

Inhibition of Angiogenesis by Non-Steroidal Anti-Inflammatory Drugs: From the Bench to the Bedside and Back

Yan Monnier, Jelena Zaric and Curzio Rüegg*

Centre Pluridisciplinaire d'Oncologie (CePO), Faculty of Biology and Medicine, University of Lausanne, CH-1011 Lausanne, Switzerland and Swiss Institute for Experimental Cancer Research (ISREC), NCCR Molecular Oncology, CH-1066 Epalinges s/Lausanne, Switzerland

Abstract: The formation of new blood vessels, a process globally referred to as angiogenesis, occurs in a number of pathological conditions, such as cancer and chronic inflammation. Recent findings indicate that cyclooxygenase-2 (COX-2), the inducible form of the cyclooxygenase (COX) isoenzymes, acts as a potent inducer of angiogenesis. Non-steroidal anti-inflammatory drugs (NSAIDs) are classical inhibitors of COX enzymes, which are widely prescribed for the treatment of inflammation, pain and fever. Selective COX-2 inhibitors (COXIBs) have been subsequently developed with the purpose to improve the safety profile of this class of therapeutics. More recently, substantial preclinical evidence demonstrated that NSAIDs and COXIBs have anti-angiogenic properties. This newly recognized activity opens the possibility of using these drugs for the treatment of angiogenesis-dependent diseases. In this article we review the most recent advances in understanding the mechanisms by which NSAIDs and COXIBs suppress angiogenesis, and we discuss their potential clinical use as anti-angiogenic drugs.

Keywords: Angiogenesis, cancer, cell adhesion, cyclooxygenase, prostaglandins, signaling, chronic inflammation,

INTRODUCTION

Over the past decade, significant advances have been made in understanding the molecular and cellular events that regulate and mediate angiogenesis [1-3]. The great interest in vascular biology and angiogenesis research is mainly driven by the potential for therapeutic interventions in angiogenesis-dependent diseases such as cancer and chronic inflammation [4, 5]. Non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors (COXIBs) are widely prescribed drugs for the treatment of pain and inflammation. They inhibit the catalytic activity of the cyclooxygenase (COX) isoenzymes and thereby block the production of prostaglandins (PGs) and thromboxanes (TXs), the two main classes of lipid-derived pro-inflammatory molecules. Increasing evidence suggests that NSAIDs and COXIBs might also prove efficient as anti-angiogenic drugs [6]. Recent clinical and experimental studies have demonstrated that COX-2, the inducible isoform of COX, plays a major role in promoting angiogenesis. NSAIDs and COXIBs are currently viewed as potential anti-angiogenic agents for the prevention and treatment of benign as well as malignant conditions dependent on angiogenesis.

Despite the hype in the COX-2 field and the hope that NSAIDs and COXIBs may be used as safe and well tolerated anti-angiogenic drugs, many basic questions remain and new ones have emerged. In the first part of this article, we briefly review the cellular and molecular mechanisms of blood vessel formation, the biological function of COX-2, its pharmacological inhibition and the recent advances in understanding the role of COX-2 in angiogenesis. In the second part, we review and discuss the rationale for the clinical use of NSAIDs and COXIBs as therapeutic agents for the prevention and treatment of cancer and other chronic inflammatory disorders. We also discuss future directions of research, including strategies to monitor their putative anti-angiogenic activity in patients.

CELLULAR AND MOLECULAR MECHANISMS OF BLOOD VESSEL FORMATION

During embryonic development, vascular progenitor cells (i.e. the hemangioblasts) give rise to committed progenitors (the angioblasts), which then differentiate into endothelial cells to form a primitive vascular plexus through a process called vasculogenesis. The primitive plexus is then remodeled into a mature vascular system through sprouting, trimming, intussusception and hierarchical branching by a process globally referred to as angiogenesis [7]. Vascular maturation also involves the recruitment of perivascular cells, the pericytes, which provide factors for endothelial cell survival and confer physical stability to the vessel wall [8]. In the adult organism, vessels are largely maintained in a quiescent state by the effects of endogenous anti-angiogenic factors (e.g. thrombospondins, extracellular matrix-derived fragments). During postnatal angiogenesis this condition is reversed, and endothelial cells proliferate, migrate and

form new capillaries in response to angiogenic factors. In contrast to physiological angiogenesis, pathological blood vessels do not undergo full maturation. For instance, tumoral vessels frequently display incomplete endothelial cell lining and are often in direct contact with tumor cells [9]. They also show disorganized recruitment of pericytes [10] and remain highly permeable and unstable. Finally, they fail to generate a hierarchically branched vascular network [11].

Many extracellular and cell surface molecules mediating and modulating angiogenesis have been identified and characterized [12-14]. They include: 1) Growth factors and growth factor receptors, such as Vascular Endothelial Growth Factors (VEGFs) and VEGF-Receptors; 2) Adhesion molecules of the integrin and cadherin families; 3) Extracellular matrix proteins, such as fibronectin, collagens and laminins; 4) Remodeling molecules and receptors (e.g. Angiopoietins/Ties, Ephrins/Ephs); 5) Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs and PAIs).

