the action of TXA_2 synthase (TXA_2S) on prostaglandin endoperoxide (PGH_2) [1, 4, 5]. TXA_2 is highly unstable in aqueous solution, where it spontaneously hydrolyzes to the biologically inactive hemiacetal thromboxane B_2 (TXB_2) with a half-life of 30 s [4]. Due to its short half-life, TXA_2 primarily functions as an autocrine or paracrine mediator in the tissues surrounding its site of production. TXA_2 is responsible for multiple biological processes through the cell surface TXA_2 receptor, or T-prostanoid (TP) receptor [6, 7]. TXA_2 biosynthesis and TP expression are elevated in numerous cardiovascular and inflammatory diseases [3, 8– 10]. It is felt that TXA_2, through its receptors, plays a very important role in the pathogenesis of acute coronary artery syndrome, vessel remodeling, thrombosis, renal, pulmonary, and atherosclerotic cardiovascular diseases primarily through its action as a potent vasoconstrictor and an inducer of platelet aggregation and activation [8, 9, 11–15]. As such TXA_2S inhibition and TP antagonism have become central to the therapy of many diseases including infarction, hypertension, stroke, and renal dysfunction [16–18]. Recently a role for TXA_2 signaling in cancer has become apparent. This review will focus on some of the more recent findings and advances with a major emphasis on understanding the functional role of TXA_2S and TP in cancer progression and metastasis.

1.1 Thromboxane A_2 synthase

The cyclooxygenase enzymes, cyclooxygenase (COX)-1 and COX-2, are responsible for the conversion of AA to PGH_2, the first step in the generation of TXA_2. TXA_2S is an endoplasmic reticulum membrane protein that belongs to the P450 epoxygenase family that catalyzes the conversion of the COX product PGH_2 to TXA_2 [19] (Fig. 1). TXA_2S was first found as a microsomal enzyme in platelets (60 kDa) and is highly expressed in lung, platelets, kidney, stomach, duodenum, colon, and spleen [20–22]. TXA_2S expression is reported to be closely associated with cardiovascular, renal, and inflammatory diseases [21, 23].

1.2 Thromboxane A_2 receptor

TXA_2 is responsible for multiple biological processes through its cell surface receptor TP. Ligation of TP by TXA_2 activates multiple downstream pathways, including phospholipase C, and raises intracellular Ca^{2+} levels leading to vasoconstriction and platelet aggregation [6, 21]. The TXA_2 receptor (TP) is a typical G-protein-coupled receptor expressed as two different isoforms in humans— TP-alpha (TPα) and TP-beta (TPβ) [24, 25]. These TP isoforms arise via alternate splicing of a single gene and share the first 328 amino acids. TPα and TPβ differ in the length of the C-terminal cytoplasmic tail with TPα shorter than the TPβ isoform (15 *versus* 79 residues) [24–26] (Fig. 2). Ligand binding sites for the TP receptor are in its extracellular region and are identical in both splice variants. TP isoforms share both common and distinct signaling pathways depending on the G protein subunits bound to their C-terminal tail. Therefore, differences in function between TPα and TPβ may manifest due to the complement of G-proteins associated with the cytoplasmic tail. TP receptors are found in a large number of tissues and cell types. TP mRNAs are expressed widely in platelets and monocytes and in the lung, liver, kidney, cardiovascular system (myocytes, vascular smooth muscle cells, and endothelium), uterus, brain, spleen, thymus, and placenta [6, 26–29].

2 Role of thromboxane A_2 and its receptors in cancer progression

In recent years, several studies have indicated functional roles for both TXA_2S and TP in cancer progression. Increased COX-2 expression has been described in a variety of human cancers and downstream metabolites of COX-2, such as TXA_2, have therefore also become of interest for their potential role in cancer progression [30– 33]. Multiple studies have documented roles for TXA_2 signaling in the essential processes of neoplastic transformation, including enhanced tumor cell motility that are key steps in cancer progression. These effects are observed in multiple cancers indicating the extensive nature of these effects and the widespread clinical applicability of targeting these pathways as adjunct therapy in cancer [30–32, 34].

2.1 Role of thromboxane A_2 signaling in prostate cancer

Prostate cancer (PCa) is one of the most common cancers among men in the USA and is the second leading cause of death in this population [35]. Strong correlations exist between diets high in fat (especially from red meat) and PCa [36–38]. As red meat is rich in AA, the presence of enzymes associated with AA metabolism might result in increased synthesis of downstream lipid mediators. Several metabolites of AA, such as 5-HETE and 12-HETE, have already been studied widely for their roles in PCa [39–42]. Numerous studies demonstrate increased COX-2 mRNA and protein expression in PCa correlate with poor prognosis [43, 44]. TXA_2 is derived from the COX product PGH_2 and, as a consequence, both TXA_2S and TP have long been hypothesized to have a possible role in prostate cancer progression.

Prostate tumor progression involves several key steps including cell survival, cell migration, cell invasion, and metastasis. Human PCa cells express functionally active TXA_2S and biosynthesize TXA_2 [45]. As the expression of COX-2 and COX-1 in prostate cancer has

been reported previously, Nie and colleagues investigated their role in synthesis of TXA_2 [45]. Treatment of PC-3 cells with both COX-1 and COX-2 inhibitors reduced TXA_2 synthesis by 95%, similar to the level achieved by direct TXA_2S antagonism. These data suggest that human PCa cells express TXA_2S and that both COX-1 and COX-2 are essential for providing the substrate for TXA_2S mediated TXA_2 biosynthesis in PCa cells [45]. Increased TXA_2S expression and activity in PCa cells augmented cell migration but had minimal effect on cell cycle progression or survival indicating that TXA_2S activity might contribute to PCa progression through modulating cell motility [45].

