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Prostacyclin Synthase, Thromboxane Synthase and Cancer

The Role of Prostacyclin Synthase and Thromboxane Synthase Signaling in the Development and Progression of Cancer

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Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; PGIS, prostacyclin synthase; PGI₂, prostacyclin; IP, prostacyclin receptor; PPAR, peroxisome proliferator activated receptor; TXS, thromboxane synthase; TXA₂, thromboxane A₂; TP, thromboxane receptor; LMWH, low molecular weight heparin.

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1. Abstract

Prostacyclin synthase and thromboxane synthase signaling via arachidonic acid metabolism affects a number of tumor cell survival pathways such as cell proliferation, apoptosis, tumor cell invasion and metastasis, and angiogenesis. However, the effects of these respective synthases differ considerably with respect to the pathways described. While prostacyclin synthase is generally believed to be pro-tumor, an anti-carcinogenic role for thromboxane synthase has been demonstrated in a variety of cancers. The balance of oppositely acting COX-derived prostanoids influences many processes throughout the body, such as blood pressure regulation, clotting, and inflammation. The $\text{PGI}_2/\text{TXA}_2$ ratio is of particular interest *in-vivo*, with the corresponding synthases shown to be differentially regulated in a variety of disease states. Pharmacological inhibition of thromboxane synthase has been shown to significantly inhibit tumor cell growth, invasion, metastasis and angiogenesis in a range of experimental models. In direct contrast, prostacyclin synthase over-expression has been shown to be chemopreventative in a murine model of the disease, suggesting that the expression and activity of this enzyme may protect against tumor development.

In this review, we discuss the aberrant expression and known functions of both prostacyclin synthase and thromboxane synthase in cancer. We discuss the effects of these enzymes on a range of tumor cell survival pathways, such as tumor cell proliferation, induction of apoptosis, invasion and metastasis, and tumor cell angiogenesis. As downstream signaling pathways of these enzymes have also been implicated in cancer states, we examine the role of downstream effectors of PGIS and TXS activity in tumor

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growth and progression. Finally, we discuss current therapeutic strategies aimed at targeting these enzymes for the prevention/treatment of cancer.

2. Introduction

Cancer causes seven million deaths annually, accounting for 12.5% of deaths worldwide. It is the second leading cause of death in the developed world and is among the three leading causes of death for adults in developed countries. It is estimated that, by 2020 there will be 16 million new cases every year, representing a 50% increase in cancer incidence [1].

The arachidonic acid pathway is responsible for the generation of a wide variety of bioactive metabolites. These metabolites, otherwise known as eicosanoids, have been shown to be involved in many different pathologies, including inflammation and cancer [2]. Arachidonic acid can be metabolised into the biologically active eicosanoids via the action of three separate groups of enzymes: cyclooxygenases (COX), lipoxygenases (LOX), and epoxygenases (cytochrome *P450*). The COX enzymes catalyse the first step in the synthesis of prostanoids from arachidonic acid [3]. COX was shown to exist as two distinct isoforms in the early 1990's. These included the constitutively expressed COX-1 and the inducible form of COX-2, associated with inflammation [4]. A third COX isoform has also recently been identified, known as COX-3 [5]. However, it has since been shown to have no COX activity and is therefore unlikely to have prostaglandin-producing activity in human tissues [6]. COX-derived prostanoids, prostaglandins and thromboxanes, are biologically active lipid mediators involved in a wide range of physiological processes such as modulation of vascular tone, the inflammatory response and gastric cytoprotection. Prostanoids have also been implicated in various disease states such as arthritis, heart disease and pulmonary hypertension [7].

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The development of cancer in both humans and experimental animals is consistently linked to an imbalance in COX signaling [8, 9]. There has been a significant interest in COX-2 and its role in the development and progression of cancer over the past number of years, with a number of clinical trials examining selective COX-2 inhibition as a potential therapeutic strategy [10-13]. COX-2 expression has been associated with a poor prognosis in a variety of cancer states [14-16]. A number of clinical trials have been carried out to examine the role of COX-2 inhibition in lung cancer chemoprevention [17-20], with further human trials ongoing. However, conflicting studies aimed at examining the role of non-specific COX or selective COX-2 inhibition will lead to difficulty interpreting these results. An inhibition in mouse lung tumorigenesis has been observed in studies using non-selective COX inhibitors [21, 22]. However, selective COX-2 inhibition with celecoxib resulted in reduced pulmonary inflammation, but no differences in tumor multiplicity and an increase in tumor size in an initiator promoter lung tumor mouse model [23]. In addition to these observations, chronic administration of selective COX-2 inhibitors at high concentrations has been associated with an increased risk of cardiovascular events, such as thrombosis, stroke, and myocardial infarction [24-26]. The mechanism whereby these drugs contribute to cardiac complications is thought to be through a disruption in the fine balance between PGI₂ and TXA₂, which regulates blood clotting. The role of these respective prostanoids in the regulation of coagulation has been demonstrated using a rat model of pulmonary hypertension [27]. It was demonstrated that COX-2 dependant PGI₂ formation limits the pulmonary hypertensive response to hypoxia *in-vivo*, partly through attenuation of TXA₂-dependant platelet activation and deposition. These observations were later confirmed in a COX-gene disrupted mouse model of pulmonary hypertension [28]. This model

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demonstrated that *COX-2* gene deletion exacerbated the pulmonary hypertensive response to hypoxia, an effect mediated at least in part through enhanced sensitivity to TXA_2 , and a resulting increase in intravascular thrombosis.

The effects of COX expression in cancer has been proposed to be related to the expression profile down-stream COX-derived prostanoids. However, the relationship of the prostanoid profile to cancer growth is not yet completely understood [29]. Recently, increased *COX-2* expression has been associated with increased levels of downstream enzymes required for prostanoid synthesis, suggesting that the tumor-promoting effects of *COX-2* overexpression may be attributable to specific downstream products of arachidonic acid metabolism [30]. The past number of years has seen considerable interest in targeting downstream effectors of the cyclooxygenase signaling pathway in cancer. Selective targeting of these downstream effectors could have the potential of avoiding the cardiovascular effects associated with selective *COX-2* inhibition, while maintaining anti-cancer properties. Previous studies have proposed that the prostaglandin biosynthesis profile of malignant cells may differ from that of normal tissue [31, 32]. Immunohistochemical examination of expression profiles of the COX enzymes and downstream enzymes of COX metabolism in lung cancer showed that *COX-2* and *TXS* expression was abundant in lung cancer, but that *PGIS* expression was absent [30]. Furthermore, endothelial cells of vessels found within or near the tumor showed extensive expression of *COX-2* and *TXS*, while endothelial cells of normal lung specimens expressed *COX-1* and *PGIS*. It was concluded that the expression pattern of these enzymes is altered

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in lung cancer tissue, compared to normal, an effect which may impact on tumor progression.

Prostacyclin synthase (PGIS) and thromboxane synthase (TXS) enzymes act downstream of COX-signaling to catalyse the formation of prostanoids prostacyclin (PGI₂) and thromboxane A₂ (TXA₂) respectively. These prostanoids exert directly opposing effects on the vasculature. While PGI₂ is a vasodilator, which activates platelets and promotes their aggregation, TXA₂ has the opposite effects. In cancer states, these enzymes and their corresponding prostanoids have been shown to have similarly contrasting effects. PGI₂ is a potent anti-mitogenic and anti-metastatic agent in cancer [33], and has been implicated as a potential chemopreventative agent in NSCLC [34, 35]. In contrast, thromboxane synthase and its product TXA₂ have been shown to promote proliferation, invasion, metastasis and angiogenesis in a variety of cancers [36-40]. The prostacyclin-thromboxane ratio is considered to be of relevance *in-vivo*, with a number of studies showing the corresponding synthases to be differentially regulated [41]. An imbalance in the generation of PGI₂ and TXA₂ has also been implicated in disease states, such as pulmonary hypertension [27, 28, 42]. This ratio is of particular importance in the cardiovascular system, where the balance between PGI₂ production in vascular endothelial cells and TXA₂ production in platelets may maintain cardiovascular homeostasis [43]. As these prostanoids and their corresponding synthases appear to have directly opposing roles in cancer development and progression, the ratio in their respective expression levels of may also be of significant importance in the disease.

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In recent years, there has been a significant rise in the number of reports examining the fractional roles of arachidonic acid-derived prostanoids and their corresponding synthases in the development and progression of cancer. A number of studies have also examined the prostanoid synthases as potential targets for chemoprevention or therapeutic intervention. In this review, we will discuss the current state of knowledge regarding the individual roles of prostacyclin synthase and thromboxane synthase in the development and progression of cancer. We will examine the involvement of these synthases and downstream signaling pathways in a range of tumor cell survival pathways such as tumor cell proliferation, programmed cell death or apoptosis, coagulation/thrombosis, invasion and metastatic spread of tumors, and finally, tumor angiogenesis. Finally, we will also assess a potential role for these enzymes as potential targets for future chemoprevention or treatment of the disease, which may be used either alone, or in combination with other agents and/or conventional therapies.

3. Arachidonic Acid Metabolism: Generation and Classification of Arachidonic Acid Derived Prostanoids.

Under normal conditions, free arachidonic acid (A.A.) is virtually undetectable. However, A.A. can be mobilized from the plasma membrane in response to a variety of growth factors, cytokines, and hormones, and converted to a range of bioactive lipids, otherwise known as eicosanoids, via cyclooxygenase (COX), lipoxygenase (LOX) or P-450 epoxygenase pathways. The key regulatory step in the COX-signaling pathway is the enzymatic conversion of arachidonate to PGG_2 , which is then reduced to an unstable endoperoxide intermediate, PGH_2 . PGH_2 is then catalytically converted to the various prostanoids via reduction, rearrangement, or isomerisation by the terminal synthase enzymes (prostaglandin-E-synthase, prostaglandin-D-synthase, prostaglandin-F-synthase, prostacyclin synthase and thromboxane synthase) (Fig. 1). The resulting prostanoid products are unstable compounds and are therefore rapidly metabolised *in-vivo* [44].

Following COX activation, prostanoids are readily generated by a number of cell types. Platelets, mast cells and monocytes/macrophages synthesize TXA_2 , PGD_2 , PGE_2 and PGF_2 , while endothelium is the major source of PGI_2 [45]. The prostanoids exert their cellular functions by binding to cell surface receptors belonging to a family of seven transmembrane domain G-protein-coupled receptors [46]. These cell surface receptors are designated IP for the PGI_2 receptor and TP for the TXA_2 receptor. In some cases, prostanoids and their metabolites bind their nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) [47].

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Selective coupling of the cyclooxygenase isoforms with the prostanoid synthases is a major event in the cyclooxygenase signaling cascade, which can have important downstream effects. Recently, it has become clear that coupling of COX-2/PGIS is the major event leading to sustained PGI₂ release, both into the circulation and the sub-endothelium, particularly in activated endothelial cells [48]. COX-2/PGIS coupling occurs at the nuclear envelope and endoplasmic reticulum, but also inside membrane microdomains, known as caveolae, where both PGIS and COX-2 have been detected [49, 50]. PGIS therefore produces PGI₂ as soon as COX-2 is induced and inserted into caveolae, nuclear envelope, or endoplasmic reticulum. COX-2 and PGIS may be functionally inter-connected, both participating to signal transduction events connected to the regulation of processes such as angiogenesis and apoptosis. An inhibitory effect of rofecoxib on PGIS activity has been reported in human umbilical vein endothelial cells and in PGIS-enriched bovine aortic microsomal fractions, an effect which was not observed using other anti-inflammatory compounds [48].

4. Prostacyclin Synthase and Thromboxane Synthase Pathways

4.1 Prostacyclin Synthase

Prostacyclin synthase cDNA is composed of 1500 nucleotides, coding for a 500 amino acid protein. PGIS was purified from bovine aorta by immunoaffinity chromatography in the late 1980's, and was reported to be a haemprotein belonging to the cytochrome P-450 family, with a molecular mass of approximately 52 kDa [48]. PGIS co-localises with COX in the endoplasmic reticulum, plasma membrane and nuclear membrane [51]. Expression of this enzyme is abundant in human tissues, particularly vascular endothelial cells and

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smooth muscle cells, as well as non-vascular cells such as neurons [52]. Its expression can be induced by TNF α [53, 54], and other inflammatory cytokines such as IL-1 [55] and IL-6 [56, 57]. Transcriptional regulation of the PGIS gene has not been reported frequently in the literature. PGIS mRNA and protein expression is increased in response to mechanical cyclical stretch in both uterine myometrial and spinal ligament cells [58, 59]. In addition, synchronized upregulation of PGIS and COX-2 has been observed in human umbilical vein endothelial cells treated with a thromboxane analogue [60].

The PGIS product, prostacyclin (PGI₂) was first discovered in 1976 using the cascade tissue perfusion technique [61]. PGI₂ has many important biologic functions. It is the most important endogenous inhibitor of platelet aggregation discovered to date, and also causes vascular relaxation [62]. PGI₂ is also anti-mitogenic and inhibits DNA synthesis in smooth muscle cells [63]. The presence of PGIS at the nuclear and endoplasmic reticular membrane suggests multiple signaling pathways for this enzyme *via* PGI₂ generation, involving both cell surface and nuclear receptors. However, the cellular signaling initiated by this class of compounds is probably the least understood of all the primary prostanoids [64]. It has recently been established that there are two main signaling pathways for prostacyclin following its production through PGIS activity. The first pathway is through the prostacyclin (IP) receptor, and the second is at the nuclear membrane via the peroxisome-proliferator activated receptors (PPARs) [65].