BIOLOGICAL ROLE OF CYCLOOXYGENASE

COX, also known as prostaglandin H synthase, is a rate-limiting enzyme that catalyses the cyclooxygenase and peroxidase reactions in the conversion of arachidonic acid to PGH₂. Specific isomerases are then required to convert PGH₂ into a series of biologically active prostanoids, PGE₂, PGF₂, PGD₂, PGI₂, and Thromboxane (TX) A₂ [15]. There are two homologous cyclooxygenase isoenzymes encoded by two distinct genes: COX-1 and COX-2 [15]. These two COX isoforms differ in many respects. COX-1 is constitutively expressed in most tissues and plays an important role in homeostasis. In particular, it is implicated in maintaining the protective lining of the stomach mucosa, regulating the renal blood flow and mediating platelet aggregation at sites of vascular injury [16]. By contrast, COX-2 is absent from most normal tissues, except for some specific regions in the brain, in the kidney and in the uterus. COX-2 expression is rapidly induced by inflammatory cytokines, such as Tumor Necrosis Factor (TNF), and Interleukin (IL) -1 or -6, in leukocytes, in particular monocytes/macrophages, as well as in stromal, epithelial and endothelial cells. Numerous oncogenic mutations in many genes, including c-src, Ras, APC, p53 and STK11/Lkb1, also stimulate COX-2 transcription. Increased amounts of COX-2 are found commonly in both precancerous or cancerous tissues [17, 18]. Furthermore, COX-2 has been shown to be induced in response to chemotherapy and radiotherapy [19, 20]. This increase in COX-2 expression results in enhanced synthesis of PGs in neoplastic and inflamed tissues [16, 21-23]. COX-2 expression has been documented in most human cancers [24, 25]. Tumor cells, but also activated stromal fibroblasts, tumor infiltrating inflammatory cells and angiogenic endothelial cells can express COX-2 [26].

Although COX-1 was not associated with tumor progression and angiogenesis [16], and was shown in many experimental models not to promote tumor growth [27], it is worth noting that constitutive COX-1 expression and overexpression was recently reported in many cancers. COX-1 was found overexpressed in ovarian cancers [28, 29] in association with enhanced production of angiogenic factors [30]. Expression of COX-1 was documented in testicular cancers [31], in the stroma of human familial adenomatous polyposis polyps [32], in non-small

*Address correspondence to this author at the Laboratory of the CePO, Swiss Institute for Experimental Cancer Research (ISREC), 155 Chemin des Boveresses, CH-1066 Epalinges, Switzerland; Tel: +41 21 692 5853; Fax: +41 21 692 5872; E-mail: curzio.ruegg@isrec.ch

Table 1. List of Publications Describing the Anti-Angiogenic Effects of NSAIDs and COXIBs. The Reports on the Anti-Angiogenic Effects *In vitro* and *In vivo* in Animal Models, are Listed Here. This List is Not Exhaustive

NSAIDs	<i>In vivo</i> effects			<i>In vitro</i> effects
	Tumor / inflammation / tissue angiogenesis	Cornea / retinal angiogenesis	Matrigel / sponge implant angiogenesis	Proliferation, migration, apoptosis
Aspirin	Tsujii [58] Yoshida [147]		Yoshida [147]	Salcedo [86] Pearce [148] Shtivelband [149] Fierro [150]
Indomethacin	Majima [50] Sawaoka [151] Chang [55] Li [152] Rozic [153] Ghosh [154] Milas [155]	Yamada [156]	Rozic [153] Amano [157] Majima [51]	Dormond [52, 83] Majima [50] Jones [62] Pearce [148] Pai [158]
Ibuprofen	Skopinska-Rozewska [139] Farrell [159]			Jones [62] Szabo [160]
Diclofenac	Seed [161, 162] Freemantle [163]			Shen [164] Seed [162]
Piroxicam	Mohammed [165, 166]			Seed [161] Salcedo [86]
COXIBs				
NS398	Liu [167, 168] Li [152] Rozic [153] Ghosh [154] Yoshida [147]	Yamada [156]	Dormond [52] Majima [50] Amano [157] Sengupta [169] Majima [51] Yoshida [147]	Dormond [52, 83] Salcedo [86]
Celecoxib	Chang [55]	Masferrer [26] Leahy [77]		
Rofecoxib	Guo [170]	Wilkinson-Berka [171]		
Nimesulide	Emanueli [172]		Majima [50] Amano [157] Tamarat [173]	

cell lung carcinomas [33], osteoid osteomas [34] and in chemically-induced rat mammary tumors [35]. These recent results warrant a re-evaluation of the role of COX-1 in cancer progression.

Recently, an acetaminophen-sensitive COX isoform produced from the COX-1 gene by alternative exon usage, was reported and termed COX-3 [36]. Its putative role in cancer and other diseases has not been investigated yet.