In a study conducted on tissue samples from 46 patients, with well-documented histological and molecular data, increased TXA_2S and COX-2 expression were observed in tumors. In the same study, TP expression was higher in malignant tissues (high-grade prostatic intraepithelial neoplasia and cancer glands) [46]. Similarly, TXA_2S mRNA and protein expression were higher in prostate carcinomas compared to matched normal tissues. In contrast, epithelial cells in non-tumoral glands displayed almost non-existent TXA_2S and TP expression [46]. The degree of TXA_2S expression correlated with the severity of prostate carcinoma lesions, with advanced stages and poorly differentiated forms having the highest expression levels [45, 46]. Moreover, a significant association between the expression of COX-2/TXA_2S/TP and higher Gleason score/pathologic stage of the tumors was observed [46]. Within the cancer tissue, expression of TXA_2S and TP were localized to areas of perineural invasion, a known mechanism by which PCa cells penetrate the prostatic capsule and spread to other tissues. Strong expression of TXA_2S along perineural tracts might be an indicator for its potential involvement in tumor cell invasion and metastasis [45–47]. The enzyme was found to be involved in motility, but not proliferation or survival, of PCa cells [45].

Tumor cell migration is an important step in the metastatic cascade. At this time, tumor cells leave the primary organ, enter the circulation, and colonize distant tissues [48]. Rho GTPases are critical for the dynamic changes in cell shape and adhesion that drive cell migration. Nie and colleagues demonstrated that the TXA_2-TP signaling axis regulated cell migration and cytoskeleton reorganization through promoting Rho-A activation [49]. Recent studies have indicated that the Gα12 family of heterotrimeric G proteins (Gα12 and Gα13) is up-regulated in PCa and that activation of Gα12 signaling promotes PCa cell invasion, through a Rho-dependent pathway. In addition, Gα12 signaling via Rho is required for TXA_2 stimulated invasion of PCa cells [50]. As TP is known to couple to the Gα12 family of heterotrimeric G proteins, the up-regulation of both TP and Gα12 in PCa suggests a possible mechanism by which TP may drive the cell migration and invasion observed in high-grade PCa. This hypothesis needs to be investigated further.

A recent report provided evidence of a novel constitutive interaction between TPα/TPβ and protein kinase C-related kinases (PRK1) [51]. PRK1 is a Rho-A effector that has been widely implicated in androgen-associated PCas and ovarian serous carcinomas [51]. It was established that PRK1 directly interacts with endogenously expressed TPα and TPβ in both PC-3 and LNCaP cells, and disruption of PRK1 by siRNA substantially impairs cell migration in response to TXA_2 agonist U46619 in these cells [51]. These findings all point towards a possible functional role of TP in cell migration in prostate cancer.

2.2 Role of thromboxane A_2 signaling in breast cancer

Breast cancer is one of the most common cancers among American women and is the second leading cause of death among them in USA [35]. In a cancer-profiling array, TXA_2S mRNA levels were increased in seven of nine breast tumors when compared to their matched normal tissues [45]. A larger study (120 patients) in normal breast and tumor tissues with well-documented histological and molecular data found transcripts of TP and TXA_2S were differentially expressed by quantitative polymerase chain reaction (qPCR) [52]. TXA_2S levels were similar in tumor and normal breast tissues; however, TXA_2S expression was significantly lower in high-grade tumors compared to low-grade tumors. By comparison, higher total TP mRNA expression was observed in the tumor (grade 3 and above) compared with normal mammary tissues [52] and may indicate higher mortality and worse prognosis. TP expression correlated with estrogen receptor status in this study (p= 0.0128) but was independent of nodal involvement or primary tumor type (ductal *versus* lobular) [52]. As in prostate cancer, recent studies suggest the increased expression of Gα12 and TP in breast tissue may activate Rho-A to promote cell motility to further exacerbate the progression of breast cancer [53]. These data strongly suggest a role for TXA_2 signaling in the development and progression of breast cancer. Moreover, the association of high TP expression in aggressive tumors with poor prognosis indicates TP may have significant prognostic value in clinical breast cancer.

Abraham et al. analyzed seven prostaglandin pathway genes for single nucleotide polymorphisms that may predispose to breast cancer and found that only PTGIS and TXA_2S polymorphisms showed modest associations [54]. Collectively, these reports suggest that TP may play a role in breast cancer; however, the significance of this role remains uncertain due to the conflicting nature of the reports. The pathogenic role is made less clear by the realization that it is not necessary for both TXA_2S and TP to be expressed in the same tissue to have a possible role in cancer progression. TXA_2 can act as paracrine mediator due to the abundant expression of TXA_2S in platelets and other cell types in the tumor microenvironment. Thus, further pre-clinical investigation into the role of TXA_2 signaling is warranted to define the true role of this bioactive lipid in breast cancer.