The IP is a cell-membrane specific, seven-transmembrane domain G-protein coupled receptor. Activation of IP by PGI₂ results in G_s protein and adenylyl cyclase activation,

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leading to the generation of cAMP and subsequent PKA activation [66]. Elevated cAMP levels and subsequent PKA activation potently inhibits the MAP kinase pathway [64] (Fig. 2A). Consistent with a major role for G_s in IP signaling, the PGI_2 analogue, Iloprost inhibited LPS-induced p42/p44 MAP kinase as well as p38 MAP kinase activation in mouse RAW 264.7 macrophages [67]. In addition, PGI_2 analogues were also found to attenuate the thromboxane receptor agonist-induced p42/44 MAP kinase activation. This study was a good example of the ability of PGI_2 to modulate TXA_2 activity, as the smooth muscle hypertrophy induced by the thromboxane receptor agonist was also reversed by the PGI_2 analogues [68]. In addition to G_s , activation of G_q and G_i proteins by IP has also been reported, with G_q -dependant PGI_2 signaling through the PKC pathway frequently observed [69]. The signaling pathways activated by PGI_2 and subsequent IP activation are shown in Fig. 2A.

PGI_2 and its analogues have also been shown to be ligands for peroxisomal-proliferator-activated receptors (PPAR) [70]. PPARs belong to the nuclear hormone receptor superfamily of regulated transcription factors and consist of three isoforms. These include $PPAR\alpha$, $PPAR\gamma$, and $PPAR\delta$ -which all bind to specific DNA sequences as heterodimers with the retinoic acid X-receptors to regulate gene transcription [71]. PGI_2 has been shown to upregulate $PPAR\delta$, indicating a novel signaling mechanism for this prostanoid [72]. Furthermore, $PPAR\delta$ has been shown to be overexpressed in colorectal cancer, an expression that is colocalised with COX-2 within the tumor [65]. PGIS and PGI_2 have also been shown to affect $PPAR\gamma$ activity. An almost two-fold induction in $PPAR\gamma$ expression has been reported following lung-specific PGIS over-expression [35]. It was recently

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observed that endogenous PGI₂ generation by co-expression of COX-2 and PGIS leads to transcriptional activation of PPAR δ . In contrast, co-expression of COX-1 and PGIS did not lead to any significant PGI₂ generation and subsequent transcriptional PPAR δ activation [65]. These findings suggest that COX-2/PGIS coupling leads to PGI₂ generation and that this PGI₂ subsequently uses PPAR δ as its receptor. In support of these observations, co-overexpression of COX-2 and PPAR δ has been reported in endometrial adenocarcinoma [73]. However, the mechanism by which the PGIS/PPAR δ pathway promotes tumor development and progression remains to be elucidated.

4.2 Thromboxane Synthase

Thromboxane A₂ synthase (TXS) also belongs to the cytochrome P-450 family [74]. The human gene for TXS was first characterised in 1994. The TXS gene contains 13 exons and is 193 kb long, the largest gene ever to be isolated. TXS mRNA has been shown to be widely expressed in human tissues, with particular abundance observed in peripheral blood leukocytes, spleen, lung and liver [75]. The TXS enzyme is a 60 kDa haemprotein that is distributed in platelets, monocytes, and several other cell types and is associated with the endoplasmic reticulum [74, 76, 77]. TXS activity was first demonstrated in platelets, and subsequently in other tissues [78]. TXS catalyses either the conversion of PGH₂ to thromboxane A₂ (TXA₂) by an isomerase reaction, or the formation of HHT (12-L-hydroxy-5, 8, 10-heptadecatrienoic acid), and malondialdehyde (MDA) by a fragmentation reaction in a 1:1:1 ratio (TXA₂: HHT: MDA). The biological functions of MDA and HHT are unknown. However, MDA can form adducts with lysine residues of proteins or with amine head groups of phospholipids [79]. MDA adducts have been detected in atherosclerotic lesions of human aorta. It has also been shown to participate in the

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formation of an endogenous DNA adduct, which may have importance in the etiology of human genetic diseases or cancer [80].

TXA₂ is a potent vasoconstrictor, bronchoconstrictor, and promoter of platelet aggregation [81, 82]. As it is rather labile, TXA₂ is rapidly hydrolyzed in aqueous solution (with a half-life of about 30 s) to form the biologically inactive product, thromboxane B₂ (TXB₂). The human TXA₂ receptor, TP, was the first eicosanoid receptor to be cloned in 1991 and is a member of the seven transmembrane G-protein-coupled receptor superfamily [83]. TPs are widely distributed in a variety of organs and are localized to both the cell membrane and intracellular structures. Two alternatively spliced variants of human TP have been identified; TP α and TP β [84]. No differences have been observed in ligand binding and coupling of the TP receptor α and β splice variants [64]. TP mRNAs are widely expressed in the lung, liver, heart, kidney, uterus and vascular cells [85]. TP receptors are functionally coupled to the heterotrimeric G_q protein. G_q protein binding leads to a subset of signaling cascades, which include phospholipase C (PLC) activation and the subsequent release of calcium and of protein kinase C activation. This signaling cascade is central to TXA₂/TP signaling, as leads to vasoconstriction and platelet aggregation [86]. However, the α -isoform can also couple to G_s and the β isoform to G_i, leading to opposing actions on cAMP synthesis [47]. The signaling cascades activated following thromboxane receptor activation are illustrated in Fig. 2B.

4.3 The Thromboxane Synthase/Prostacyclin Synthase Balance in Health and Disease

The balance of oppositely-acting prostanoids produced by tissues has a major influence many processes throughout the body, such as blood pressure regulation, clotting, sleep, labour, and inflammatory responses [41]. The balance between circulating PGI_2 and TXA_2 levels is considered to be the most important regulator of vascular haemostasis. The prostacyclin-thromboxane ratio is thought to be of particular importance *in-vivo*, with several studies showing the corresponding synthases to be differentially regulated [41]. The relationship between prostacyclin and thromboxane A_2 was initially proposed when prostacyclin was first discovered in 1976 [76] and has since been confirmed in several experimental models [27, 28, 87]. An imbalance in the PGI_2 - TXA_2 ratio is thought to underlie many pathological conditions, such as pulmonary hypertension [42], and the pregnancy-associated disorder, pre-eclampsia [88]. As these prostanoids and their corresponding synthases have been shown to have directly opposing roles in a variety of cancer states, the balance in the expression profiles of these synthases/prostanoids may be of critical importance in the development and progression of cancer (Fig. 3). While numerous studies have examined the fractional roles of these prostanoid synthases (and their downstream signaling pathways) in cancer, there have been no specific studies to examine the hypothesis that an imbalance in the expression levels of these enzymes may underlie tumor growth. The opposing roles of these prostanoid synthases and their downstream signaling pathways will be further discussed throughout this review.

5. The Role of Prostacyclin Synthase and Thromboxane Synthase in Cancer

5.1 PGIS and TXS expression in normal and malignant tissue

Prostacyclin Synthase

A number of studies have demonstrated the prostanoid biosynthesis profile of malignant cells to be different to from that of normal tissue [31, 32]. A dramatic loss in PGIS mRNA levels was observed in primary human lung tumor samples, relative to matched normal controls [89]. These findings are in agreement with those of a previous report of decreased PGIS mRNA and immunohistochemical protein expression as well as very low levels of $\text{PGF}_{1\alpha}$ (the stable metabolite of PGI_2) in lung tumors compared to normal surrounding tissue [90]. Gene expression analysis of NSCLC showed a loss of PGIS content in human lung adenocarcinoma samples. However, a small subset of adenocarcinoma patients whose lung tumors retained PGIS expression were found to have significantly enhanced survival. A statistically significant correlation was also observed between positive PGIS staining of lung tumor tissue and patient survival. It was therefore suggested that the detection of PGIS in tissue samples had strong prognostic value in predicting patient survival [91]. While the PGIS product, PGI_2 , is one of the most abundant prostanoids in normal lung, it is only produced in very small amounts by human non-small cell lung cancers [32]. Comparative immunohistochemical analyses of non-small cell lung cancer (NSCLC) and normal human lung tissue have shown that, while tumors were typically positive for TXS expression, PGIS expression was undetectable [30]. In contrast, a moderate PGIS expression was observed in endothelial cells from pulmonary vessels of corresponding normal tissue.

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Studies carried out in our lab showed that PGIS protein expression was significantly reduced or lost in NSCLC protein samples, relative to matched normal controls (Fig. 4A; unpublished data), suggesting that expression of this enzyme may be important for chemoprevention.

The precise mechanisms responsible for the down-regulation of PGIS in cancer are unclear, although several potential mechanisms have been investigated. Using transcription reporter assays, it was observed that single nucleotide polymorphisms in the PGIS promoter can affect transcriptional activity in human lung cancer [92]. PGIS expression was silenced in several lung cancer cell lines by CpG methylation (sites were mapped across the variable number of tandem repeats in the promoter and CpGs within intron and exon 1). FISH analysis demonstrated that lung cancer cell lines and tissues do not exhibit a loss of the PGIS genomic region, but have multiple copies. These observations suggest that an individual's PGIS promoter haplotype may have a significant impact on a patient's predisposition for lung cancer. They also suggest an epigenetic mechanism, namely CpG methylation, which may be responsible for the down-regulation in PGIS expression.

Little is known regarding the expression pattern of the prostacyclin receptor (IP) in cancer. Real-time analysis of 62 colorectal tumors and adjacent normal tissue (n=48) revealed overall IP expression to be significantly reduced in tumor samples, relative to normal [93]. In support of these observations, the IP gene was not expressed at detectable levels in NSCLC cells, suggesting that expression of this receptor is not essential for tumor development and progression [89]. It has been demonstrated that PGI₂ could serve as a

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ligand for PPAR δ . mRNA expression of this nuclear receptor was increased in colorectal tumor samples, relative to matched normal controls [65]. However, the functional consequence of this increase in PPAR δ expression is still unclear.

Thromboxane Synthase

An overall increase in TXS expression has been observed in tumor tissues, relative to normal, suggesting a potential role for this enzyme in tumorigenesis and/or progression of cancer. In normal lung tissue, TXS was found to be moderately expressed in bronchial epithelial cells and weakly expressed in vascular smooth muscle cells [30]. Overexpression of TXS has been observed in a variety of cancers including papillary thyroid carcinoma [36], prostate cancer [39, 94], colorectal cancer [37], and renal carcinoma [38]. Increased TXS expression at mRNA level has been reported in renal cell carcinoma, breast carcinoma, prostate cancer and uterine cancer when compared with matched normal tissues [39]. In a cancer profiling array, TXS mRNA levels were increased in 12 out of 14 cases of renal carcinoma, 7 out of 9 cases of breast carcinoma, 2 out of 3 cases of prostate cancer, and 5 out of 7 cases of uterine cancer, suggesting a role for this enzyme in the development and progression of cancer [95]. Recent observations in our laboratory have found TXS expression to be increased in NSCLC protein samples, relative to matched normal controls (Fig. 4B; unpublished data), suggesting that this enzyme may be a therapeutic target in the disease. The observed overexpression of TXS in these NSCLC samples, in the face of a loss of PGIS expression may also be of clinical significance in the disease. As far as we are aware, this is the first observation of a loss of PGIS expression in tumor protein samples, with a corresponding increase in TXS expression. While the PGIS/TXS balance has been implicated in a range of disease states, it may also be of significant importance in cancer.

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This relative expression patterns and roles of these opposing synthases and their corresponding prostanoids in cancer therefore require further investigation.

In bladder cancer patients, overexpression of TXS was associated with a significant reduction in survival [38]. TXS expression was increased in tumor specimens of advanced stage and grade in prostate cancer, and particularly in areas of perineural invasion. It was suggested that the activity of thromboxane synthase is dependent on COX-2 and, to a lesser extent, COX-1 to supply the substrate PGH₂ [39, 94]. A recent study in breast cancer patients demonstrated TXS to be expressed at significantly low levels in high grade tumors and in patients with a predicted poor clinical outcome. In contrast, TP expression was commonly observed in breast tumors, particularly in aggressive tumors. TP levels were also negatively associated with disease-free survival, indicating that this receptor, and not its corresponding synthase, may be a prognostic factor in the disease [96]. TP expression was significantly increased in colorectal tumor samples, relative to matched normal controls, lending support to previous observations in breast cancer [93]. However, expression was not linked to tumor stage or tumor cell differentiation, suggesting that expression of this receptor is not prognostic in colorectal cancer. More recently, studies were undertaken to determine the individual contributions of the thromboxane receptors in bladder cancer. Significant overexpression of TP- β was observed in the epithelial and stromal compartments of bladder cancer tissue, relative to control tissue, without a concomitant significant overexpression of TP- α [97]. TP- β overexpression was also correlated with a significantly poorer overall patient survival ($p < 0.005$). Therefore, while expression of TXS

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appears to be increased in a variety of cancer states, the role of this enzyme and its corresponding receptor in patient survival remain to be completely elucidated.

Levels of the TXA₂ metabolite, TXB₂, were significantly increased in tumor tissues, relative to non-tumor controls. Of particular interest, TXB₂ concentration was higher in samples from patients that smoked, when compared to those that did not smoke, suggesting that cigarette smoking influences the generation of this metabolite in lung cancer [98]. In support of these observations, smoking was previously shown to significantly increase TXB₂ production in both gastric and lung tissues [99]. Increased TXB₂ generation has also been observed in peri-tumoral tissue of laryngeal cancer patients, compared to healthy mucosa. In the same patient group, the ratio between TXB₂ and 6-keto-PGF_{1α} generation was found to be almost two-fold higher in tumor tissue, peritumor tissue, and metastatic and non-metastatic lymph nodes relative to control tissue, lending support to the notion of an imbalance between TXB₂ and 6-keto-PGF_{1α} generation in cancer, promoting metastatic spread [100].