CYCLOOXYGENASE INHIBITION BY NSAIDs AND COXIBs

Two classes of COX inhibitors are currently available for use in humans: pan-COX inhibitors, globally referred to as NSAIDs, and selective COX-2 inhibitors, or COXIBs. [37, 38]. NSAIDs inhibit both COX-1 and COX-2 but generally bind more effectively to the COX-2 active site. COXIBs have very little effects on COX-1, and thereby preferentially suppress PGs and TXs production at inflammatory sites. The therapeutic benefits of NSAIDs are due to the suppression of COX-2 activity in injured or inflamed tissues. On the other hand, all the NSAIDs-related complications such as gastrointestinal ulceration, platelet dysfunction and renal failure are mostly due to COX-1 inhibition in non-inflamed, healthy tissue. Approximately 1% of chronic NSAIDs users will develop serious and potentially lethal gastrointestinal complications during treatment. Patients that suffer from arthritis or other chronic inflammatory diseases are particularly at risk [39, 40].

COXIBs have been subsequently developed with the purpose to improve the safety profile of this class of therapeutics. Thus, COX-2

inhibitors were expected to retain the beneficial effects of classical NSAIDs, in particular the anti-inflammatory and analgesic activities, without the deleterious effects on gastrointestinal mucosa, renal plasma flow and platelet aggregation. However, while it is now demonstrated that prolonged COXIBs usage is associated with a significant reduction of gastrointestinal complications compared to NSAIDs [41-43], it appears that most of the undesired effects on kidney function are retained [43]. This observation is consistent with the recently reported constitutive expression of COX-2 in the renal medulla [44], and in the macula densa [45]. Since, in addition to the kidney, low level of COX-2 expression was observed in additional tissues and organs, such as the brain and the uterus [22, 46], it is conceivable that long-term administration of COXIBs may be associated with potential side effects in these organs.

Unanticipated severe thrombotic cardiovascular complications were observed in a rofecoxib-based study in patients with arthritis and musculoskeletal pain (the VIGOR, Vioxx Gastrointestinal Outcomes Research, Study, 8076 patients). The meta-analysis of this study, revealed that patients without coronary artery disease had a 2.38 greater risk of developing a thrombotic cardiovascular event (myocardial infarction, unstable angina, cardiac thrombus, cardiac arrest, sudden death, ischemic stroke, and transient ischemic attacks) under rofecoxib treatment compared to patients treated with NSAIDs [47]. In a similar study based on celecoxib (CLASS, Celecoxib Long-term Arthritis Safety, Study, 8059 patients), however, there was no significant difference in cardiovascular event (myocardial infarction, stroke, and death) rates between celecoxib and NSAIDs-treated patients. The reasons for this increased risk in the

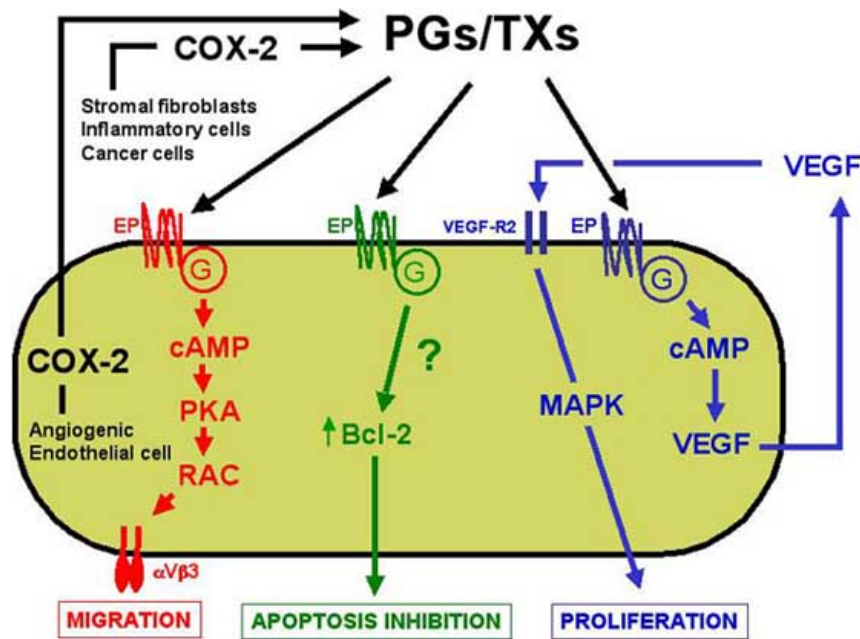


Fig. (1). Proposed integrative model for the role of COX-2 in angiogenesis. COX-2-derived prostaglandins and thromboxanes are released in the microenvironment of tumoral or inflamed tissues. Prostaglandins and thromboxanes promote angiogenesis by binding to E-prostane surface receptors (EP) on endothelial cells and stimulating signaling pathways that promote cell proliferation, migration and inhibition of apoptosis. See text for detailed description of pathways and NSAIDs/COXIBs effects.

Abbreviations: cAMP, cyclic adenosine monophosphate; COX, cyclooxygenase; EP, E-prostane receptor; MAPK, mitogen activated protein kinase; PG, prostaglandin; PKA, protein kinase A; TX, thromboxane; VEGF, vascular endothelial growth factor, VEGF-R2, VEGF-receptor-2;

VIGOR study are not fully clear and may be complex (including drug-specific effects) [48]. These data call for caution about the risk of cardiovascular events with COX-2 inhibitors. Therefore, long-term preventive treatments with COXIBs should not be performed outside well-designed clinical trials as long as clear benefits in relevant endpoints have not been demonstrated.