2.3 Role of thromboxane A_2 signaling in lung cancer

Lung cancer is the second leading cause of cancer in the USA every year and more people die from lung cancer than breast, colon, and prostate cancers combined [35]. The role of COX-2 and prostaglandins in lung cancer is now attracting considerable attention from cancer biologists and the public. A study conducted on 48 samples of non-small cell lung cancer (NSCLC) and matched normal lung tissues observed specific cellular expression patterns of the COX-isoenzymes and terminal synthases of prostanoid synthesis. Increased COX-2 and simultaneous down-regulation of COX-1 expression in NSCLC were identified by immunohistochemistry [55]. Moreover, high levels of TXB_2, the stable metabolite of TXA_2, have been detected in human lung cancer tissues indicating increased TXA_2S activity in lung cancer [56]. An extension of the aforementioned work, in a larger sample size, compared the prostaglandin biosynthesis pathways in small cell lung carcinoma and NSCLC and further correlated their observations with angiogenesis and metastasis [57]. Expression

of TXA_2S, COX-1, COX-2, and microsomal prostaglandin-E synthase were significantly higher in the metastatic cases of NSCLC as compared to non-metastatic cases [57].

TPα expression has been documented in five out of six NSCLC cell lines [58]. Moreover, A549 cells with ectopic TPα expression exhibited greater tumor growth and increased vascularization than the control A549 cells when implanted into nude mice [58]. Over-expression of COX-2 in lung tumors has been widely reported and factors that may cause over-expression of COX-2 in lung tumors are not completely understood. Activation of TPα with the TXA_2 mimetic IBOP induces the expression of COX-2 through activation of four signaling pathways (extracellular signal-regulated kinase (ERK), p38 MAPK, JAK, and β-catenin). In addition, transcription factors such as NFκB, cAMP response element-binding (CREB), C/EBP, and Stat3 are downstream signaling molecules that interact with the COX-2 promoter and play important roles in TPα-mediated expression of COX-2 [59].

Strong evidence suggests that Nurr1 is critical for the proliferation of lung carcinoma cells (H157) through regulation of cyclin D1 expression [60]. Studies have indicated that TP signaling induces Nurr1 expression through a mechanism that bypasses the epidermal growth factor receptor (EGFR) pathway. Moreover, TP agonist-induced proliferation in lung cancer cells promoted cyclin D1 expression through induction of Nurr1 [60]. Nie and colleagues showed that pulmonary metastasis of intravenously injected Lewis lung carcinoma (LLC) is attenuated by TXA_2S inhibitors. Moreover, oral administration of TXA_2S inhibitors CI or furegrelate reduced the number and size of metastatic colonies in the lung after injection of LLC cells [61]. These data confirm that TXA_2S activity is required for development of lung tumor metastasis and that TXA_2S inhibitors are potential anti-metastatic agents. These findings shed new light on the contribution of TPα signaling to lung tumorigenesis.

Evading apoptosis (or programmed cell death) is a hallmark of most types of cancer [62] and is a key step in cancer progression. TXA_2S expression inhibits apoptosis in lung cancer cells and TXA_2S inhibition induces apoptosis in NSCLC cells. Moreover, two specific TXA_2 antagonists enhance the effects of cisplatin (a chemotherapeutic agent) [63]. The mechanism by which TXA_2 signaling enhances cell survival and proliferation in lung cancer cells involves reduction of nuclear p27 levels which mitigates apoptosis [64]. While the mechanism inducing apoptosis is not fully defined treating NSCLC cells with the TXA_2S inhibitor 1-BI induces ROS generation and decreases NF-κB activity and nuclear translocation by decreasing IκBα phosphorylation [65].

Given the well-established link between smoking and lung cancer and the role for TXA_2 signaling in lung cancer, it will be obvious to examine if TXA_2 signaling mediates the smoking–lung cancer connection. Indeed, it was shown that significantly higher TXB_2 production in lung cancer tissues from smokers compared to cancer tissues from non-smokers [66]. To examine the causal role for TXA_2 signaling in lung cancer, Huang and colleagues recently correlated TXA_2S, TXB_2, and carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers and non-smokers. As expected, significantly higher TXA_2S expression was observed in tumor tissues compared to non-tumor tissues from the same patient. A more interesting link to NNK could be inferred from the observation that

significantly fewer tumors from non-smokers showed TXA_2S expression as compared to tumors from smokers. Further, 100% of tissues from smokers (tumors and non-tumoral tissue) were positive for TXA_2S expression unlike non-smokers where 50% of non-tumoral and 25% of tumoral tissues were negative for TXA_2S expression. NNK increased TXA_2S, TXA_2 synthesis, and TP activation *in vivo* and in lung cancer cells *in vitro*. The increased TXA_2 may subsequently activate CREB through the PI3K/Akt and ERK pathways, thereby contributing to the promotion of survival and growth of lung cancer cells by NNK [67]. Moreover, specific TXA_2S inhibitors and TP antagonists abolished growth and induced apoptosis in lung cancer cells [67] indicating the importance of TXA_2- to NNK-induced tumor growth in lung cancer.

These data were sufficiently provocative that Cathcart and colleagues examined the expression of TXA_2S in NSCLC in 204 patients, to identify if TXA_2S was a prognostic and/or survival factor [68]. They also examined the role of TXA_2S in mediating the migration and growth of NSCLC cells *in vitro*. Though they observed no prognostic role for TXA_2S in NSCLC, they confirmed that both the plasma and protein level of TXA_2S and TXB_2 were significantly higher in tumor tissues than the matched “normal” tissues [68]. Equally their model system confirmed that enhanced TXA_2S expression promotes proliferation, motility, invasiveness, and survival of cancer cells. Inhibition of TXA_2S with selective inhibitors induced apoptosis confirming that it is indeed a potential therapeutic target in NSCLC [68].