5.2 Effect of PGIS and TXS signaling on tumor growth

One of the main hallmarks of cancer states is the acquisition of limitless cellular replicative potential, or increased (and unopposed) proliferation of tumor cells. It has been hypothesized that changes in prostanoid profile affect cancer growth. This theory was tested in a colon cancer mouse model, where colon adenocarcinoma cells overexpressing either PGIS or TXS were inoculated into BALB/c mice. Tumors derived from TXS transformants grew at a significantly faster rate, whereas tumors derived from PGIS transformants demonstrated opposing effects. These effects were reversed by the

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administration of specific PGIS and TXA₂ receptor inhibitors [101]. These observations suggest that PGIS and its prostanoid PGI₂ are anti-cancer, while TXS and its prostanoid TXA₂ are pro-cancer in these cells. These findings also lend support to the theory that tumor growth and progression may be controlled by the balance in expression of these enzymes and respective prostanoids (Fig. 3). PGIS over-expression protected against lung cancer development in a variety of murine tumorigenesis models, including tobacco smoke exposure. In the carcinogenesis models used for PGIS overexpressing animals, an increase in PGI₂ production was consistently required for chemoprevention to occur [34, 35]. Expression of PPAR is increased in lung cancer and has been proposed to play a key role in malignant transformation [102]. Eicosanoids such as PGI₂ have been shown to modulate PPAR activity. PGI₂ analogues can activate the PPAR δ receptor and have been shown to inhibit the growth of the A-549 NSCLC cell line [103]. Both PGIS and PGI₂ may also modulate PPAR γ activity with an almost two-fold induction in PPAR γ expression observed following lung-specific PGIS overexpression (Keith et al., 2004). Ligand-activated PPAR γ expression also results in growth arrest of human NSCLC cell lines [104], and growth inhibition *in-vitro* and *in-vivo* in gastric cancer [105]. In contrast, treatment of these cells with a PPAR δ agonist did not affect the growth of these cells, suggesting that the effects of PPAR activation on tumor growth are not universal for all receptor types and cancers.

While one study has shown that the IP receptor is not involved in colon-tumor formation in AOM-treated mice [106], there is little else known about the role of PGI₂ and its corresponding receptor in the disease. Overexpression of PGIS provided equal protection against Carcinogen-induced lung tumor incidence in mice lacking IP, in mice heterozygous

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for IP expression or in mice expressing IP, suggesting that the protective effects of PGI₂ are not mediated through IP activation [107]. In the same study, Iloprost, a stable PGI₂ analogue, activated PPAR γ in non-transformed bronchial epithelial cells and a subset of NSCLC cell lines. Transgenic mice overexpressing PPAR γ also developed fewer lung tumors. These findings suggest that PPAR γ may be a critical target for PGI₂-mediated lung cancer chemoprevention. This study is in accordance with earlier observations that ligand activation of PPAR γ suppressed both *in-vitro* and *in-vivo* gastric cancer growth [105]. In addition to PPAR γ , PGI₂-mediated activation of PPAR δ has also been shown to play a role in the negative growth of lung cancer cell lines [103]. However, these studies are in contrast to that carried out in colorectal cells, with PGI₂ shown to activate PPAR δ , leading to a subsequent acceleration in intestinal tumor growth in *Apc*^{Min/+} mice [108]. Therefore, while the PGI₂-mediated PPAR activation has been clearly implicated in the regulation of tumor growth, the roles of the individual PPAR subtypes in cancer is still unclear and requires further investigation.

The role of thromboxane synthase expression in tumor growth has been investigated in a variety of tumor states [37, 38, 101, 109]. Inhibition of TXS protein expression using an antisense oligonucleotide inhibited proliferation of colorectal cancer cells. This effect could be rescued by direct addition of a stable thromboxane A₂ analogue [37]. In bladder cancer, treatment of two cell lines with both TXS inhibitors and TP antagonists significantly reduced tumor cell growth [38]. In addition, molecular inhibition of TXS expression by siRNA resulted in a significant reduction in tumor cell growth in bladder cancer cell lines, leading support to previous observations [109]. Recent studies carried out in our laboratory

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demonstrated a significant ($p < 0.05$) reduction in tumor cell growth of two NSCLC cell lines following 24 h treatment with the selective TXS inhibitor, ozagrel (Fig. 5), suggesting that this enzyme may be a survival factor in the disease.

The thromboxane A₂ receptor (TP) has been shown to induce cell proliferation in several cell lines. A recent report has shown that TP activation by its agonist I-BOP, induced expression of Nurr1, stimulating human lung cancer cell proliferation [110]. Nurr1 is an orphan nuclear receptor, which has been implicated in cell proliferation, differentiation, and apoptosis. These observations therefore provide strong evidence that this receptor may mediate TP-agonist induced proliferation in lung cancer cells. In bladder cancer cells, TP- β receptor expression, but not TP- α promoted a significant increase in cellular growth rate [97]. In contrast to these observations, disruption of the TP receptor was not found to affect colon formation in AOM-treated mice [106], suggesting that the role of TXS signaling in cancer growth is some-what contradictory and requires further investigation.

5.3 Regulation of tumor cell death by PGIS and TXS pathways

Early tumor growth is dependant on the balance between increased tumor cell proliferation and decreased cell death. A second hallmark of cancer required for sustained tumor growth, involves the evasion of apoptosis or programmed cell death. Apoptosis may be defined as an active, energy-dependant, well-defined process whereby cells commit suicide. It is a physiological process by which unwanted or damaged cells are eliminated during tissue development and haemostasis [111]. Impaired apoptosis is thought to be a crucial step in tumor growth. Insufficient apoptosis can confer multiple selective advantages on tumor cells, allowing them to persist in a hostile environment, escape death,

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and evolve into more aggressive clones. Many anti-cancer agents, including radiation, induce apoptosis as a mechanism of killing tumor cells [112, 113]. A functional link between the induction of apoptosis and interference with the eicosanoid biosynthesis pathway has been established. In support of this, one of the most widely investigated and supported potential mechanisms for the anti-neoplastic effects of COX-2 inhibition is the induction of tumor cell apoptosis [114].

Very few studies have been carried out to examine the role of PGIS in apoptosis, particularly tumor cell apoptosis. The PGIS-derived prostanoid, PGI₂, has been shown to use PPAR δ to modulate apoptotic processes. Intracellular PGI₂, formed by expressing PGIS in human embryonic kidney 293 cells has been shown to promote apoptosis through PPAR δ activation. In contrast, treatment of these cells with extra-cellular PGI₂ or dibutyl cAMP resulted in the opposite effects [115]. PPAR δ activation promotes keratinocyte differentiation in response to inflammatory stimuli, while activated PPAR δ in the skin appears to regulate genes associated with apoptosis [116]. PGI₂-mediated activation of PPAR δ leads to binding of activated PPAR δ to specific PPAR response elements (PPRE) of target genes. One of the genes upregulated by PPAR δ has recently been identified as *14-3-3epsilon*. An increase in cytosolic 14-3-3epsilon protein expression has been shown to enhance sequestration of the pro-apoptotic Bad protein, thereby regulating apoptosis [117]. PGI₂ expression was found to prevent nitric oxide-induced megakaryocyte apoptosis. This effect was inhibited when adenylyl cyclase activity was suppressed and partially reversed following protein kinase A inhibition [118]. Further downstream of PGI₂, activation of the PGI₂ ligand, PPAR γ , has been associated with an upregulation in pro-apoptotic genes *bad*

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and *p53*, and downregulation of anti-apoptotic genes *bcl2* and *bcl-xl*, suggesting a link between PPAR activation and induction of apoptosis [105].

The role of TXS in tumor cell apoptosis has been more extensively studied. Treatment of human glioma cells with the specific TXS inhibitor, furegulate led to caspase activation, DNA fragmentation, subsequent cell death, providing a rationale for therapeutic intervention. The data provided suggested that the apoptosis induced by TXS inhibition may predominantly involve a mitochondrial pathway, which may also explain the extended incubation time before cell death [119]. Specific TXS inhibition in these cells also had a synergistic effect on apoptosis induction by camptothecin [120]. More recently, treatment of bladder cancer cells with the TXS inhibitors ozagrel or furegulate induced an apoptotic effect, determined by an increase in caspase-3 activation and subsequent cell death and decreased survivin expression [109]. Moreover, pharmacological and molecular inhibition of TXS increased tumor cell sensitivity to the chemotherapeutic agents, cisplatin and paclitaxel. TXS inhibitors have also been shown to induce apoptosis in human vascular endothelial cells (HUVEC), as demonstrated by DNA fragmentation assay [121]. Recent studies carried out in our laboratory have shown that selective TXS inhibition leads to an increase in tumor cell apoptosis in NSCLC cell lines. This was demonstrated by High Content Screening (HCS) following 24 h selective TXS inhibition with ozagrel. This observation was further validated by two separate DNA fragmentation assays; DNA laddering and cell death detection ELISA (data not shown). Selective TXS inhibition has been shown to significantly induce apoptosis in a NSCLC cell line and a small cell lung carcinoma (SCLC) cell line, lending support to our observations. This effect was associated

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with an elevation in nuclear p27, an atypical tumor suppressor, which is normally sequestered in the nucleus [122]. Downstream of TXS, blockade of TXA₂ production enhances cisplatin-induced apoptosis in NSCLC cell lines by up-regulating the expression of proteins Ice and Ced-3 homolog (ICH-1L) [123].

Increased TXB₂ levels were associated with increased lipid peroxidation and BCl₂ expression, suggesting that TXB₂ generation may promote tumor formation and inhibit apoptosis in lung cancer and also suggesting a mechanism for the pro-carcinogenic effects of TXA₂ expression [98].

5.4 PGIS, TXS, coagulation and cancer

Thrombosis is one of the most common complications in cancer, representing a frequent cause of cancer-associated mortality [124]. Thrombosis represents the second most common cause of death in cancer patients [125]. Platelet abnormality and thromboembolic disorders affect 15-20% of all cancer patients, while platelet activation and aggregation have been shown to facilitate tumor angiogenesis and metastasis [126, 127]. Furthermore, post-mortem studies have revealed an incidence of thrombosis in nearly 50% of cancer patients [128]. Until recently, cancer and the treatment of cancer were merely assumed to be proximate causes of the increased risk for thrombosis, while thrombosis itself was not considered a molecular event in oncogenesis [129]. However, more recently, evidence has been presented linking cancer development, tumor angiogenesis and metastasis to thrombosis formation. Up-regulation of thrombosis-associated genes, *PAI-1* (plasminogen activator inhibitor type-1) and *COX-2* have been associated with the development of liver cancer [130], while expression of tissue factor (TF) has been proposed to be an important

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effector of the tumorigenic and angiogenic phenotype in colorectal cancer cells [131]. Alterations in the expression profiles of down-stream effectors of COX metabolism (particularly those regulating thrombosis) may be associated with cancer states. An imbalance in the PGI₂-TXA₂ ratio in favour of TXA₂ production, may promote an increase in thrombosis, which has been implicated in the development and progression of cancer.

Targeting of clotting intermediates in cancer may therefore be a unique approach to future treatment of cancer. Numerous cell-signaling cascades are triggered by the generation of these pro-coagulant molecules, which are thought to influence tumor cell migration, adhesion, cell-cell interaction, replication, and angiogenesis [129]. Anticoagulant therapy is both safe and effective for prophylaxis and treatment of venous thromboembolism (VTE; includes both deep vein thrombosis (DVT) and pulmonary embolism (PE)) in cancer patients. Of the available anticoagulants, low-molecular weight heparins (LMWH) are the preferred treatment option for prophylaxis and treatment of VTE. LMWHs were developed to overcome some of the limitations associated with both warfarin and UFH [133]. The results of the MALT (malignancy and low-molecular weight heparin therapy) trial have indicated that LMWH therapy may favourably prolong survival [134]. Similar observations were made with the FAMOUS (fragmin advanced malignancy outcome study) trial [135]. Additionally, LMWH has been shown to enhance the efficacy of chemotherapeutic drugs in both lung cancer [136] and pancreatic cancer [137].

Recently, the relationship between cancer-related thrombosis and biomarkers has been examined. Among the biomarkers showing promise as predictive factors of thrombosis

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included markers of platelet activation [138]. The mixed TXS inhibitor/receptor antagonist, BM-567 effectively reduced tumor cell-induced platelet aggregation (TCIPA) in osteogenic sarcoma cells [139]. In addition to selective TXS inhibition, broad-spectrum coagulation inhibitors have been tested in large clinical trials, displaying the ability not only to prevent clotting disorders associated with cancer, but also cancer itself [140, 141]. Recently, treatment of a NSCLC cell line (A-549) with dalteparin, a type of LMWH, was shown to dose and time-dependantly inhibit cell viability. Dalteparin also caused arrest of the cells in G1 phase, inducing them to early apoptosis [142]. In a separate study in our lab, treatment of a NSCLC cell line with un-fractionated heparin down-regulated TXS protein expression (Fig. 6; unpublished result), implicating TXS as a target of these inhibitors and supporting the hypothesis that targeting of this pro-coagulant enzyme may be a potential therapeutic strategy for the treatment of cancer.

Investigation of platelet activation in pulmonary cancer revealed serum TXB₂ levels to be significantly increased in patients, relative to matched controls, indicating *in-vivo* platelet activation in these patients [143]. This observation implicates thromboxane in cancer-associated thrombosis, and provides further evidence that targeting of this clotting intermediate may also negatively impact on cancer growth (*via* its effects on proliferation, apoptosis, angiogenesis, invasion and metastasis, as discussed in other sections of this review).