ROLE OF CYCLOOXYGENASE-2 IN ANGIOGENESIS

There is growing experimental evidence indicating that COX-2 and PGs promote angiogenesis, including tumor angiogenesis, and that NSAIDs and COXIBs inhibit this process [13, 49]. NSAIDs and COXIBs suppressed FGF-2-induced angiogenesis in a rat model of corneal and sponge implant angiogenesis as well as in the Matrigel plug model [26, 50-52]. Transgenic overexpression of COX-2 in basal epidermal cells enhanced tumor progression upon exposure to mutagenic agents [53], while overexpression of COX-2 in murine mammary cells caused breast tumor formation following multiple pregnancies [54]. In both models, tumor progression was associated with enhanced angiogenesis, while NSAIDs and COXIBs suppressed angiogenesis and tumor formation. Moreover, using the same model of transgenic mice over-expressing COX-2 in mammary glands, Chang *et al.* demonstrated a correlation between known angiogenic regulatory genes (VEGF, Ang2, Flt-1, Tie2) and COX-2 over-expression [55]. The reports in which anti-angiogenic activity of NSAIDs and COXIBs have been originally described are listed in Table 1.

The cellular and molecular mechanisms by which COX-2 promotes angiogenesis are being progressively uncovered. COX-2 stimulates angiogenesis through at least three main mechanisms: by inducing endothelial cell proliferation, by enhancing their survival and resistance to apoptosis, and by stimulating cell adhesion, spreading and migration (See Fig. (1)). In the following section we summarize the experimental evidences for the contribution of COX-2 in promoting angiogenesis.

COX-2 in Cellular Proliferation

Among the various growth factors implicated in blood vessels formation, Vascular Endothelial Growth Factor (VEGF) emerged as the most specific and the important one [3, 14, 56]. VEGF promotes endothelial cell proliferation, migration, and survival and causes a rapid increase in vascular permeability, all features associated with angiogenesis [57]. For example, VEGF is produced during cancer progression in response to tumor hypoxia and following activation of

oncogenes (e.g. Ras) or inhibition of tumor suppressor genes (e.g. p53, von Hippel Lindau protein). Recently, numerous reports have demonstrated that COX-2 activity and PGs production are important regulators of VEGF expression and biological function. Tsujii *et al.* have originally shown that COX-2 expression in colon cancer cells induced enhanced synthesis of VEGF in endothelial cells through an autocrine mechanism, and this effect was inhibited by NS-398, a COX-2 specific inhibitor [58]. This observation was confirmed in a large number of different tumor types (see Table 1). Mice lacking the COX-2 gene have deficient VEGF production while treatment of wild-type fibroblasts with a COX-2 inhibitor suppressed VEGF production [59]. Consistent with the idea that COX-2 regulates VEGF expression, exogenous administration of PGs increased VEGF production in many different cells [60, 61]. Moreover, Jones *et al.* demonstrated that the inhibition of COX-2 prevented the VEGF-R2 mediated activation of the mitogen activated protein kinase (MAPK) pathway, thereby inhibiting proliferation in endothelial cells [62]. Interestingly, it has been recently shown that VEGF itself can upregulate COX-2 expression in human endothelial cells [63]. This finding suggests a possible positive feedback mechanism between COX-2 and VEGF, reminiscent of a positive feed-back loop previously reported between COX-2/PGE₂ and EGF-R signaling. EGF stimulates COX-2 expression and PGE₂ production *via* EGF-R and ERK-p38 signaling [64]. In turn, PGE₂ causes EGF receptor transphosphorylation and enhances ERK activation and cell proliferation [65].

Based on these data, one can propose a general mechanism for the regulation of endothelial cell proliferation by COX-2 (See Fig. (1)). When COX-2 is induced within a tumoral or an inflamed tissue, increased PGs/TXs are generated and bind to cell surface receptors belonging to the family of seven-transmembrane G protein-coupled rhodopsin-type receptors, designed as EP1, EP2, EP3, EP4. Upon stimulation with PGs/TXs, these receptors induce changes in the level of second messengers (cAMP, free Ca⁺⁺) [66]. As a result, elevated levels of cAMP, a known downstream event of the EP2 and EP4 receptors activation, induce VEGF production. VEGF is then released within the tissue and acts through an autocrine/paracrine manner on endothelial cells through the VEGF-R2 (and VEGF-R1) mediated MAPK activation [1, 67].