2.4 Role of thromboxane A_2 signaling in colon cancer

Colorectal cancer is one of the leading causes of cancer-related deaths in the United States [35]; however, early diagnosis often leads to a complete cure. As in the case of prostate cancer, studies have shown strong correlation between high-fat diets, associated with consumption of red meat, and colon cancer [69]. Consumption of foods rich in AA likely increases synthesis of prostaglandins, such as TXA_2, in the presence of enzymes associated with AA metabolism. TXA_2S and TXA_2 biosynthesis are increased in colon cancer and cause detrimental effects by promoting TP signaling [70]. The recognition that COX signaling was a strong pro-cancer signal for colon tumors is not recent and the correlated expression of COX-2 with colon carcinoma [71] has reinforced the causal relationship between the two. Epidemiologic data showed that up to 40% reduction of mortality in colorectal cancer patients with regular nonsteroidal anti-inflammatory drugs (NSAIDs) use as compared to non-NSAID consumers [71]. Gene transfer of TXA_2S increased colon cancer cell growth *in vivo* and enhanced angiogenesis [72] (see below). Strong evidence of a role for TXA_2S in colorectal carcinoma was provided when a marked over-expression was observed in different grades of colorectal tumors compared to the paired-normal tissues. The same study found increased expression of TXA_2S in colon cancer cell lines and that abrogating TXA_2 signaling with TXA_2S inhibitors, TXA_2S anti-sense as well as TP antagonists reduced proliferation of the colon cancer cell lines [73, 74]. qPCR was used to quantify TP transcript expression from 62 tumors and adjacent normal colon tissues. Results indicated that TP was expressed at significantly higher levels in tumors compared to normal tissues but displayed lower levels of TP expression in cultured colorectal cancer cell lines (HT-29 and HCA-7) [73]. The aforementioned studies have started to carve a role for TXA_2

signaling in colon cancer; however, the data presented are all from studies with small sample size and need to be validated in a larger cohort of patients with colon cancer.

2.5 Role of thromboxane A_2 signaling in brain cancer

Gliomas are brain malignancies of glial cell origin, such as oligodendroglioma, that are notorious for recurrence and the rate at which they progress to high-grade malignancies [75]. The capacity of glial tumor cells to migrate and diffusely infiltrate normal brain makes the disease notoriously difficult to cure by surgical eradication. Thus, identification of genes associated with invasion may offer novel strategies for anti-invasive therapies. This prompted McDonough and colleagues to assess glioma cell lines with a migration advantage on a glioma-derived extracellular matrix [76]. The outcome was a genetically stable strain that showed a migration advantage, a slightly arrested growth rate, anchorage-independent growth and was comparable to the parental cells in their tumorigenicity in athymic nude mice. This migration-advantaged strain showed increased TXA_2S expression [76]. When treated with TXA_2S antagonists (such as dazmegrel and furegrelate) the migration of the strain with enhanced mobility was normalized to the rate of the parental cells, suggesting direct regulation of cellular motility by TXA_2S [76]. Giese and colleagues showed TXA_2S expression to be present in a large panel of glioma cell lines but not in normal human astrocytes [77]. Moreover, TXA_2S expression was observed in the parenchyma of glial tumors and in reactive astrocytes but not in quiescent astrocytes and oligodendroglia of normal brain. A wide range of TXB_2 formation in glioma cell lines was observed, the relative expression of which correlated with migration rates of these cells. Not surprisingly, TXA_2S-specific inhibitors effectively blocked the migration advantage and decreased intercellular adhesion in glioma cells [77]. This was the first indication that TXA_2 signaling may play a crucial role in glial tumors and represent a novel strategy for anti-invasive therapies.

Giese and colleagues also revisited phospholipid biosynthesis in glioma by assessing the metabolic activity of the upstream enzymes COX-1 and COX-2. In comparison to inhibiting TXA_2S, the inhibition of COX isoforms produced no significant shift in the phenotype of glioma, despite the robust expression of COX in all grades of glioma [77]. This may highlight a functionally antagonistic role for other downstream metabolites of COX in cancer. In addition, TXA_2S inhibition with furegrelate induced caspase activation, DNA fragmentation, and eventual apoptosis only in glioma-derived cells but not normal astrocytes or fibroblast [77]. These same effects of TXA_2S inhibition were also observed on endothelial cells indicating that anti-TXA_2S therapy might sensitize the invasive glioma cells, and angiogenic endothelium to apoptosis [77].

Diagnosis of invasive glioma is often too late for anti-invasive therapy to be effective. Thus, the identification of TXA_2S inhibitors as regulators of glioma cell motility is of little consequence unless the invasive cells are also rendered susceptible to cyto-reductive treatments. In this regard TXA_2 antagonism shows great promise as an adjunct to standard therapy. Pre-treatment of glioma cells with furegrelate, a specific TXA_2S inhibitor, increased radiation sensitivity of cultured glioma cells [78]. The present standard of care in glioblastoma (the most malignant grade 4 glioma) is surgical resection followed by

combined chemo-radiation therapy [79]. Schmidt and colleagues presented compelling data indicating that a single administration of furegrelate to *in vivo* orthotopic glioblastoma in mice significantly reduced tumor size, tumor cell proliferation, and angiogenesis and increased apoptotic cell death [80]. Combining TXA_2S inhibition with an alkylating agent (BCNU) also showed a significant survival effect on an ectopic mouse model of glioma [80]. The ultimate proof of principle for the inclusion of TXA_2S inhibition in glioma therapy will be to demonstrate significant improvements to clinically valid treatment regimens (such as radiation therapy followed by temozolomide treatment) in orthotopic mouse models. If successful, TXA_2S inhibition is likely to be quickly implemented as an adjunct therapy in clinical trials, which raises hopes of finding an effective treatment for glioblastoma patients especially for those with unmethylated MGMT promoter [81].