5.5 Regulation of tumor cell angiogenesis by PGIS and TXS pathways

Angiogenesis may be defined as the process of generating new capillaries from a pre-existing blood supply. Although indispensable in development, angiogenesis is highly regulated, and persistence of angiogenesis in adults is usually linked with disease, including cancer, chronic inflammatory disease and diabetic retinopathy [144, 145]. The induction of angiogenesis is necessary for the supply of oxygen and nutrients to tumors >2 mm in diameter, and is therefore essential for successful tumor growth [146]. In order to grow and metastasize, solid tumors secrete a range of pro-angiogenic factors, which tip the delicate balance in favour of angiogenesis [147]. The prostanoids and their corresponding synthases can contribute, at least in part, to tumor development *via* their role in the regulation of angiogenesis.

Gene transfer of *TXS* and *PGIS* has been shown to alter tumor angiogenesis and tumor growth in a murine colon-cancer model. While tumors from *TXS* transformants demonstrated an increased growth rate and more abundant vasculature, tumors from *PGIS* transformants presented with the opposite effects. This study provides further evidence to support the theory that an imbalance in the expression of these synthases and corresponding prostanoids may underlie tumor growth and progression (Fig. 3). The authors suggested that the profile of downstream COX-derived prostanoids in tumor cells may be a determinant for tumor development [101].

PGI₂ has been shown to induce pro-angiogenic VEGF expression in rat intestinal epithelial cells. Correspondingly, PGI₂ expression, as well as *PGIS* expression was induced

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by Ha-Ras(V-12), suggesting a signalling mechanism for PGI₂ and subsequent VEGF production [148]. The recent discovery of the PPAR δ nuclear receptor for PGI₂ suggests a significant new role for this prostanoid. PPAR δ activation has been implicated in the control of endothelial cell functions [149]. Prostacyclin generation by PGIS leads to angiogenesis through the activation of the PPAR δ receptor [65]. It has previously been reported that COX-2-derived prostacyclin promotes embryo implantation in the mouse uterus via PPAR δ receptor activation [150].

Thromboxane synthase inhibitors strongly inhibited capillary tube formation in human vascular endothelial cells [121]. Both the number of branches from nodal areas and the length of tube-like structures decreased following treatment with the selective TXS inhibitor, in a dose dependant manner. Evidence for the role of TXA₂ in angiogenesis is some-what contradictory, with both inhibition and induction of angiogenesis being documented. The thromboxane A₂ mimetic, U-46619 has been shown to stimulate endothelial cell migration. Inhibition of TXA₂ synthesis, which was stimulated by basic fibroblast growth factor or VEGF, reduced endothelial cell migration, implicating TXA₂ in angiogenesis [40]. The TXA₂ receptor antagonist SQ29548 was shown to inhibit COX-2 dependent microvascular endothelial cell migration and corneal angiogenesis [151]. However, anti-angiogenic roles for thromboxane have also been described, including induction of endothelial cell apoptosis, inhibition of migration, vascular tube formation and intercellular communication [152-154].

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Pharmacological inhibition of COX-2 inhibited TXA₂ generation, endothelial cell migration, and fibroblast growth factor-induced corneal angiogenesis. Both of these parameters were also inhibited by treatment with a TP antagonist. U-46619, a TP agonist was shown to restore both migration and angiogenesis responses invoked by COX-2 inhibition [151]. TXA₂ has been shown to act as a potent stimulator of angiogenesis, both directly, and by inducing VEGF (vascular endothelial growth factor) and PDGF (platelet-derived growth factor) secretion from platelets following aggregation [155, 156].

The thromboxane receptor (TP) has been found to influence angiogenesis in a lung tumor model of the disease, possibly by affecting endothelial cell migration [40]. More recently, it was reported that the sustained activation of TP, or its blockade significantly inhibited prostate tumor cell migration. [157]. Dual TXS/TP inhibitors have been shown to dose-dependantly inhibit endothelial cell migration in chemotaxis assays. In addition, pre-treatment of endothelial cells with these original dual inhibitors significantly attenuated TP agonist-induced intracellular Ca²⁺ pool mobilization, suggesting a mechanistic link between TXS/TP inhibition and reduced endothelial cell migration [158].

5.6 The role of PGIS and TXS signaling in tumor cell invasion and metastasis

Tumor invasion is initiated by receptor-mediated adhesion of tumor cells to matrix proteins, followed by a second phase of matrix-breakdown by tumor-secreted proteases. This process creates an intra-cellular space into which invading cells can migrate [159]. Tumor spread from the primary site to distant organs is the most clinically important property of malignant tumors and is known as metastasis. Metastasis allows the cancer to

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survive surgical excision of the primary tumor, and is responsible for increases in tumor burden and increasing difficulty in its clinical management [160]. Tumor cell invasion is essential for the dissemination of metastatic cells across extracellular matrices and spread to distant organ sites. Tumor cell metastasis has been implicated in most cancer deaths [147]. The higher the microvessel count is in areas of highest vessel density, the lower the overall survival rate of the patients [161]. Studies suggest that the prostanoids and their corresponding synthases can play distinct roles in tumor progression and metastasis [95].

Alterations in the $\text{PGI}_2/\text{TXA}_2$ ratio (via the activity of the corresponding synthases) has been hypothesized to play an important role in increasing the metastatic potential of the tumor [162]. Prostacyclin has been shown to significantly reduce metastasis formation in a lewis lung carcinoma injected murine model. Treatment with PGI_2 significantly reduced initial lewis lung carcinoma cell density. In addition, lung weight was reduced by over 50% and the number of visible metastatic nodes by over 90% ($p < 0.05$) [163]. PGI_2 has also been shown to reduce the growth of lung micrometastases [164].

Thromboxane synthase inhibition resulted in a significant reduction (60% reduction in migration compared to vehicle controls) in endothelial cell migration in HUVEC *in-vitro* [121]. Specific TXS inhibition has been shown to suppress growth and reduce invasion and migration of bladder cancer cells [38]. Glioma cell migration was blocked following treatment with specific TXS inhibitors. A concomitant reduction in TXB_2 generation was also reported here, implicating the TXS pathway as an important regulator of glioma motility [165].

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The role of thromboxane synthase in tumor metastases has been well studied [38, 96, 119, 121, 165, 166]. TXS expression was associated with increased micro-vessel density (a prognostic factor, predictive of metastasis and poor survival) and metastasis in patients presenting with NSCLC [167]. A TXS inhibitor has been shown to block colorectal carcinoma metastasis in an *in-vivo* model of the disease [168]. TXS has also been shown to be involved with renal cell carcinoma metastasis. Early *in-vivo* studies with TXS inhibitors have failed to report any beneficial effects on metastasis or spread to the lymph nodes [169]. However, when used in combination with a TP antagonist, TXS inhibitors have been found to inhibit metastasis formation from tail vein injected B16a cells, as well as spontaneous metastasis formation from subcutaneous B16a and Lewis lung carcinoma tumors [162]. Several other studies have demonstrated a potential role for TXS in promoting tumor invasion and metastasis [37, 39, 96, 101]. Increased TXS expression was associated with an increase in tumor cell invasion in an astrocytoma cell line [170]. Furthermore, other studies have shown that inhibition of TXA₂ generation can inhibit tumor cell migration as well as trefoil peptide-stimulated tumor cell invasion [39, 171].

A potential role for the thromboxane receptor in tumor cell invasion and metastasis has also recently been examined. In prostate cancer cells, migration was significantly inhibited by either sustained activation of TP or by inhibition of TP activation, suggesting that TP activation is tightly controlled during cell migration [157]. More recently again, it was observed that TP- β receptor expression, but not TP- α increased the migratory and invasive capacity of bladder cancer cells, suggesting a role for this receptor subtype in bladder cancer [97]. The observations of these studies suggest that the contribution of this receptor

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to tumor cell invasion and metastasis is unclear. Further studies are therefore required to clarify the contribution of TP to this cell survival pathway.

5.7 PGIS and TXS as Potential Targets for Future Targeted Therapies/Chemoprevention

There has been a significant interest in COX-2 and its role in the development and progression of cancer over the past number of years, with a number of clinical trials examining selective COX-2 inhibition as a potential therapeutic strategy. A number of studies have shown a correlation between COX-2 expression and poor prognosis in NSCLC [14-16]. In addition, COX-2 inhibitors have been shown to modulate existing cancer therapies in NSCLC [172]. However, selective COX-2 inhibitors have recently been associated with a potentially unfavourable side-effect profile [173]. In addition, data from murine studies evaluating the role of non-selective or selective COX-2 inhibition have failed to yield overwhelmingly positive results. Surprisingly, COX-2 inhibition with celecoxib leads to no change in tumor multiplicity and an increase in tumor size in an initiator-promoter model of lung tumorigenesis [23]. Increased COX-2 expression is associated with increased levels of downstream enzymes required for prostanoid synthesis [30]. The tumor-promoting effects of COX-2 overexpression may therefore be attributable to specific downstream products of arachidonic acid metabolism. The selective targeting of downstream effectors of the cyclooxygenase signaling pathway may therefore be a promising approach for the treatment of cancer, with the potential of avoiding the cardiovascular effects associated with selective COX-2 inhibition, while maintaining anti-angiogenic and anti-cancer properties.

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Based on preclinical studies, a multicenter double-blind phase II study has been carried out to investigate the use of Iloprost (an oral prostacyclin analogue) for chemoprevention of non-small cell lung cancer. Patients on the trial were assessed for the histologic progression of endobronchial dysplasia. Former smokers who received iloprost demonstrated a statistically significant improvement in histologic lung tissue measurements following six months of treatment. Based on these observations, the authors concluded that iloprost is a promising agent for cancer chemoprevention, and warrants testing in a larger phase III trial [174]. In addition to PGI₂, PPAR γ activation is an alternative mechanism, which may be ready for future chemoprevention trials [107].

Specific thromboxane synthase inhibition has been shown to suppress tumor cell growth, and reduce migration and invasion in a number of cancer types [37-39]. More recently, specific TXS inhibition has also been shown to induce apoptosis in bladder cancer cells, with a concomitant activation of caspase-3 and a reduction in survivin protein levels [109]. Treatment of human glioma cells with the specific TXS inhibitor furegulate lead to caspase activation, DNA fragmentation, subsequent cell death, providing a rationale for therapeutic intervention [119]. Therefore, simply reducing TXS synthesis may have some anti-proliferative effects in cancer cells.

TXS inhibitors impede the metabolic pathways of cyclic endoperoxides into thromboxane, which indirectly increases the formation of prostaglandins [121]. Inhibitors of this pathway may interfere with migration and render invasive cells susceptible to apoptosis. TXS inhibitors, thromboxane receptor antagonists, and drugs combining both

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properties have been developed by several pharmaceutical companies since the early 1980's. Several compounds have been launched on the market and are under clinical evaluation [175]. Among these, ozagrel (OKY-046; 3-[4-(1H-imidazol-1-ylmethyl)phenyl]-2E-propenic acid) is a 1-alkyl imidazole derivative, which acts as a selective thromboxane synthase inhibitor. While the precise mechanism of action is unclear, this drug appears to inhibit the enzyme by competing with the PGH_2 substrate to sit on the coordinate site of the haem moiety of the enzyme [176]. Ozagrel was the first thromboxane modulator released in the market (in Japan, 1992) for the treatment of bronchial asthma [177]. This selective TXS inhibitor has also been studied in clinical trials for pre-eclampsia, cerebral vasospasm, and cerebral infarction [178]. Ozagrel is therefore an interesting pharmacological tool to evaluate the role of thromboxane synthase and TXA_2 production in both physiologic and pathologic states [175, 179]. A recent report has demonstrated that dual TXA_2 inhibitors (which display dual inhibitory activity of both TXS and TP) exhibit anti-angiogenic properties in human endothelial cells, suggesting that this pathway may be an attractive target for anti-angiogenic/anti-cancer therapies [158]. In addition to this, TP antagonists have been shown to sensitize non-small cell lung cancer cells to cisplatin in several studies. This effect may be partly explained by decreased Na^+ -, K^+ -ATPase activity, leading to reduced intracellular cisplatin accumulation into cells following treatment [180, 181].

6. Summary/Conclusion

Extensive research has been carried out to examine the roles of prostacyclin synthase, thromboxane synthase, and their corresponding signaling pathways in the development and progression of cancer. Evidence from the literature suggests that PGIS may be chemopreventative in cancer. This theory is currently being investigated in the iloprost chemoprevention trial. It is thought that PGIS may protect against cancer development by inhibiting tumor growth, angiogenesis, invasion and metastasis. These effects have been proposed to be mediated through the PPAR receptors, although this theory requires further investigation.

While an abundance of studies have been carried out to examine the role of PGIS in cancer, TXS has been far more extensively studied in this disease. Research carried out to date shows that TXS is over-expressed in a range of cancer states, and implicates this enzyme as a potential target for treatment of the disease. TXS has been proposed to contribute to tumor development and progression through its effect on a range of tumor survival pathways such as growth, apoptosis inhibition, thrombosis, angiogenesis and invasion and metastasis. The role of this enzyme in these cell survival pathways has been well documented in the literature, suggesting that it may be a valuable target for therapeutic intervention studies. To date there are no known clinical trials aimed at examining the effects of TXS inhibition in cancer, either alone, or in combination with conventional chemotherapy. In addition to TXS, a number of promising studies have been carried out examining the role TP in the development and progression of cancer. While the research

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into this novel therapeutic target is still fairly new, the results to date are promising and warrant further investigation.

While PGIS and TXS appear to have very contrasting roles in cancer, it is possible that the balance in the expression of these enzymes may also have clinical relevance. The balance in the expression of their respective prostanoids (PGI_2 and TXA_2) has been implicated in a range of disease states. To date, there have been no studies carried out to directly examine this theory, although overexpression of these enzymes has been shown to have directly opposing effects on tumor growth and angiogenesis in colorectal cancer. Recently in our lab, an overexpression of TXS was observed in a panel of NSCLC tumor protein samples, relative to matched normal controls, with a concomitant reduction or loss in PGIS expression in the same sample set. In addition, the ratio of PGIS/TXS expression was found to be significantly lower in NSCLC patient samples, relative to matched normal controls, suggesting that the balance in the expression/activity of these enzymes may of clinical importance in the disease. This is a potentially significant hypothesis, which warrants further investigation.