COX-2 in Prevention of Apoptosis

There is significant evidence demonstrating that COX-2 expression suppresses apoptosis in many cell types and that COXIBs enhance it [68-70]. Most of the evidence for an anti-apoptotic effect of COX-2 comes from studies performed on cancer cell lines. For example, Mc Ginty *et al.*

demonstrated that induction of COX-2 expression in PC-12 pheochromocytoma cells prevented apoptosis induced by nerve growth factor withdrawal [71]. COX-2 was shown to protect cells against apoptosis through direct intracellular as well as autocrine or paracrine mechanisms [72]. Two molecular pathways involved in the anti-apoptotic effects of COX-2 have been described: the Bcl-2 and the nitric oxide (NO) pathways [72]. Sheng *et al.* demonstrated that COX-2 expression in colon cancer cells correlated with an increase in the expression of Bcl-2, the prototypic anti-apoptotic member of the Bcl family of proteins, decreased cytochrome-c release from mitochondria, and decreased caspase-9 activation, whereas treatment with COXIBs caused a decrease in Bcl-2 expression and increased apoptosis [73]. Using a differential expression array in the PC-12 cells, Chang *et al.* showed that COX-2 activity stimulated the expression of the dynein light chain and was followed by inhibition of neuronal nitric oxide synthase activity, thereby preventing apoptosis [74]. Furthermore, they showed that the nitric oxide (NO) pathway was downstream of PGE₂, as addition of exogenous PGE₂ reproduced the effect. In addition to the above mechanisms, a recent study reported that treatment of human prostate cancer cells with the COX-2-specific inhibitor celecoxib induced apoptosis by blocking activation of protein kinase B (PKB/Akt), a critical regulator of cell survival [75], independently of the effects on Bcl-2 [76]. At this point, while it appears clear that COX-2 inhibitors can induce endothelial cell apoptosis *in vivo* [77], the exact mechanisms involved remain mostly to be elucidated. In addition, it is possible that these mechanisms are cell specific. For instance, in contrast to PC-12 cells, NO is a potent survival factor for endothelial cells [78], and COXIBs-mediated inhibition of the PKB pathway may induce endothelial cell death by suppressing NOS-3 activity, a direct target of PKB [75], and by decreasing NO production [79].

COX-2 in Cell Motility and Adhesion

Vascular integrin adhesion molecules are important regulators of physiological as well as pathological angiogenesis. Integrins are cell surface heterodimeric receptors which bind to extracellular matrix proteins to provide the physical interaction necessary for cell adhesion, migration and positioning [80]. By interacting with the extracellular matrix, they also trigger signaling events, which, in concert with growth factor receptor-mediated signals, provide essential cues for cell survival, proliferation and differentiation [13, 81]. Among the known integrins, α V β 3 has emerged as one of the most critically involved in regulating tumor angiogenesis [81, 82].

Recently, COX-2 has been shown to play a role in endothelial cell migration and angiogenesis by regulating the function of the integrin α V β 3 [52]. The inhibition of COX-2 activity in endothelial cells by NSAIDs and COXIBs suppressed α V β 3-dependent endothelial cell spreading and migration *in vitro* and FGF-2-induced angiogenesis *in vivo* [52]. This positive effect of COX-2 on the α V β 3-spreading and migration was mediated by the activation of Cdc42 and Rac, two proteins belonging to the Rho family of GTPases that regulate cytoskeletal organization and cell migration. The NSAIDs and COXIBs effects were reversed by the administration of exogenous PGE₂. Furthermore, besides stimulating cell spreading, PGE₂ also accelerated α V β 3-mediated endothelial cell adhesion. The endothelial cells used in these experiments (i.e. HUVEC) expressed the PGE₂ receptors EP₂ and EP₄ and selective EP₂ and EP₄ receptor agonists (i.e. butaprost and PGE₁ alcohol, respectively), accelerated HUVEC adhesion and spreading with similar kinetics as observed for PGE₂. α V β 3-mediated adhesion (and PGE₂ stimulation) induced a transient rise in cAMP levels, and activation of protein kinase A (PKA). A cell permeable cAMP analogue 8-brcAMP accelerated adhesion, promoted Rac activation and cell spreading in the presence of COX-2 inhibitors, while a pharmacological inhibitor of PKA completely blocked α V β 3-mediated adhesion. A constitutive active Rac mutant (L61Rac) rescued α V β 3-dependent spreading in the presence of NS398, but did not accelerate adhesion, and a dominant negative Rac mutant (N17Rac) suppressed spreading without affecting adhesion. In contrast, α 5 β 1-mediated HUVEC adhesion, Rac activation and cell spreading were not affected by COX-2 inhibition [83].

Based on these results, a model was proposed to explain the effects of COX-2 on cell adhesion, spreading and migration (Fig. (1)). COX-2-derived PGE₂ binds to the EP₂ and EP₄ prostane receptors at the surface of the endothelial cells and thereby trigger the activation of adenylate cyclase. The cAMP burst that follows adenylate cyclase activation, accelerates α V β 3-mediated endothelial cell adhesion through a cAMP-PKA pathway and promotes α V β 3-dependent spreading via cAMP-PKA-dependent activation of Rac. Consistent with the notion that Rac has an

important function in angiogenesis, it was demonstrated that endothelial cell chemotaxis induced by VEGF required Rac activation [84], and that inhibition of its effector p21-activated kinase (PAK)-1 suppressed endothelial tube formation *in vitro* and angiogenesis *in vivo* [85].

Recently it has been reported that PGE₂ mediates the angiogenic effects of FGF-2 and VEGF in human microvascular endothelial cells by inducing the up-regulation of the chemokine receptor CXCR4 [86]. Inhibition of COX-2 in FGF-2 or VEGF-stimulated endothelial cells resulted in a decrease in CXCR4 expression. Since chemokines and chemokine-receptors stimulate integrin-mediated migration, this observation corroborates the role of COX-2 and PGE₂ in stimulating endothelial cell migration [86].