2.6 Role of thromboxane A_2 signaling in bladder cancer

Over the last decade, there has been an increasing awareness of the role of prostanoids in the development and progression of invasive bladder cancer. Susceptibility to invasive bladder cancer correlates with the −765G>C mutation in the COX-2 promoter [82]. This polymorphism results in an under active promoter and lower COX-2 expression. This change likely causes liberated AA to shunt into a COX-1-based pathway and potentially results in greater synthesis of prostaglandins like TXA_2. TXA_2 release is not observed from unstimulated or challenged “normal” urothelial cells [83] and TXA_2S release and TXA_2S expression are very low in normal bladder tissue [84, 85]. Conversely, TXA_2S expression is an average of 11.6-fold higher (range 5–18-fold) in invasive bladder cancer and transformed bladder epithelial cell lines exhibit greater expression than non-transformed immortalized cells [85]. Focal TXA_2S expression is first observed in carcinoma *in situ* and becomes widespread in invasive and high-grade carcinoma [84]. In both bladder cancer tissue and cell lines, TXA_2S expression is transcriptionally regulated. TXA_2S expression correlates significantly with tumor grade, stage, and overall survival and is an independent marker of invasiveness in the tumor [85].

TP expression is also increased in invasive bladder cancer compared to “normal” adjacent and remote bladder tissue. TP expression is increased at the protein, but not them RNA, level prompting speculation that post-translational mechanisms stabilize the protein [85]. However, the increased TP expression in bladder cancer was documented using antibodies/probes that bind the common domain. More recently, the significance of individual TP isoforms in bladder cancer has been investigated. Increased expression of the humanspecific isoform TPβ, but not TPα, is enhanced in epithelial and stromal compartments in invasive bladder cancer and correlates with cancer grade. Like expression of TXA_2S, TP expression, and in particular TPβ expression, correlates well with increased growth rate, invasiveness, and shorter survival time [86]. TPβ expression was only observed in bladder cancer cell lines and inhibition of TPβ produces apoptosis associated with suppression of ERK and focal adhesion kinase (FAK) signaling. Conversely, ectopic expression of TPβ in “normal” bladder epithelium stimulates growth, migration, and invasive potential [86]. Moreover, it enables “normal” bladder cancer to form xenografts in nude mice that result in highly de-differentiated tumors [86]. TP signaling through the Gα12 family was implicated in the enhanced motility of bladder cancer but no further information was provided on the

pathways responsible for proliferation. This is one of the few cancers for which roles of the two TP isoforms have been identified and provides evidence for distinct roles for TPα and TPβ in the pathogenesis of cancer.

While the changes in TXA_2/TP expression are pronounced in bladder cancer, the real question is: Does targeting this pathway arrest/perturb tumor growth? Cells overexpressing TXA_2S or TP grow at an accelerated rate and are highly invasive [85, 86]. Pharmacological antagonism of TXA_2S or TP *in vitro* slows proliferation of bladder cancer cell lines two- to three fold and ablates migration and invasion [85, 86]. Further, depriving bladder cancer cells from TP signaling (either pharmacological inhibition or small interfering RNA (siRNA) knockdown approaches) promotes caspase processing and the onset of apoptosis [84] and sensitizes the cells to the effects of other chemotherapeutic agents including paclitaxel and cisplatin [84]. These data suggest that enhanced TXA_2 levels due to TXA_2S overexpression were activating TP-dependent pathways of cell proliferation.

2.7 Modulation of endothelial cell migration/angiogenesis by thromboxane A_2

Neovascularization, or the formation of new blood vessels, is central to the pathogenesis of most solid tumors and their sequelae, including metastasis. It occurs through a number of mechanisms including angiogenesis, vasculogenesis, and intussusception (reviewed in [87]). The level of new blood vessel growth is dependent upon the balance between proand anti-angiogenic factors present in the tissue. TXA_2 has both pro- and anti-angiogenic effects in multiple experimental systems. We have found that TP stimulation reduces spontaneous endothelial cell (EC) migration by 58% and *in vitro* capillary formation by 85% [88] as well as abrogating the pro-angiogenic effects of vascular endothelial growth factor (VEGF)-A [89] and fibroblast growth factor-2 (FGF-2) [90] *in vitro* and *in vivo*. Moreover, TP stimulation results in the destruction of established EC networks through increased apoptosis [91]. The regulatory pathways for each of the effects are highly stimulus specific and share few features in common. Antagonism of nitric oxide and FAK [89], stabilization of p53/ antagonism of integrin αvβ3 [90], and inhibition of intercellular communication [88] are just some of the mechanisms employed by TXA_2 to prevent angiogenesis. A number of reports from other groups support the concept that TXA_2 is an anti-angiogenic stimulus. TXA_2 suppresses angiogenesis and promotes vascular degeneration in the retina through induction of calpain-dependent neuro-retinovascular EC death [92]. TP stimulation mediates EC apoptosis associated with diabetes [93, 94] and acts in an additive/synergistic manner with platelet releasate [95] and neurokinin B [96] to promote EC injury and inhibit angiogenesis. In addition, the TP ligands isoprostane 8-iso-$PGF_{2\alpha}$, 8-iso-PGE_2, and 8-iso-PGA_2 all inhibit spontaneous and VEGF-induced migration and differentiation of human coronary ECs *in vitro* and sprouting angiogenesis from cardiac explants *ex vivo* without influencing apoptosis [97]. These effects are sensitive to inhibition of Rho kinase suggesting deranged actin metabolism was involved, later proven by alterations to stress fiber formation. Collectively, these data support the hypothesis that TXA_2 may discourage re-vascularization of infarcted/ hypoxic tissue and may promote the regression of vessels through the induction of apoptosis in exposed vascular beds. Thus, blocking TP seems an appropriate course of action in diseases such as myocardial infarction, where re-vascularization is to be encouraged.