In conclusion, PGIS and TXS are promising chemoprevention agents/therapeutic targets for cancer. In addition, as these enzymes appear to have directly opposing roles in cancer states, the ratio of their expression levels may also be a determining factor for therapy and patient survival.

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References

- [1] W.H.O., Global action against cancer, World Health Organisation and International Union Against Cancer, 2005, pp. 1-24.
- [2] W.L. Smith, The eicosanoids and their biochemical mechanisms of action, *Biochem J* 259 (1989) 315-324.
- [3] B. Hinz, K. Brune, Cyclooxygenase-2--10 years later, *J Pharmacol Exp Ther* 300 (2002) 367-375.
- [4] T.D. Warner, J.A. Mitchell, Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic, *Faseb J* 18 (2004) 790-804.
- [5] N.V. Chandrasekharan, H. Dai, K.L. Roos, N.K. Evanson, J. Tomsik, T.S. Elton, D.L. Simmons, COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression, *Proc Natl Acad Sci U S A* 99 (2002) 13926-13931.
- [6] J.A. Snipes, B. Kis, G.S. Shelness, J.A. Hewett, D.W. Busija, Cloning and characterization of cyclooxygenase-1b (putative cyclooxygenase-3) in rat, *J Pharmacol Exp Ther* 313 (2005) 668-676.
- [7] P. Needleman, P.C. Isakson, Selective inhibition of cyclooxygenase-2, *Science and Medicine* (1998) 26-35.
- [8] M. Cuendet, J.M. Pezzuto, The role of cyclooxygenase and lipoxygenase in cancer chemoprevention, *Drug Metabol Drug Interact* 17 (2000) 109-157.
- [9] A.J. Dannenberg, K. Subbaramaiah, Targeting cyclooxygenase-2 in human neoplasia: rationale and promise, *Cancer Cell* 4 (2003) 431-436.
- [10] E.S. Antonarakis, E.I. Heath, J.R. Walczak, W.G. Nelson, H. Fedor, A.M. De Marzo, M.L. Zahurak, S. Piantadosi, A.J. Dannenberg, R.T. Gurganus, S.D. Baker, H.L. Parnes, T.L. DeWeese, A.W. Partin, M.A. Carducci, Phase II, randomized, placebo-controlled trial of neoadjuvant celecoxib in men with clinically localized prostate cancer: evaluation of drug-specific biomarkers, *J Clin Oncol* 27 (2009) 4986-4993.
- [11] T. Dragovich, H. Burris, 3rd, P. Loehrer, D.D. Von Hoff, S. Chow, S. Stratton, S. Green, Y. Obregon, I. Alvarez, M. Gordon, Gemcitabine plus celecoxib in patients

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- with advanced or metastatic pancreatic adenocarcinoma: results of a phase II trial, *Am J Clin Oncol* 31 (2008) 157-162.
- [12] A. Jakobsen, J.P. Mortensen, C. Bisgaard, J. Lindebjerg, S.R. Rafaelsen, V.O. Bendtsen, A COX-2 inhibitor combined with chemoradiation of locally advanced rectal cancer: a phase II trial, *Int J Colorectal Dis* 23 (2008) 251-255.
- [13] V.A. Papadimitrakopoulou, W.N. William, Jr., A.J. Dannenberg, S.M. Lippman, J.J. Lee, F.G. Ondrey, D.E. Peterson, L. Feng, A. Atwell, A.K. El-Naggar, C.O. Nathan, J.I. Helman, B. Du, B. Yueh, J.O. Boyle, Pilot randomized phase II study of celecoxib in oral premalignant lesions, *Clin Cancer Res* 14 (2008) 2095-2101.
- [14] H. Achiwa, Y. Yatabe, T. Hida, T. Kuroishi, K. Kozaki, S. Nakamura, M. Ogawa, T. Sugiura, T. Mitsudomi, T. Takahashi, Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinomas, *Clin Cancer Res* 5 (1999) 1001-1005.
- [15] F.R. Khuri, H. Wu, J.J. Lee, B.L. Kemp, R. Lotan, S.M. Lippman, L. Feng, W.K. Hong, X.C. Xu, Cyclooxygenase-2 overexpression is a marker of poor prognosis in stage I non-small cell lung cancer, *Clin Cancer Res* 7 (2001) 861-867.
- [16] J. Brabender, J. Park, R. Metzger, P.M. Schneider, R.V. Lord, A.H. Holscher, K.D. Danenberg, P.V. Danenberg, Prognostic significance of cyclooxygenase 2 mRNA expression in non-small cell lung cancer, *Ann Surg* 235 (2002) 440-443.
- [17] M.J. Edelman, D. Watson, X. Wang, C. Morrison, R.A. Kratzke, S. Jewell, L. Hodgson, A.M. Mauer, A. Gajra, G.A. Masters, M. Bedor, E.E. Vokes, M.J. Green, Eicosanoid modulation in advanced lung cancer: cyclooxygenase-2 expression is a positive predictive factor for celecoxib + chemotherapy--Cancer and Leukemia Group B Trial 30203, *J Clin Oncol* 26 (2008) 848-855.
- [18] M.J. Fidler, A. Argiris, J.D. Patel, D.H. Johnson, A. Sandler, V.M. Villaflor, J.t. Coon, L. Buckingham, K. Kaiser, S. Basu, P. Bonomi, The potential predictive value of cyclooxygenase-2 expression and increased risk of gastrointestinal hemorrhage in advanced non-small cell lung cancer patients treated with erlotinib and celecoxib, *Clin Cancer Res* 14 (2008) 2088-2094.
- [19] R. Mutter, B. Lu, D.P. Carbone, I. Csiki, L. Moretti, D.H. Johnson, J.D. Morrow, A.B. Sandler, Y. Shyr, F. Ye, H. Choy, A phase II study of celecoxib in

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- combination with paclitaxel, carboplatin, and radiotherapy for patients with inoperable stage IIIA/B non-small cell lung cancer, *Clin Cancer Res* 15 (2009) 2158-2165.
- [20] M.S. O'Reilly, L. Holmgren, Y. Shing, C. Chen, R.A. Rosenthal, M. Moses, W.S. Lane, Y. Cao, E.H. Sage, J. Folkman, Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma, *Cell* 79 (1994) 315-328.
- [21] T.W. Moody, J. Leyton, H. Zakowicz, T. Hida, Y. Kang, S. Jakowlew, L. You, L. Ozbun, H. Zia, J. Youngberg, A. Malkinson, Indomethacin reduces lung adenoma number in A/J mice, *Anticancer Res* 21 (2001) 1749-1755.
- [22] N. Rioux, A. Castonguay, Prevention of NNK-induced lung tumorigenesis in A/J mice by acetylsalicylic acid and NS-398, *Cancer Res* 58 (1998) 5354-5360.
- [23] L.R. Kisley, B.S. Barrett, L.D. Dwyer-Nield, A.K. Bauer, D.C. Thompson, A.M. Malkinson, Celecoxib reduces pulmonary inflammation but not lung tumorigenesis in mice, *Carcinogenesis* 23 (2002) 1653-1660.
- [24] C. Bombardier, L. Laine, A. Reicin, D. Shapiro, R. Burgos-Vargas, B. Davis, R. Day, M.B. Ferraz, C.J. Hawkey, M.C. Hochberg, T.K. Kvien, T.J. Schnitzer, Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group, *N Engl J Med* 343 (2000) 1520-1528, 1522 p following 1528.
- [25] R.S. Bresalier, R.S. Sandler, H. Quan, J.A. Bolognese, B. Oxenius, K. Horgan, C. Lines, R. Riddell, D. Morton, A. Lanus, M.A. Konstam, J.A. Baron, Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial, *N Engl J Med* 352 (2005) 1092-1102.
- [26] S.D. Solomon, J.J. McMurray, M.A. Pfeffer, J. Wittes, R. Fowler, P. Finn, W.F. Anderson, A. Zauber, E. Hawk, M. Bertagnolli, Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention, *N Engl J Med* 352 (2005) 1071-1080.
- [27] G.P. Pidgeon, R. Tamosiuniene, G. Chen, I. Leonard, O. Belton, A. Bradford, D.J. Fitzgerald, Intravascular thrombosis after hypoxia-induced pulmonary hypertension: regulation by cyclooxygenase-2, *Circulation* 110 (2004) 2701-2707.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [28] M.C. Cathcart, R. Tamosiuniene, G. Chen, T.G. Neilan, A. Bradford, K.J. O'Byrne, D.J. Fitzgerald, G.P. Pidgeon, Cyclooxygenase-2-linked attenuation of hypoxia-induced pulmonary hypertension and intravascular thrombosis, *J Pharmacol Exp Ther* 326 (2008) 51-58.
- [29] V.W. Yang, J.M. Shields, S.R. Hamilton, E.W. Spannhake, W.C. Hubbard, L.M. Hyland, C.R. Robinson, F.M. Giardiello, Size-dependent increase in prostanoid levels in adenomas of patients with familial adenomatous polyposis, *Cancer Res* 58 (1998) 1750-1753.
- [30] L. Ermert, C. Dierkes, M. Ermert, Immunohistochemical expression of cyclooxygenase isoenzymes and downstream enzymes in human lung tumors, *Clin Cancer Res* 9 (2003) 1604-1610.
- [31] T.L. McLemore, W.C. Hubbard, C.L. Litterst, M.C. Liu, S. Miller, N.A. McMahon, J.C. Eggleston, M.R. Boyd, Profiles of prostaglandin biosynthesis in normal lung and tumor tissue from lung cancer patients, *Cancer Res* 48 (1988) 3140-3147.
- [32] W.C. Hubbard, M.C. Alley, G.N. Gray, K.C. Green, T.L. McLemore, M.R. Boyd, Evidence for prostanoid biosynthesis as a biochemical feature of certain subclasses of non-small cell carcinomas of the lung as determined in established cell lines derived from human lung tumors, *Cancer Res* 49 (1989) 826-832.
- [33] K.V. Honn, B. Cicone, A. Skoff, Prostacyclin: a potent antimetastatic agent, *Science* 212 (1981) 1270-1272.
- [34] R.L. Keith, Y.E. Miller, Y. Hoshikawa, M.D. Moore, T.L. Gesell, B. Gao, A.M. Malkinson, H.A. Golpon, R.A. Nemenoff, M.W. Geraci, Manipulation of pulmonary prostacyclin synthase expression prevents murine lung cancer, *Cancer Res* 62 (2002) 734-740.
- [35] R.L. Keith, Y.E. Miller, T.M. Hudish, C.E. Girod, S. Sotto-Santiago, W.A. Franklin, R.A. Nemenoff, T.H. March, S.P. Nana-Sinkam, M.W. Geraci, Pulmonary prostacyclin synthase overexpression chemoprevents tobacco smoke lung carcinogenesis in mice, *Cancer Res* 64 (2004) 5897-5904.
- [36] S. Kajita, K.H. Ruebel, M.B. Casey, N. Nakamura, R.V. Lloyd, Role of COX-2, thromboxane A2 synthase, and prostaglandin I2 synthase in papillary thyroid carcinoma growth, *Mod Pathol* 18 (2005) 221-227.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [37] H. Sakai, T. Suzuki, Y. Takahashi, M. Ukai, K. Tauchi, T. Fujii, N. Horikawa, T. Minamimura, Y. Tabuchi, M. Morii, K. Tsukada, N. Takeguchi, Upregulation of thromboxane synthase in human colorectal carcinoma and the cancer cell proliferation by thromboxane A₂, *FEBS Lett* 580 (2006) 3368-3374.
- [38] O. Moussa, J.S. Yordy, H. Abol-Enein, D. Sinha, N.K. Bissada, P.V. Halushka, M.A. Ghoneim, D.K. Watson, Prognostic and functional significance of thromboxane synthase gene overexpression in invasive bladder cancer, *Cancer Res* 65 (2005) 11581-11587.
- [39] D. Nie, M. Che, A. Zacharek, Y. Qiao, L. Li, X. Li, M. Lamberti, K. Tang, Y. Cai, Y. Guo, D. Grignon, K.V. Honn, Differential expression of thromboxane synthase in prostate carcinoma: role in tumor cell motility, *Am J Pathol* 164 (2004) 429-439.
- [40] D. Nie, M. Lamberti, A. Zacharek, L. Li, K. Szekeres, K. Tang, Y. Chen, K.V. Honn, Thromboxane A₂ regulation of endothelial cell migration, angiogenesis, and tumor metastasis, *Biochem Biophys Res Commun* 267 (2000) 245-251.
- [41] R.J. Helliwell, L.F. Adams, M.D. Mitchell, Prostaglandin synthases: recent developments and a novel hypothesis, *Prostaglandins Leukot Essent Fatty Acids* 70 (2004) 101-113.
- [42] B.W. Christman, C.D. McPherson, J.H. Newman, G.A. King, G.R. Bernard, B.M. Groves, J.E. Loyd, An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension, *N Engl J Med* 327 (1992) 70-75.
- [43] J.R. Vane, Biomedicine. Back to an aspirin a day?, *Science* 296 (2002) 474-475.
- [44] D. Wang, R.N. DuBois, Measurement of eicosanoids in cancer tissues, *Methods Enzymol* 433 (2007) 27-50.
- [45] N.V. Bogatcheva, M.G. Sergeeva, S.M. Dudek, A.D. Verin, Arachidonic acid cascade in endothelial pathobiology, *Microvasc Res* 69 (2005) 107-127.
- [46] A.N. Hata, R.M. Breyer, Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation, *Pharmacol Ther* 103 (2004) 147-166.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [47] A. Alfranca, M.A. Iniguez, M. Fresno, J.M. Redondo, Prostanoid signal transduction and gene expression in the endothelium: role in cardiovascular diseases, *Cardiovasc Res* 70 (2006) 446-456.
- [48] C. Griffoni, E. Spisni, A. Strillacci, M. Toni, M.M. Bachschmid, V. Tomasi, Selective inhibition of prostacyclin synthase activity by rofecoxib, *J Cell Mol Med* 11 (2007) 327-338.
- [49] J.Y. Liou, W.G. Deng, D.W. Gilroy, S.K. Shyue, K.K. Wu, Colocalization and interaction of cyclooxygenase-2 with caveolin-1 in human fibroblasts, *J Biol Chem* 276 (2001) 34975-34982.
- [50] E. Spisni, C. Griffoni, S. Santi, M. Riccio, R. Marulli, G. Bartolini, M. Toni, V. Ullrich, V. Tomasi, Colocalization prostacyclin (PGI₂) synthase--caveolin-1 in endothelial cells and new roles for PGI₂ in angiogenesis, *Exp Cell Res* 266 (2001) 31-43.
- [51] W.L. Smith, D.L. DeWitt, M.L. Allen, Bimodal distribution of the prostaglandin I₂ synthase antigen in smooth muscle cells, *J Biol Chem* 258 (1983) 5922-5926.
- [52] I. Siegle, T. Klein, M.H. Zou, P. Fritz, M. Komhoff, Distribution and cellular localization of prostacyclin synthase in human brain, *J Histochem Cytochem* 48 (2000) 631-641.
- [53] M. Murakami, I. Kudo, K. Inoue, Molecular nature of phospholipases A₂ involved in prostaglandin I₂ synthesis in human umbilical vein endothelial cells. Possible participation of cytosolic and extracellular type II phospholipases A₂, *J Biol Chem* 268 (1993) 839-844.
- [54] S. Hara, A. Miyata, C. Yokoyama, H. Inoue, R. Brugger, F. Lottspeich, V. Ullrich, T. Tanabe, Isolation and molecular cloning of prostacyclin synthase from bovine endothelial cells, *J Biol Chem* 269 (1994) 19897-19903.
- [55] S. Fleisher-Berkovich, A. Danon, IL-1 α but not IL-1 β -induced prostaglandin synthesis is inhibited by corticotropin-releasing factor, *Cytokine* 11 (1999) 239-243.
- [56] D. Chevalier, C. Cauffiez, C. Bernard, J.M. Lo-Guidice, D. Allorge, F. Fazio, N. Ferrari, C. Libersa, M. Lhermitte, J.C. D'Halluin, F. Broly, Characterization of new mutations in the coding sequence and 5'-untranslated region of the human prostacyclin synthase gene (CYP8A1), *Hum Genet* 108 (2001) 148-155.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [57] A. Miyata, S. Hara, C. Yokoyama, H. Inoue, V. Ullrich, T. Tanabe, Molecular cloning and expression of human prostacyclin synthase, *Biochem Biophys Res Commun* 200 (1994) 1728-1734.
- [58] H. Ohishi, K. Furukawa, K. Iwasaki, K. Ueyama, A. Okada, S. Motomura, S. Harata, S. Toh, Role of prostaglandin I₂ in the gene expression induced by mechanical stress in spinal ligament cells derived from patients with ossification of the posterior longitudinal ligament, *J Pharmacol Exp Ther* 305 (2003) 818-824.
- [59] D. Korita, N. Sagawa, H. Itoh, S. Yura, M. Yoshida, K. Kakui, M. Takemura, C. Yokoyama, T. Tanabe, S. Fujii, Cyclic mechanical stretch augments prostacyclin production in cultured human uterine myometrial cells from pregnant women: possible involvement of up-regulation of prostacyclin synthase expression, *J Clin Endocrinol Metab* 87 (2002) 5209-5219.
- [60] G.E. Caughey, L.G. Cleland, J.R. Gamble, M.J. James, Up-regulation of endothelial cyclooxygenase-2 and prostanoid synthesis by platelets. Role of thromboxane A₂, *J Biol Chem* 276 (2001) 37839-37845.
- [61] S. Bunting, R. Gryglewski, S. Moncada, J.R. Vane, Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation, *Prostaglandins* 12 (1976) 897-913.
- [62] S. Moncada, R. Gryglewski, S. Bunting, J.R. Vane, An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation, *Nature* 263 (1976) 663-665.
- [63] P. Libby, S.J. Warner, G.B. Friedman, Interleukin 1: a mitogen for human vascular smooth muscle cells that induces the release of growth-inhibitory prostanoids, *J Clin Invest* 81 (1988) 487-498.
- [64] C.L. Bos, D.J. Richel, T. Ritsema, M.P. Peppelenbosch, H.H. Versteeg, Prostanoids and prostanoid receptors in signal transduction, *Int J Biochem Cell Biol* 36 (2004) 1187-1205.
- [65] R.A. Gupta, T. J., W.F. Krause, M. Geraci, T.M. Willson, S.K. Dey, Prostacyclin mediated activation of peroxisome proliferator-activated receptor delta in colorectal