Finally, it is noteworthy that another COX-2 metabolite, TXA₂, was shown to be involved in endothelial cell migration and angiogenesis, although its role remains controversial. Daniel *et al.* demonstrated that a selective COX-2 antagonist inhibited TXA₂ expression, endothelial cell migration, and FGF-induced corneal angiogenesis. Moreover, endothelial cell migration and corneal vascularization were similarly inhibited by a TXA₂ receptor antagonist [87]. These effects were reversed by a TXA₂ agonist, regardless of the presence of a COX-2 inhibitor. In another study, however, TXA₂ was shown to inhibit endothelial cell migration and vascular tube formation [88]. The reason for these opposing effects of TXA₂ on angiogenesis remains unclear at this point.

USE OF NSAIDS AND COXIBS AS ANTI-ANGIOGENIC DRUGS

The fact that COX-2 acts as a potent inducer of angiogenesis, suggests the possible use of COX-2 inhibitors as anti-angiogenic agents. The epidemiological and clinical data showing the benefits of NSAIDs and COXIBs for the prevention and treatment of angiogenesis-related pathologies support this hypothesis. Most of the results obtained so far on this specific matter stemmed from cancer research. For this reason, we will mainly focus the following discussion on the rationale for the use of NSAIDs and COXIBs as anti-angiogenic agents in the treatment of tumoral conditions, and briefly mention their potential implication in the management of chronic inflammatory diseases.

Use of COX-2 inhibitors in Cancer Prevention and Treatment

Initial empirical evidence that NSAIDs have anti-cancer effects originated from epidemiological studies [89, 90]. These studies demonstrated that regular (i.e. 5 to 7 times per week) and prolonged (> 2 years) intake of Aspirin and other NSAIDs decreased the relative risk of developing colorectal cancer by approximately 50%. While the evidence for protection against cancers, in particular of the gastrointestinal tract (i.e. colorectal, stomach, and esophageal cancer) is quite convincing, data on possible protective effects against other cancers, in particular breast, prostate, lung, and skin are less conclusive. In 1993, a pioneering randomized, double-blind, placebo-controlled study performed on patients with Familial Adenomatous Polyposis (FAP) demonstrated that chronic administration of the NSAID sulindac caused a significant regression in the number and size of colonic and rectal adenomas [91]. The first direct demonstration that specific targeted inhibition of COX-2 may prevent development of colorectal cancer came later on from a study also performed in patients with FAP. Treatment with the COX-2 specific inhibitor celecoxib during a 6 months period, resulted in a significant reduction of the number and size of polyps in these patients [92, 93]. Based on those data, the logical extension of these studies was to assess whether COXIBs may protect against cancer development in patients with other premalignant lesions associated with COX-2 overexpression, such as bronchial metaplasia, Barrett esophagus, oral leukoplakia or dysplastic nevi. Several large randomized trials addressing these questions in high-risk populations are currently ongoing. In order to improve our understanding on the mechanisms associated with a positive or negative outcome, it will be important to associate these trials with biological, biochemical and molecular endpoints. In particular, drug effects on the tumor should be specifically monitored (see below). It is important to stress, that even if these trials give a positive result, long-term preventive treatments with COXIBs bear the risk of potential serious side effects and should therefore be clearly restricted to high-risk patients. It is noteworthy that, while COX-2 has well established pro-angiogenic effects, it is not possible to assess at this time how much of the protective activity of COX-2 inhibitors against human cancer is due to suppression of tumor-associated angiogenesis.

The observation that NSAIDs and COXIBs are effective as cancer-preventive agents, suggested the possibility that these drugs could also be

used for the treatment of established tumors. Indeed, COX-2 is over-expressed in most cancers [17, 94] and anti-cancer treatments can induce *de novo* expression or overexpression of COX-2 in cancer cell lines [23]. In preclinical experiments, inhibition of tumor growth was observed upon treatment with COXIBs alone (Reviewed in [95]). It will be unlikely, however, that similar dramatic effects will occur in humans. The potential therapeutic efficacy of COXIBs should therefore be primarily tested in combination with chemotherapy or radiotherapy. The fact that some chemotherapeutic agents (microtubule interfering drugs) and radiotherapy have been shown to induce COX-2 expression strengthens this approach and suggests that COXIBs might be used to prevent potential COX-2-related pro-tumoral side-effects [19, 20]. In contrast, the administration of COXIBs alone should be limited to studies addressing very specific questions (e.g. identification of surrogate markers and target genes in response to COX-2 inhibitions). Pre-clinical studies have shown that anti-angiogenic drugs given at different stages of tumor progression (i.e. pre-malignant lesions, rapidly expanding small tumors or large end-stage cancers) produce distinct efficacy profiles. Optimal response was seen in rapidly growing small tumors [96]. Should this also apply to COXIBs, then efficacy depends largely on the tumor stage. For this reason, it may be important that clinical trials are not restricted to advanced stage cancer only. Some of the ongoing clinical trials with COXIBs have been recently discussed [23, 97-99].