Conversely, a number of groups have shown TXA_2 is pro-angiogenic. Robust TXA_2S expression is observed in the tumor-associated endothelial cells of lung cancer but not normal lung tissue [57]. These findings support the view that TXA_2 may promote angiogenesis, thereby accelerating cancer progression. Some of the most convincing data have correlated the overexpression of TXA_2 synthase in tumor cells with enhanced angiogenesis, shortened survival time, and increased tumor growth rate [72]. However, an important limitation of these findings was that the authors did not distinguish whether the effects of TXA_2 on angiogenesis were due to direct actions on endothelial cells or an autocrine effect on the tumor cells. Indeed, Tai and colleagues [58] reported that TXA_2 stimulation of human lung cancer cells enhances production of the pro-angiogenic protein VEGF, a key stimulus for tumor angiogenesis. Stimulation of TPα increased VEGF expression at both the mRNA and protein levels via a mechanism involving activation of ERK, PKA, EGFR, and Src kinases. Xenografts of A549-TPα cells induced greater tumor growth and increased vascularization in nude mice than control A549 cells [58]. However, evidence of increased neovascularization was only macroscopic and could have easily resulted from hemorrhage into the growing tumors. These data indicate that the autocrine effects of TP on the tumor itself are not just a regulator of tumor growth but also a significant regulator of neovascularization.

TXA_2 also has direct pro-angiogenic effects on the endothelium. Pro-angiogenic factors (FGF-2 and VEGF) promote TXA_2 release from EC up to five fold [61]. TXA_2 stimulation promotes sprouting in corneal neovascularization and rat aortic ring assays, induces vascularization of the rodent ovary, and stimulates the differentiation and migration of endothelial cells *in vitro* [46, 61, 72, 98, 99]. Furthermore, antagonizing TP/TXA_2 synthesis attenuates endothelial chemotaxis to FGF-2 and VEGF, differentiation of endothelium on matrigel *in vitro*, and angiogenesis in the corneal neovascularization and rat aortic ring assays [61, 100] [98]. These activities are not overcome by incubation with VEGF [100] indicating the strength of the inhibition of angiogenesis by these agents and/or essential contribution of TXA_2 to the pro-angiogenic effects of other factors. These antagonists do not affect EC viability or adhesion indicating their effects on quiescent ECs would be minimal [100]. If these data hold true, then the inhibition of TXA_2 synthesis or TP signaling during diseases, such as cancer, would prevent vessel formation, slowing tumor growth, and prolonging survival.

TPα and TPβ have almost identical coupling to heterotrimeric G-proteins resulting in misplaced complacency regarding their distinct roles in disease. We believe the dichotomy in the reported effects of TP stimulation on angiogenesis result from the use of models that lack TPβ expression. The relative importance of both TP isoforms to EC biology, especially TPβ, is largely unexplored. Our data also show that expressing TPβ in the endothelium from TP null mice inhibits migration and differentiation *in vitro*, and transgenic mice overexpressing TPβ in endothelial cells display reduced angiogenesis in matrigel plug models[89, 90]; however, only humans have TPβ with both rat and mouse TP similar to the human TPα isoform (78% homology). Thus, the small animal models upon which the pro-angiogenic properties of TXA_2 are based are flawed as they do not account for TPβ making comparisons difficult. More importantly, our data indicate that the anti-angiogenic

properties of TPβ dominate in EC expressing both isoforms [89, 90]. The primary deficit in the original report of the TPβ transgenic mouse was decreased placental size with microscopic evidence of ischemia [101]. Our seminal observations extend these findings and suggest that vessel formation in the developing placenta was most likely suppressed by TPβ. Further, the amplified signaling of TPα-TPβ heterodimers in response to isoprostanes [102] may be the reason for the highly potent nature of these TP agonists as anti-angiogenic compounds [97]. Thus, the existence of two TP isoforms in humans may have implications for the role of TXA_2 in vascular remodeling in angiogenic diseases such as cancer.