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- cancer, *Proceedings of the National Academy of Sciences of the United States of America* 97 (2000) 13275-13280.
- [66] P.W. Shaul, B. Kinane, M.A. Farrar, L.M. Buja, R.R. Magness, Prostacyclin production and mediation of adenylate cyclase activity in the pulmonary artery. Alterations after prolonged hypoxia in the rat, *J Clin Invest* 88 (1991) 447-455.
 - [67] C.J. Lo, M. Fu, F.R. Lo, Prostaglandin I₂ analogue, iloprost, down regulates mitogen-activated protein kinases of macrophages, *J Surg Res* 76 (1998) 159-164.
 - [68] D.A. Jones, C.W. Benjamin, D.A. Linseman, Activation of thromboxane and prostacyclin receptors elicits opposing effects on vascular smooth muscle cell growth and mitogen-activated protein kinase signaling cascades, *Mol Pharmacol* 48 (1995) 890-896.
 - [69] I. Schwaner, S. Offermanns, K. Spicher, R. Seifert, G. Schultz, Differential activation of Gi and Gs proteins by E- and I-type prostaglandins in membranes from the human erythroleukaemia cell line, HEL, *Biochim Biophys Acta* 1265 (1995) 8-14.
 - [70] B.M. Forman, J. Chen, R.M. Evans, Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta, *Proc Natl Acad Sci U S A* 94 (1997) 4312-4317.
 - [71] J. DiRenzo, M. Soderstrom, R. Kurokawa, M.H. Ogliastro, M. Ricote, S. Ingre, A. Horlein, M.G. Rosenfeld, C.K. Glass, Peroxisome proliferator-activated receptors and retinoic acid receptors differentially control the interactions of retinoid X receptor heterodimers with ligands, coactivators, and corepressors, *Mol Cell Biol* 17 (1997) 2166-2176.
 - [72] H. Lim, S.K. Dey, A novel pathway of prostacyclin signaling-hanging out with nuclear receptors, *Endocrinology* 143 (2002) 3207-3210.
 - [73] B.J. Tong, J. Tan, L. Tajeda, S.K. Das, J.A. Chapman, R.N. DuBois, S.K. Dey, Heightened expression of cyclooxygenase-2 and peroxisome proliferator-activated receptor-delta in human endometrial adenocarcinoma, *Neoplasia* 2 (2000) 483-490.
 - [74] T. Tanabe, V. Ullrich, Prostacyclin and thromboxane synthases, *J Lipid Mediat Cell Signal* 12 (1995) 243-255.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [75] A. Miyata, C. Yokoyama, H. Ihara, S. Bandoh, O. Takeda, E. Takahashi, T. Tanabe, Characterization of the human gene (TBXAS1) encoding thromboxane synthase, *Eur J Biochem* 224 (1994) 273-279.
- [76] P. Needleman, S. Moncada, S. Bunting, J.R. Vane, M. Hamberg, B. Samuelsson, Identification of an enzyme in platelet microsomes which generates thromboxane A₂ from prostaglandin endoperoxides, *Nature* 261 (1976) 558-560.
- [77] Y. Tone, A. Miyata, S. Hara, S. Yukawa, T. Tanabe, Abundant expression of thromboxane synthase in rat macrophages, *FEBS Lett* 340 (1994) 241-244.
- [78] M. Haurand, V. Ullrich, Isolation and characterization of thromboxane synthase from human platelets as a cytochrome P-450 enzyme, *J Biol Chem* 260 (1985) 15059-15067.
- [79] K. Uchida, Current status of acrolein as a lipid peroxidation product, *Trends Cardiovasc Med* 9 (1999) 109-113.
- [80] A.K. Chaudhary, M. Nokubo, G.R. Reddy, S.N. Yeola, J.D. Morrow, I.A. Blair, L.J. Marnett, Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver, *Science* 265 (1994) 1580-1582.
- [81] C.D. Funk, Prostaglandins and leukotrienes: advances in eicosanoid biology, *Science* 294 (2001) 1871-1875.
- [82] B. Samuelsson, M. Goldyne, E. Granstrom, M. Hamberg, S. Hammarstrom, C. Malmsten, Prostaglandins and thromboxanes, *Annu Rev Biochem* 47 (1978) 997-1029.
- [83] M. Hirata, Y. Hayashi, F. Ushikubi, Y. Yokota, R. Kageyama, S. Nakanishi, S. Narumiya, Cloning and expression of cDNA for a human thromboxane A₂ receptor, *Nature* 349 (1991) 617-620.
- [84] M.K. Raychowdhury, M. Yukawa, L.J. Collins, S.H. McGrail, K.C. Kent, J.A. Ware, Alternative splicing produces a divergent cytoplasmic tail in the human endothelial thromboxane A₂ receptor, *J Biol Chem* 270 (1995) 7011.
- [85] S.M. Miggin, B.T. Kinsella, Expression and tissue distribution of the mRNAs encoding the human thromboxane A₂ receptor (TP) alpha and beta isoforms, *Biochim Biophys Acta* 1425 (1998) 543-559.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [86] R.M. Breyer, C.K. Bagdassarian, S.A. Myers, M.D. Breyer, Prostanoid receptors: subtypes and signaling, *Annu Rev Pharmacol Toxicol* 41 (2001) 661-690.
- [87] Y. Cheng, S.C. Austin, B. Rocca, B.H. Koller, T.M. Coffman, T. Grosser, J.A. Lawson, G.A. FitzGerald, Role of prostacyclin in the cardiovascular response to thromboxane A₂, *Science* 296 (2002) 539-541.
- [88] J.L. Mills, R. DerSimonian, E. Raymond, J.D. Morrow, L.J. Roberts, 2nd, J.D. Clemens, J.C. Hauth, P. Catalano, B. Sibai, L.B. Curet, R.J. Levine, Prostacyclin and thromboxane changes predating clinical onset of preeclampsia: a multicenter prospective study, *JAMA* 282 (1999) 356-362.
- [89] M. Kreutzer, T. Fauti, K. Kaddatz, C. Seifart, A. Neubauer, H. Schweer, M. Komhoff, S. Muller-Brusselbach, R. Muller, Specific components of prostanoid-signaling pathways are present in non-small cell lung cancer cells, *Oncol Rep* 18 (2007) 497-501.
- [90] P. Nana-Sinkam, H. Golpon, R.L. Keith, R.J. Oyer, S. Sotto-Santiago, M.D. Moore, W. Franklin, R.A. Nemenoff, M.W. Geraci, Prostacyclin in human non-small cell lung cancers, *Chest* 125 (2004) 141S.
- [91] R.S. Stearman, L. Dwyer-Nield, L. Zerbe, S.A. Blaine, Z. Chan, P.A. Bunn, Jr., G.L. Johnson, F.R. Hirsch, D.T. Merrick, W.A. Franklin, A.E. Baron, R.L. Keith, R.A. Nemenoff, A.M. Malkinson, M.W. Geraci, Analysis of orthologous gene expression between human pulmonary adenocarcinoma and a carcinogen-induced murine model, *Am J Pathol* 167 (2005) 1763-1775.
- [92] R.S. Stearman, M.C. Grady, P. Nana-Sinkam, M. Varella-Garcia, M.W. Geraci, Genetic and epigenetic regulation of the human prostacyclin synthase promoter in lung cancer cell lines, *Mol Cancer Res* 5 (2007) 295-308.
- [93] A. Gustafsson, E. Hansson, U. Kressner, S. Nordgren, M. Andersson, C. Lonnroth, K. Lundholm, Prostanoid receptor expression in colorectal cancer related to tumor stage, differentiation and progression, *Acta Oncol* 46 (2007) 1107-1112.
- [94] T. Dassel, X. de Leval, L. de Leval, B. Pirotte, V. Castronovo, D. Waltregny, Activation of the thromboxane A₂ pathway in human prostate cancer correlates with tumor Gleason score and pathologic stage, *Eur Urol* 50 (2006) 1021-1031; discussion 1031.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [95] M.T. Wang, K.V. Honn, D. Nie, Cyclooxygenases, prostanoids, and tumor progression, *Cancer Metastasis Rev* (2007).
- [96] G. Watkins, A. Douglas-Jones, R.E. Mansel, W.G. Jiang, Expression of thromboxane synthase, TBXAS1 and the thromboxane A2 receptor, TBXA2R, in human breast cancer, *Int Semin Surg Oncol* 2 (2005) 23.
- [97] O. Moussa, A.W. Ashton, M. Fraig, E. Garrett-Mayer, M.A. Ghoneim, P.V. Halushka, D.K. Watson, Novel role of thromboxane receptors beta isoform in bladder cancer pathogenesis, *Cancer Res* 68 (2008) 4097-4104.
- [98] G.G. Chen, T.W. Lee, J.H. Yip, H. Xu, I.K. Lee, T.S. Mok, T.D. Warner, A.P. Yim, Increased thromboxane B(2) levels are associated with lipid peroxidation and Bcl-2 expression in human lung carcinoma, *Cancer Lett* 234 (2006) 193-198.
- [99] H.J. Fahn, L.S. Wang, S.H. Kao, S.C. Chang, M.H. Huang, Y.H. Wei, Smoking-associated mitochondrial DNA mutations and lipid peroxidation in human lung tissues, *Am J Respir Cell Mol Biol* 19 (1998) 901-909.
- [100] S. Pinto, L. Gori, O. Gallo, S. Boccuzzi, R. Paniccia, R. Abbate, Increased thromboxane A2 production at primary tumor site in metastasizing squamous cell carcinoma of the larynx, *Prostaglandins Leukot Essent Fatty Acids* 49 (1993) 527-530.
- [101] P. Pradono, R. Tazawa, M. Maemondo, M. Tanaka, K. Usui, Y. Saijo, K. Hagiwara, T. Nukiwa, Gene transfer of thromboxane A(2) synthase and prostaglandin I(2) synthase antithetically altered tumor angiogenesis and tumor growth, *Cancer Res* 62 (2002) 63-66.
- [102] L. Michalik, B. Desvergne, W. Wahli, Peroxisome-proliferator-activated receptors and cancers: complex stories, *Nat Rev Cancer* 4 (2004) 61-70.
- [103] K. Fukumoto, Y. Yano, N. Virgona, H. Hagiwara, H. Sato, H. Senba, K. Suzuki, R. Asano, K. Yamada, T. Yano, Peroxisome proliferator-activated receptor delta as a molecular target to regulate lung cancer cell growth, *FEBS Lett* 579 (2005) 3829-3836.
- [104] V.G. Keshamouni, R.C. Reddy, D.A. Arenberg, B. Joel, V.J. Thannickal, G.P. Kalemkerian, T.J. Standiford, Peroxisome proliferator-activated receptor-gamma