Use of COX-2 Inhibitors in Chronic Inflammatory Conditions

Apart from the fact that COXIBs might play a role in cancer prevention and treatment in the future, there are some data indicating that their anti-angiogenic properties could also represent an additional benefit in the treatment of chronic inflammatory disorders, such as in rheumatoid arthritis (RA). RA is a chronic systemic inflammatory disease of unknown origin that principally attacks the joints, producing a non-suppurative synovitis. Morphologically, RA is characterized by an inflammatory lesion in the synovial membrane that can progress to form a proliferative tissue, the pannus, that disrupt the joint and the adjacent bone. Increased vascular density owing to vasodilatation and angiogenesis is a hallmark of this condition [100, 101], and has been shown to play a key role in the development and progression of the disease. Indeed, studies performed on animal models of RA demonstrated that inhibition of angiogenesis, using $\alpha V\beta 3$ integrin antagonists or endostatin gene transfer, produced a decrease in inflammation and disease progression [102-104]. While neovascularisation in RA may be driven by the increased requirement of oxygen and nutrients from the proliferative synovial pannus, it is likely that the sustain production of angiogenic factors secondary to the inflammatory events in the synovium mainly drives the process. Since COX-2 is up-regulated in the RA synovium [105], one may assume that locally produced prostanoids may actively contribute to promote new vessels formation in the tissue. In support of this hypothesis, Woods *et al.* demonstrated that COX-2 expression in RA synovial fibroblasts and in microvascular endothelial cells promoted angiogenic activity in diseased synovium and that treatment of these cells with the selective COX-2 inhibitor rofecoxib inhibited this process [106]. Therefore, these results raise the question whether some of the beneficial effects of NSAIDs and COXIBs on RA may involve inhibition of angiogenesis. However, these drugs do not prevent pannus formation and have no curative effects on RA progression. This suggests that NSAIDs or COXIBs may not be sufficient on their own to inhibit angiogenesis in the synovium, or that inhibition of angiogenesis alone does not result in inhibition of disease progression. Whether anti-angiogenic agents, alone or in association with NSAIDs or COXIBs, may provide an efficient or additional control of neovascularization in RA and stop disease progression is an open question that deserves further investigation.

It has been recently shown that NSAIDs or COXIBs, similarly to specific anti-angiogenesis therapy (i.e. VEGF-R2 antagonist or vasostatin), had no significant impact on the healing of incisional wounds, dermal angiogenesis or recovery of tensile strength [107, 108]. These observations raise the possibility that stromal tissue-remodeling events may be less dependent on angiogenesis than cancer progression, and thereby are not, or significantly less affected by anti-angiogenic drugs.

MONITORING THE ANTI-ANGIOGENIC EFFECTS OF COXIBS IN PATIENTS

One main challenge in the field of angiogenesis, is to specifically monitor angiogenesis and the anti-angiogenic effects of a drug *in vivo*. This problem also exists for monitoring the anti-angiogenic activity of NSAIDs and COXIBs. To date, there are no *bona fide* validated molecular

or cellular markers to monitor angiogenesis or the drug-mediated inhibition of the process. Ideally, such surrogate markers should be specific for the drug and allow to measure the anti-angiogenic effects of COXIBs, independently of their anti-inflammatory effects. To this regard, it should be clarified that measuring PGE₂ levels in patients under COXIB therapy is not a direct way to monitor its anti-angiogenic effect. For example, in a recently published celecoxib phase II trial, patients with non-small cell lung cancer were treated preoperatively with chemotherapy and celecoxib. The increased PGE₂ levels found within tumor tissue were normalized after treatment with celecoxib (400 mg BID) [109]. This observation indicates that the COXIB dose used was sufficient to inhibit COX-2 activity at the target site and is therefore reassuring and gratifying, but it does not demonstrate any potential anti-angiogenic effects.

Many efforts are currently under way in many laboratories, including ours, to identify and validate surrogate markers of angiogenesis. The heterogeneity of the process of angiogenesis itself (many different molecules and mechanisms involved in vessel formation), the different degree of contribution of angiogenesis to individual pathologies, and the changes of angiogenesis-dependence during disease progression, indicate that the determination of several different parameters, rather than a single one, will be necessary to effectively assess angiogenesis in patients. We would like here to briefly review the different available approaches and tools that might prove useful in the future to identify such markers. For a more comprehensive review and discussion on this topic, we refer the reader to recently published articles [95, 110, 111].

Imaging-Based Techniques

They are commonly used for diagnostic purposes, as a baseline before therapy and to monitor response to treatment. Thanks to tremendous improvement in instruments, enhancing agents and data processing, it is now possible to monitor regional blood flow, regional blood volume, mean transit time, permeability and pO₂ [112, 113]. Since these methods have a sufficient spatial and temporal resolution, they are ideal candidates for monitoring tissue perfusion and drug efficacy in clinical trials.

Contrast-enhanced dynamic Magnet Resonance Imaging (CED-MRI) and computer tomography (CED-CT) are already used to these purposes. CED-MRI was used to correlate immunohistological data and clinical outcome. In the vast majority of the cases, a good correlation with immunohistological assessment of tumor angiogenesis was observed [114]. In some situations CED-MRI analysis was a better predictor of disease outcome than histological evaluation of angiogenesis [115] or of circulating VEGF levels [116]. CED-CT is routinely used to measure cerebral blood flow, blood volume, mean transit time and vascular permeability in patients after ischemic brain lesions [117]. In a recent paper it was reported that tissue VEGF, microvascular density (MVD) and lymph node involvement in patients with lung adenocarcinoma correlated well with perfusion data obtained by CED-CT [118].