The mechanisms by which the two TP isoforms regulate angiogenesis are still somewhat unclear. TPα does not share the same angioregulatory activity as TPβ indicating that the divergent tail residues are the source of these properties. The tail of TPβ contains multiple sites that could regulate receptor signaling but few are well characterized. Residues TPβ355–TPβ366 and TPβ337–TPβ344 are important for ligand-induced [103] and tonic [104] internalization and mediate interactions with Nm23-H2 [105] and Rab11 [106]. These data indicate the protein interactions of the two tails are different and have binding sites for signaling molecules that may regulate angiogenesis. Further, Kinsella and colleagues recently reported PRK1 and angio-associated migratory cell protein as the first non-G-protein signaling proteins that couple to TP isoforms that have direct effects on cell motility [51, 107]. These data highlight the role of non-G-protein-mediated mechanisms in the regulation of angiogenesis by TP isoforms and further strengthen the notion of divergent pathological roles for TPα and TPβ in disease.

3 Summary and future directions

In summary, these findings suggest TXA_2S and TP not only play an important role in cardiovascular disorders but also in cancer progression and metastasis. Over the past decade, several advances have been made in the field of TXA_2 research that have significantly increased our knowledge and understanding of the underlying mechanisms of TXA_2S and TP signaling. COX-2 expression has been found to be upregulated in a variety of cancers and TXA_2S and TXA_2 being a downstream metabolite of COX-2 is also over expressed in various cancers. Cancer progression involves several key steps such as angiogenesis, cell survival, cell migration, cell invasion, and metastasis; studies have shown that both TXA_2S and TP play important roles in one or more of these key process. It is of great importance to further determine whether and how prostanoids, such as TXA_2, mediate the effects of COX-2 in cancer, potentially leading to a more targeted approach for cancer prevention and treatment. The failure of existing TXA_2S inhibitors and TXA_2 antagonists to show robust clinical benefit in treatment of cardiovascular disorders has largely been due to co-incident use of NSAIDs, such as aspirin, as part of the standard of care. Conversely, current therapeutic regimens for cancer incorporate few agents in this class making TXA_2S/TP antagonism an attractive target with potentially significant therapeutic benefit. Moreover, in contrast to NSAIDs, TXA_2S/TP inhibitors do not prevent other COX-derived anti-tumor products, such as PGI_2, with beneficial effects from being synthesized [108, 109]. Hence, future work should focus on the advantages of directly targeting TXA_2S and/or antagonizing TP for its functional role in cancer progression.

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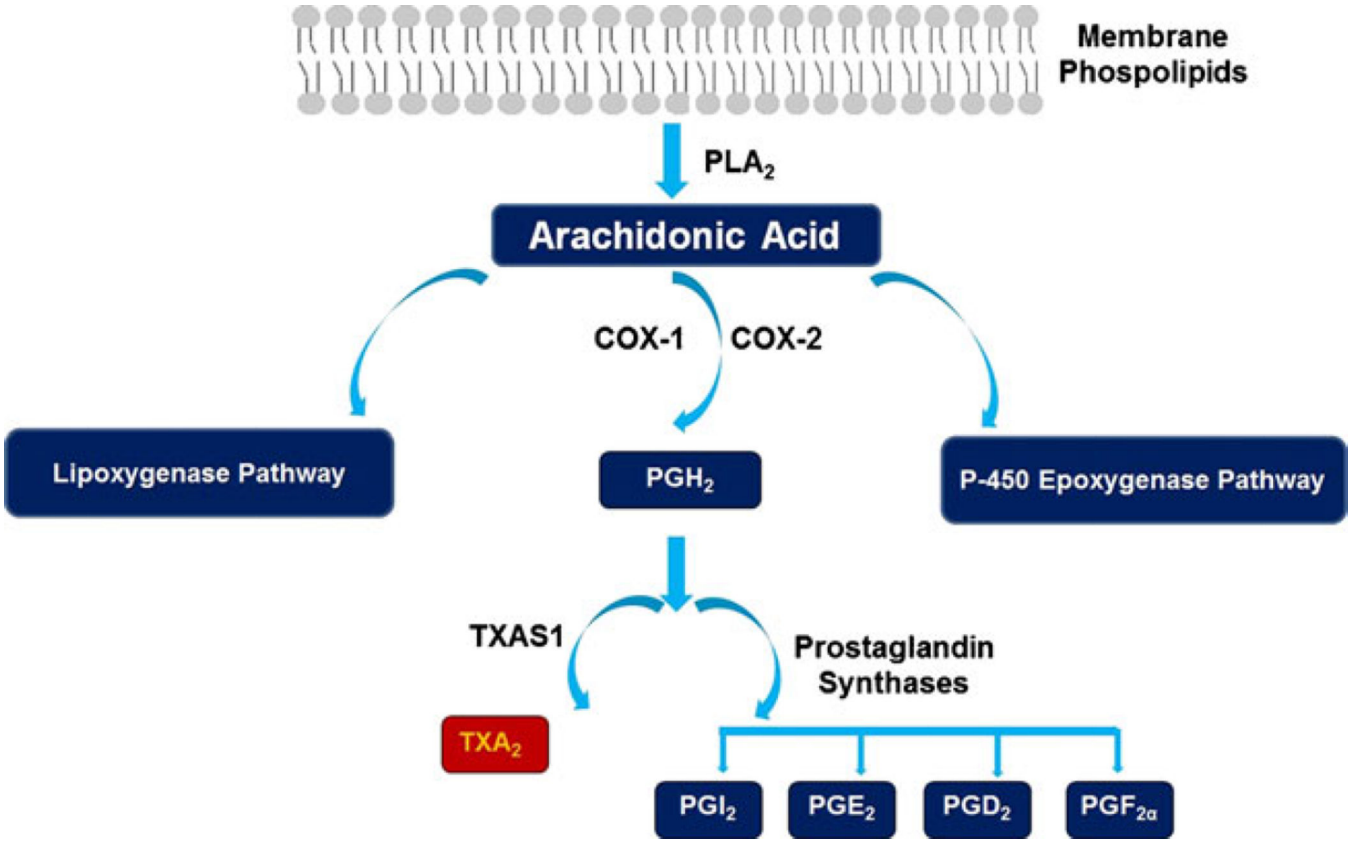

Fig. 1.