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- activation inhibits tumor progression in non-small-cell lung cancer, *Oncogene* 23 (2004) 100-108.
- [105] W.K. Leung, A.H. Bai, V.Y. Chan, J. Yu, M.W. Chan, K.F. To, J.R. Wu, K.K. Chan, Y.G. Fu, F.K. Chan, J.J. Sung, Effect of peroxisome proliferator activated receptor gamma ligands on growth and gene expression profiles of gastric cancer cells, *Gut* 53 (2004) 331-338.
- [106] M. Mutoh, K. Watanabe, T. Kitamura, Y. Shoji, M. Takahashi, T. Kawamori, K. Tani, M. Kobayashi, T. Maruyama, K. Kobayashi, S. Ohuchida, Y. Sugimoto, S. Narumiya, T. Sugimura, K. Wakabayashi, Involvement of prostaglandin E receptor subtype EP(4) in colon carcinogenesis, *Cancer Res* 62 (2002) 28-32.
- [107] R. Nemenoff, A.M. Meyer, T.M. Hudish, A.B. Mozer, A. Snee, S. Narumiya, R.S. Stearman, R.A. Winn, M. Weiser-Evans, M.W. Geraci, R.L. Keith, Prostacyclin prevents murine lung cancer independent of the membrane receptor by activation of peroxisomal proliferator--activated receptor gamma, *Cancer Prev Res (Phila Pa)* 1 (2008) 349-356.
- [108] D. Wang, H. Wang, Y. Guo, W. Ning, S. Katkuri, W. Wahli, B. Desvergne, S.K. Dey, R.N. DuBois, Crosstalk between peroxisome proliferator-activated receptor delta and VEGF stimulates cancer progression, *Proc Natl Acad Sci U S A* 103 (2006) 19069-19074.
- [109] O. Moussa, J.M. Riker, J. Klein, M. Fraig, P.V. Halushka, D.K. Watson, Inhibition of thromboxane synthase activity modulates bladder cancer cell responses to chemotherapeutic agents, *Oncogene* (2007).
- [110] X. Li, H.H. Tai, Activation of Thromboxane A2 Receptors Induces Orphan Nuclear Receptor Nurr1 Expression and Stimulates Cell Proliferation in Human Lung Cancer Cells, *Carcinogenesis* (2009).
- [111] M. Perl, C.S. Chung, A. Ayala, Apoptosis, *Crit Care Med* 33 (2005) S526-529.
- [112] S.H. Kaufmann, W.C. Earnshaw, Induction of apoptosis by cancer chemotherapy, *Exp Cell Res* 256 (2000) 42-49.
- [113] L. Milas, V. Gregoire, N. Hunter, B. Kurdoglu, K. Mason, R. Meyn, Radiation-induced apoptosis in tumors: effect of radiation modulating agents, *Adv Exp Med Biol* 400B (1997) 559-564.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [114] J.E. Castelao, R.D. Bart, 3rd, C.A. DiPerna, E.M. Sievers, R.M. Bremner, Lung cancer and cyclooxygenase-2, *Ann Thorac Surg* 76 (2003) 1327-1335.
- [115] T. Hatae, M. Wada, C. Yokoyama, M. Shimonishi, T. Tanabe, Prostacyclin-dependent apoptosis mediated by PPAR delta, *J Biol Chem* 276 (2001) 46260-46267.
- [116] N.S. Tan, L. Michalik, N. Noy, R. Yasmin, C. Pacot, M. Heim, B. Fluhmann, B. Desvergne, W. Wahli, Critical roles of PPAR beta/delta in keratinocyte response to inflammation, *Genes Dev* 15 (2001) 3263-3277.
- [117] K.K. Wu, J.Y. Liou, Cyclooxygenase inhibitors induce colon cancer cell apoptosis Via PPARdelta --> 14-3-3epsilon pathway, *Methods Mol Biol* 512 (2009) 295-307.
- [118] R.G. Pozner, S. Negrotto, L.P. D'Atri, M.L. Kotler, M.A. Lazzari, R.M. Gomez, M. Schattner, Prostacyclin prevents nitric oxide-induced megakaryocyte apoptosis, *Br J Pharmacol* 145 (2005) 283-292.
- [119] K. Yoshizato, S. Zapf, M. Westphal, M.E. Berens, A. Giese, Thromboxane synthase inhibitors induce apoptosis in migration-arrested glioma cells, *Neurosurgery* 50 (2002) 343-354.
- [120] F. Kurznel, C. Hagel, S. Zapf, H. Meissner, M. Westphal, A. Giese, Cyclo-oxygenase inhibitors and thromboxane synthase inhibitors differentially regulate migration arrest, growth inhibition and apoptosis in human glioma cells, *Acta Neurochir (Wien)* 144 (2002) 71-87.
- [121] J. Jantke, M. Ladehoff, F. Kurznel, S. Zapf, E. Kim, A. Giese, Inhibition of the arachidonic acid metabolism blocks endothelial cell migration and induces apoptosis, *Acta Neurochir (Wien)* 146 (2004) 483-494.
- [122] K.C. Leung, M.K. Hsin, J.S. Chan, J.H. Yip, M. Li, B.C. Leung, T.S. Mok, T.D. Warner, M.J. Underwood, G.G. Chen, Inhibition of thromboxane synthase induces lung cancer cell death via increasing the nuclear p27, *Exp Cell Res* (2009).
- [123] M. Fujimura, K. Kasahara, H. Shirasaki, U. Heki, K. Iwasa, A. Ueda, T. Matsuda, Up-regulation of ICH-1L protein by thromboxane A2 antagonists enhances cisplatin-induced apoptosis in non-small-cell lung-cancer cell lines, *J Cancer Res Clin Oncol* 125 (1999) 389-394.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [124] N. Goldenberg, S.R. Kahn, S. Solymoss, Markers of coagulation and angiogenesis in cancer-associated venous thromboembolism, *J Clin Oncol* 21 (2003) 4194-4199.
- [125] R.L. Bick, Cancer-associated thrombosis: focus on extended therapy with dalteparin, *J Support Oncol* 4 (2006) 115-120.
- [126] K.V. Honn, B.F. Sloane, Prostacyclin, thromboxanes, and hematogenous metastasis, *Adv Prostaglandin Thromboxane Leukot Res* 12 (1983) 313-318.
- [127] H.M. Pinedo, H.M. Verheul, R.J. D'Amato, J. Folkman, Involvement of platelets in tumour angiogenesis?, *Lancet* 352 (1998) 1775-1777.
- [128] M.B. Donati, Cancer and thrombosis, *Haemostasis* 24 (1994) 128-131.
- [129] F.R. Rickles, Mechanisms of cancer-induced thrombosis in cancer, *Pathophysiol Haemost Thromb* 35 (2006) 103-110.
- [130] C. Boccaccio, G. Sabatino, E. Medico, F. Girolami, A. Follenzi, G. Reato, A. Sottile, L. Naldini, P.M. Comoglio, The MET oncogene drives a genetic programme linking cancer to haemostasis, *Nature* 434 (2005) 396-400.
- [131] J.L. Yu, L. May, V. Lhotak, S. Shahrzad, S. Shirasawa, J.I. Weitz, B.L. Coomber, N. Mackman, J.W. Rak, Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis, *Blood* 105 (2005) 1734-1741.
- [132] E. Grad, M. Golomb, N. Koroukhov, J.A. Lawson, C. Lotan, G.A. Fitzgerald, H.D. Danenberg, Aspirin reduces the prothrombotic activity of C-reactive protein, *J Thromb Haemost* 7 (2009) 1393-1400.
- [133] A.A. Khorana, Cancer and thrombosis: implications of published guidelines for clinical practice, *Ann Oncol* (2009).
- [134] C.P. Klerk, S.M. Smorenburg, H.M. Otten, A.W. Lensing, M.H. Prins, F. Piovella, P. Prandoni, M.M. Bos, D.J. Richel, G. van Tienhoven, H.R. Buller, The effect of low molecular weight heparin on survival in patients with advanced malignancy, *J Clin Oncol* 23 (2005) 2130-2135.
- [135] A.K. Kakkar, M.N. Levine, Z. Kadziola, N.R. Lemoine, V. Low, H.K. Patel, G. Rustin, M. Thomas, M. Quigley, R.C. Williamson, Low molecular weight heparin, therapy with dalteparin, and survival in advanced cancer: the fragmin advanced malignancy outcome study (FAMOUS), *J Clin Oncol* 22 (2004) 1944-1948.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [136] M. Altinbas, H.S. Coskun, O. Er, M. Ozkan, B. Eser, A. Unal, M. Cetin, S. Soyuer, A randomized clinical trial of combination chemotherapy with and without low-molecular-weight heparin in small cell lung cancer, *J Thromb Haemost* 2 (2004) 1266-1271.
- [137] F. Icli, H. Akbulut, G. Utkan, B. Yalcin, D. Dincol, A. Isikdogan, A. Demirkazik, H. Onur, F. Cay, A. Buyukcelik, Low molecular weight heparin (LMWH) increases the efficacy of cisplatin plus gemcitabine combination in advanced pancreatic cancer, *J Surg Oncol* 95 (2007) 507-512.
- [138] A.A. Khorana, Liebman, H.A., White, R.H., Won, T., Lyman, G.H., The risk of venous thromboembolism in patients with cancer., *ASCO Education Book* 26 (2008) 240-248.
- [139] X. de Leval, V. Benoit, J. Delarge, F. Julemont, B. Masereel, B. Pirotte, M.P. Merville, J.L. David, J.M. Dogne, Pharmacological evaluation of the novel thromboxane modulator BM-567 (II/II). Effects of BM-567 on osteogenic sarcoma-cell-induced platelet aggregation, *Prostaglandins Leukot Essent Fatty Acids* 68 (2003) 55-59.
- [140] A.K. Kakkar, M.N. Levine, Thrombosis and cancer: implications beyond Trousseau, *J Thromb Haemost* 2 (2004) 1261-1262.
- [141] G.A. Petralia, N.R. Lemoine, A.K. Kakkar, Mechanisms of disease: the impact of antithrombotic therapy in cancer patients, *Nat Clin Pract Oncol* 2 (2005) 356-363.
- [142] X. Chen, W. Xiao, X. Qu, S. Zhou, The effect of dalteparin, a kind of low molecular weight heparin, on lung adenocarcinoma A549 cell line in vitro, *Cancer Invest* 26 (2008) 718-724.
- [143] D. Prisco, R. Paniccia, M. Coppo, M. Filippini, I. Francalanci, T. Brunelli, P. Comeglio, R. Abbate, Platelet activation and platelet lipid composition in pulmonary cancer, *Prostaglandins Leukot Essent Fatty Acids* 53 (1995) 65-68.
- [144] J. Folkman, Angiogenesis in cancer, vascular, rheumatoid and other disease, *Nat Med* 1 (1995) 27-31.
- [145] P. Carmeliet, R.K. Jain, Angiogenesis in cancer and other diseases, *Nature* 407 (2000) 249-257.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [146] D. Hanahan, J. Folkman, Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis, *Cell* 86 (1996) 353-364.
- [147] R.S. Herbst, A. Onn, A. Sandler, Angiogenesis and lung cancer: prognostic and therapeutic implications, *J Clin Oncol* 23 (2005) 3243-3256.
- [148] F.G. Buchanan, W. Chang, H. Sheng, J. Shao, J.D. Morrow, R.N. DuBois, Up-regulation of the enzymes involved in prostacyclin synthesis via Ras induces vascular endothelial growth factor, *Gastroenterology* 127 (2004) 1391-1400.
- [149] D. Bishop-Bailey, Peroxisome proliferator-activated receptors in the cardiovascular system, *Br J Pharmacol* 129 (2000) 823-834.
- [150] H. Lim, R.A. Gupta, W.G. Ma, B.C. Paria, D.E. Moller, J.D. Morrow, R.N. DuBois, J.M. Trzaskos, S.K. Dey, Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARdelta, *Genes Dev* 13 (1999) 1561-1574.
- [151] T.O. Daniel, H. Liu, J.D. Morrow, B.C. Crews, L.J. Marnett, Thromboxane A2 is a mediator of cyclooxygenase-2-dependent endothelial migration and angiogenesis, *Cancer Res* 59 (1999) 4574-4577.
- [152] Y. Gao, R. Yokota, S. Tang, A.W. Ashton, J.A. Ware, Reversal of angiogenesis in vitro, induction of apoptosis, and inhibition of AKT phosphorylation in endothelial cells by thromboxane A(2), *Circ Res* 87 (2000) 739-745.
- [153] A.W. Ashton, R. Yokota, G. John, S. Zhao, S.O. Suadicani, D.C. Spray, J.A. Ware, Inhibition of endothelial cell migration, intercellular communication, and vascular tube formation by thromboxane A(2), *J Biol Chem* 274 (1999) 35562-35570.
- [154] A.W. Ashton, J.A. Ware, Thromboxane A2 receptor signaling inhibits vascular endothelial growth factor-induced endothelial cell differentiation and migration, *Circ Res* 95 (2004) 372-379.
- [155] J.S. Rhee, M. Black, U. Schubert, S. Fischer, E. Morgenstern, H.P. Hammes, K.T. Preissner, The functional role of blood platelet components in angiogenesis, *Thromb Haemost* 92 (2004) 394-402.
- [156] T. Arisato, T. Hashiguchi, K.P. Sarker, K. Arimura, M. Asano, K. Matsuo, M. Osame, I. Maruyama, Highly accumulated platelet vascular endothelial growth factor in coagulant thrombotic region, *J Thromb Haemost* 1 (2003) 2589-2593.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [157] D. Nie, Y. Guo, D. Yang, Y. Tang, Y. Chen, M.T. Wang, A. Zacharek, Y. Qiao, M. Che, K.V. Honn, Thromboxane A2 receptors in prostate carcinoma: expression and its role in regulating cell motility via small GTPase Rho, *Cancer Res* 68 (2008) 115-121.
- [158] X. de Leval, T. Dassel, J.M. Dogne, D. Waltregny, A. Bellahcene, V. Benoit, B. Pirotte, V. Castronovo, Evaluation of original dual thromboxane A2 modulators as antiangiogenic agents, *J Pharmacol Exp Ther* 318 (2006) 1057-1067.
- [159] A. Giese, M.A. Loo, M.D. Rief, N. Tran, M.E. Berens, Substrates for astrocytoma invasion, *Neurosurgery* 37 (1995) 294-301; discussion 301-292.
- [160] I.J. Fidler, L.M. Ellis, The implications of angiogenesis for the biology and therapy of cancer metastasis, *Cell* 79 (1994) 185-188.
- [161] N. Weidner, J.P. Semple, W.R. Welch, J. Folkman, Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma, *N Engl J Med* 324 (1991) 1-8.
- [162] K.V. Honn, Inhibition of tumor cell metastasis by modulation of the vascular prostacyclin/thromboxane A2 system, *Clin Exp Metastasis* 1 (1983) 103-114.
- [163] K.C. Cuneo, A. Fu, K.L. Osusky, L. Geng, Effects of vascular endothelial growth factor receptor inhibitor SU5416 and prostacyclin on murine lung metastasis, *Anticancer Drugs* 18 (2007) 349-355.
- [164] M. Schirner, M.R. Schneider, Inhibition of metastasis by cicaprost in rats with established SMT2A mammary carcinoma growth, *Cancer Detect Prev* 21 (1997) 44-50.
- [165] A. Giese, C. Hagel, E.L. Kim, S. Zapf, J. Djawaheri, M.E. Berens, M. Westphal, Thromboxane synthase regulates the migratory phenotype of human glioma cells, *Neuro Oncol* 1 (1999) 3-13.
- [166] H.S. Kim, H.R. Youm, J.S. Lee, K.W. Min, J.H. Chung, C.S. Park, Correlation between cyclooxygenase-2 and tumor angiogenesis in non-small cell lung cancer, *Lung Cancer* 42 (2003) 163-170.
- [167] A. Yoshimoto, K. Kasahara, A. Kawashima, M. Fujimura, S. Nakao, Characterization of the prostaglandin biosynthetic pathway in non-small cell lung cancer: a comparison with small cell lung cancer and correlation with angiogenesis, angiogenic factors and metastases, *Oncol Rep* 13 (2005) 1049-1057.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [168] I. Yokoyama, S. Hayashi, T. Kobayashi, M. Negita, M. Yasutomi, K. Uchida, H. Takagi, Prevention of experimental hepatic metastasis with thromboxane synthase inhibitor, *Res Exp Med (Berl)* 195 (1995) 209-215.
- [169] I.F. Stamford, P.B. Melhuish, M.A. Carroll, C.J. Corrigan, S. Patel, A. Bennett, Survival of mice with NC carcinoma is unchanged by drugs that are thought to inhibit thromboxane synthesis or increase prostacyclin formation, *Br J Cancer* 54 (1986) 257-263.
- [170] W. McDonough, N. Tran, A. Giese, S.A. Norman, M.E. Berens, Altered gene expression in human astrocytoma cells selected for migration: I. Thromboxane synthase, *J Neuropathol Exp Neurol* 57 (1998) 449-455.
- [171] S. Rodrigues, Q.D. Nguyen, S. Faivre, E. Bruyneel, L. Thim, B. Westley, F. May, G. Flatau, M. Mareel, C. Gespach, S. Emami, Activation of cellular invasion by trefoil peptides and src is mediated by cyclooxygenase- and thromboxane A2 receptor-dependent signaling pathways, *FASEB J* 15 (2001) 1517-1528.
- [172] T. Hida, K. Kozaki, H. Muramatsu, A. Masuda, S. Shimizu, T. Mitsudomi, T. Sugiura, M. Ogawa, T. Takahashi, Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytotoxicity of various anticancer agents in non-small cell lung cancer cell lines, *Clin Cancer Res* 6 (2000) 2006-2011.
- [173] E.J. Topol, Failing the public health--rofecoxib, Merck, and the FDA, *N Engl J Med* 351 (2004) 1707-1709.
- [174] R.L. Keith, Oral iloprost for the chemoprevention of lung cancer, 13th World Conference on Lung Cancer Abstracts (2009).
- [175] J.M. Dogne, X. de Leval, J. Hanson, M. Frederich, B. Lambermont, A. Ghuysen, A. Casini, B. Masereel, K.H. Ruan, B. Pirotte, P. Kolh, New developments on thromboxane and prostacyclin modulators part I: thromboxane modulators, *Curr Med Chem* 11 (2004) 1223-1241.
- [176] R.F. Shen, H.H. Tai, Thromboxanes: synthase and receptors, *J Biomed Sci* 5 (1998) 153-172.
- [177] M. Nakazawa, K. Iizuka, A. Ujiie, S. Hiraku, S. Ohki, [Research and development of ozagrel, a highly selective inhibitor of TXA2 synthase], *Yakugaku Zasshi* 114 (1994) 911-933.