Generation of the prostanoids through metabolism of arachidonic acid. Arachidonic acid can be metabolized into different bioactive lipids by one of three distinct signaling pathways; the cyclooxygenase (COX), the lipoxygenase, and the P-450 epoxygenase pathways. The COX-isoforms-COX-1 and COX-2 are responsible for the conversion of AA to PGH_2, the first step in the generation of TXA_2. Thromboxane synthase (TXA_2S) then catalyzes the conversion of the COX product, PGH_2 to TXA_2

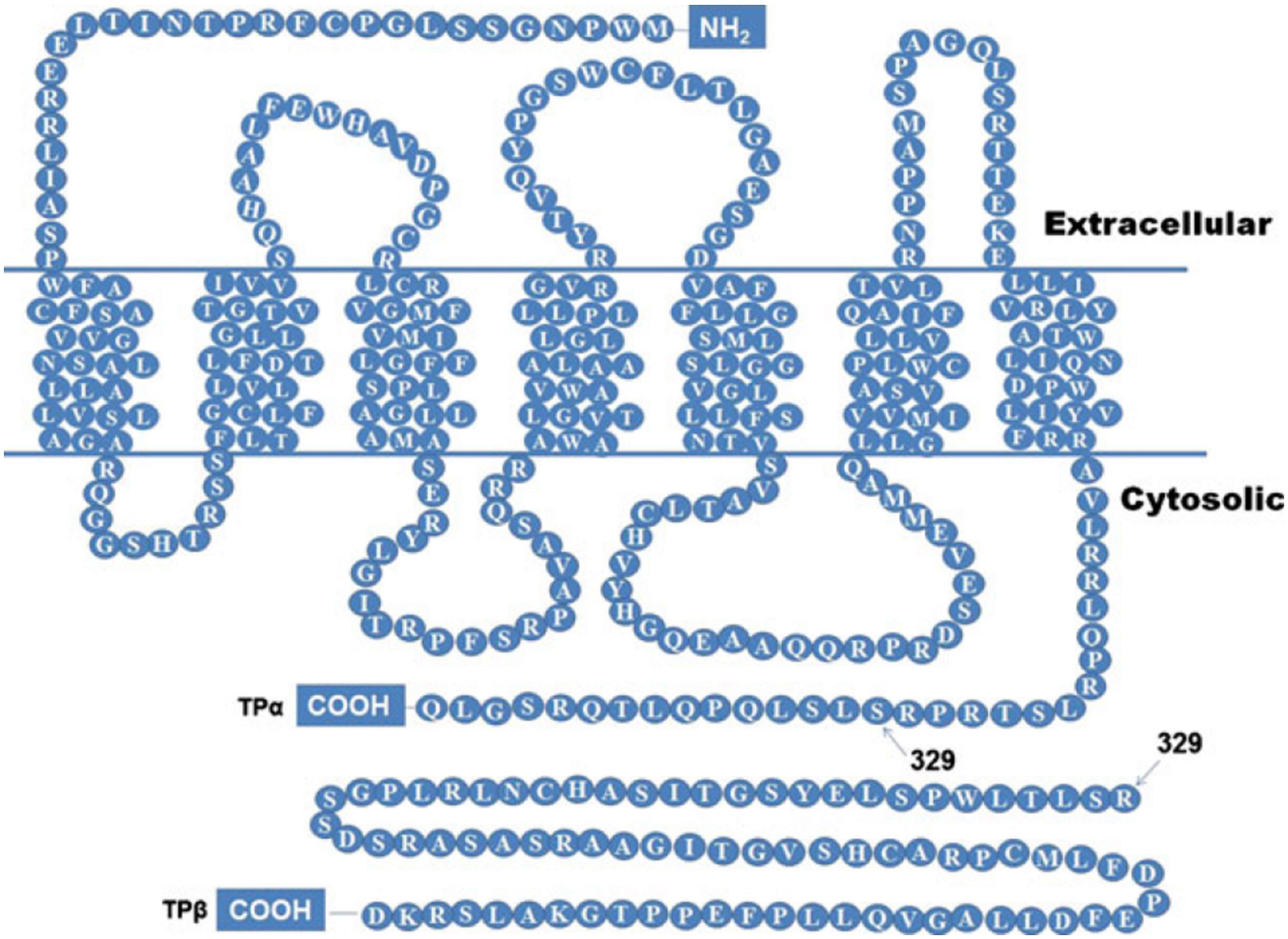

Fig. 2.

Structures of TPα and TPβ receptor proteins. The TXA_2 receptor (TP) is a typical G-protein-coupled receptor that is expressed as two different isoforms in humans—*TP*α and *TP*β. TP isoforms share the first 328 amino acids but differ in the length of the C-terminal cytoplasmic tail with TPα shorter than the TPβ isoform (15 *versus* 79 residues) [24, 25]