Prostacyclin Synthase, Thromboxane Synthase and Cancer

- [178] S. Rolin, B. Masereel, J.M. Dogne, Prostanoids as pharmacological targets in COPD and asthma, *Eur J Pharmacol* 533 (2006) 89-100.
- [179] Y. Ishitsuka, H. Moriuchi, K. Hatamoto, C. Yang, J. Takase, S. Golbidi, M. Irikura, T. Irie, Involvement of thromboxane A₂ (TXA₂) in the early stages of oleic acid-induced lung injury and the preventive effect of ozagrel, a TXA₂ synthase inhibitor, in guinea-pigs, *J Pharm Pharmacol* 56 (2004) 513-520.
- [180] T. Bando, M. Fujimura, K. Kasahara, T. Matsuda, Role of thromboxane receptor on the intracellular accumulation of cis-diamminedichloroplatinum(II) in non-small-cell but not in small-cell lung cancer cell lines, *Anticancer Res* 18 (1998) 1079-1084.
- [181] K. Kasahara, M. Fujimura, T. Bando, K. Shibata, H. Shirasaki, T. Matsuda, Modulation of sensitivity to cis-diamminedichloroplatinum (II) by thromboxane A₂ receptor antagonists in non-small-cell lung cancer cell lines, *Br J Cancer* 74 (1996) 1553-1558.

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Figure Legend:

Fig. 1 Generation of the prostanoids via the arachidonic acid pathway. Arachidonic acid is metabolized via one of three distinct signaling pathways; the cyclooxygenase (COX), the lipoxygenase (LOX), and the P-450 epoxygenase pathways. The COX-isoforms convert arachidonic acid to unstable cyclic endoperoxides PGG₂ and PGH₂, which are then further converted to the prostanoids via the activity of cell specific synthases.

Fig. 2 Downstream signaling pathways activated by prostacyclin and thromboxane A₂. A) Prostacyclin-activated signaling pathways. IP receptor activation generally leads to G_s coupling, although G_q can also be activated. G_s activation leads to downstream activation of PKA, which subsequently inhibits activation of the MAP kinase pathway (indicated by the red X) B) Thromboxane A₂-activated signaling pathways. Activation of the TP receptors generally leads to coupling with G_q, resulting in an increase in calcium release and PKC activation. In addition, the α -isoform can also couple to G_s and the β -isoform to G_i, resulting in directly opposing effects on adenylate cyclase activity (indicated by the red X) and subsequent contrasting actions on cAMP synthesis.

Fig. 3 It has been hypothesized that tumor development and progression may be modulated by the balance in the expression and activity of prostacyclin synthase, thromboxane synthase, and their down-stream prostanoid products. PGIS has been shown to be anti-tumor in a variety of cancer types, while a pro-tumor role for TXS has been frequently identified. Relative expression patterns of these enzymes in malignant tissue provides further evidence to support this theory.

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Fig. 4A Expression of PGIS in a retrospective panel of human tumor/normal matched protein samples. PGIS expression was absent or significantly reduced in both adenocarcinoma samples and squamous cell carcinoma samples, relative to matched normal controls (n=5/group).

Fig. 4B Expression of TXS in a retrospective panel of human tumor/normal matched protein samples. TXS expression was generally increased in adenocarcinoma samples and squamous cell carcinoma samples, relative to matched normal controls (n=5/group).

Fig. 5 The effect of selective TXS inhibition in tumor cell proliferation in NSCLC cell lines. A significant reduction in tumor cell proliferation/survival was observed in both adenocarcinoma (A-549; Fig. 5A) and squamous cell carcinoma (SKMES-1; Fig. 5B) cell lines following 24 h selective TXS inhibition with ozagrel (* $p < 0.05$ vs control, # $p < 0.001$ vs control; n=3).

Fig. 6 The effect of low-molecular weight heparin (LMWH) on TXS expression in A-549 cells. Treatment of A-549 cells with un-fractionated LMWH (concentration range: 0.25 U/mL, 0.5 U/mL, 1 U/mL) for 24 h led to a reduction in TXS expression, suggesting that this enzyme is a target for this class of anti-coagulant drugs.

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Fig. 1

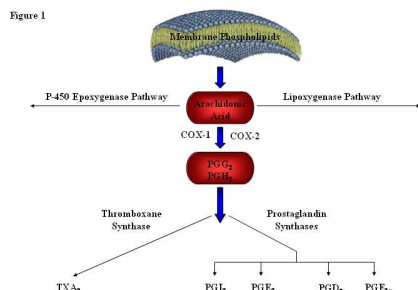
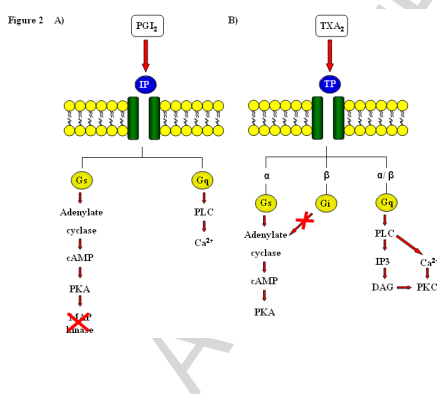


Fig. 2



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Fig. 3

Figure 3

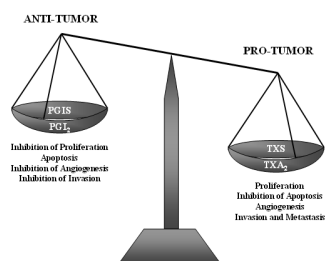


Fig. 4A

Figure 4

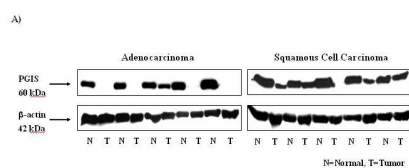
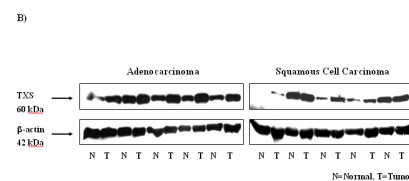


Fig. 4B

Figure 4



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Fig. 5

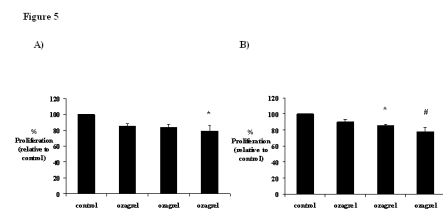


Fig. 6