Nuclear medicine techniques, such as position emission tomography (PET) and Single Photon Emission Computer Tomography (SPECT) can also be used to study tissue blood flow, blood volume and vascular permeability. Different nuclides with different chemico-physical properties are available to monitor blood flow (e.g. ¹⁵O-labelled water) or blood volume (e.g. ^{94m}Tc-labelled erythrocytes) [119]. Furthermore, ¹⁸F-deoxyglucose (FDG) is a validated tracer to monitor cellular metabolism in tumors and is a very sensitive technique to detect metabolic changes in response to therapy.

Ultrasound-based techniques have been used to investigate tumor angiogenesis in animals [120] and patients [121]. The improvements in the sensitivity and resolution of ultrasonography equipment, the coupling with Doppler technology (i.e. 'Power Doppler') and the introduction of intravenous contrast agents and harmonic imaging analysis, made it possible to efficiently visualize vessels and monitor blood flow in many organs and tissues, including tumors [122, 123]. Power Doppler ultrasonography was used to monitor tumor perfusion in diverse primary solid cancers. For example, the determined vascular index correlated well with MVD in early breast cancer [121] or with MVD, lymph-node and distant metastasis in rectal carcinoma [124].

In conclusion, imaging-based techniques represent the most advanced and promising strategy to the non-invasive measurement and quantification of tumor angiogenesis.

Cellular Markers

Circulating vascular endothelial cells of putative vessel wall origin can be detected in peripheral blood of healthy individuals [125]. In humans,

increased frequency of circulating endothelial cells (CEC) were reported in patients with cardiovascular disorders (e.g. acute coronary syndromes and chronic venous insufficiency) [126, 127] as well as in breast cancer and lymphoma patients [128]. Recently, bone marrow-derived circulating endothelial cell progenitors (ECP) were detected in the peripheral blood of tumor-bearing mice and were shown to actively contribute to tumor angiogenesis and to tumor progression in the same animals [129, 130]. To date, however, it is not clear whether the same mobilization occurs in human cancer. If this was the case, ECP may represent a unique 'diagnostic window' to monitor the angiogenic status of a patient before and during anti-angiogenic therapy.

Biochemical and Molecular Markers

Serum or plasma levels of many vascular growth factors (e.g. VEGF, FGF-2 or HGF) were shown to have prognostic value and to correlate with disease progression in many tumors (reviewed in [95]). Except for measurement of urine FGF-2 to monitor the treatment of infantile hemangiomas with IFN α [131], circulating vascular growth factors turned out to be useless as markers of drug activity or efficacy in solid tumors [132].

Circulating cell surface vascular molecules released by proliferating endothelial cells, such as cell adhesion molecules, growth factor receptors or fragments of matrix/perivascular proteins, may be valuable indicators of active angiogenesis [133]. The circulating levels of several molecules have already been investigated, and preliminary results indicate that some of them may be potential candidate as surrogate markers of angiogenesis. For example, elevated levels of soluble VEGF-R1/Flt-1 (sFlt-1) were detected in the serum of colorectal and breast cancer patients [134]. Increasing levels of soluble VCAM-1 (sVCAM-1) were found in serum of women with progressing advanced breast cancer compared to women with stable disease [135].

FUTURE PERSPECTIVES

The research developments and achievements in the understanding of the role of COX-2 and prostaglandins in promoting angiogenesis and the detailed characterization of molecular effects of NSAIDs and COXIBs on cellular signaling pathways suggest some possible future developments.

Inhibition of PGE₂

Using human head and neck xenograft tumors expressing COX-2 and producing high levels of PGE₂, Zweifel *et al.* recently showed that specific inhibition of PGE₂ activity by a neutralizing antibody mimicked the anti-tumor activity observed after celecoxib treatment [27]. This result demonstrates that inhibition of PGE₂ by neutralizing antibodies could be an alternative way to suppress COX-2 mediated angiogenic and tumor-promoting activity.

Inhibition of PG Receptors

It was recently reported that EP-3-deficient mice have deficient tumor angiogenesis and reduced tumor growth compared to wild type mice, whereas an EP-3 receptor agonist enhanced angiogenesis in a sponge model [136]. This defect was associated with impaired production of VEGF by host cells. EP-2-deficient mice also had decreased angiogenesis, while mice deficient for other PGs receptors had normal angiogenic activities [136]. These results open the possibility of interfering with EP receptor function to counteract the angiogenic and PGs-dependent effects of COX-2. Since there are eight different PGs receptors (four PGE₂ receptors) [137], it may be possible to selectively target EP expressed on angiogenic endothelial cells as alternative to COXIBs, thereby circumventing the potential side effects associated with the inhibition of COX-2 dependent PGs synthesis in healthy tissues.

Non-COX-2 COXIB targets

While it is clear that COX-2 promotes angiogenesis and tumor progression, and that inhibition of COX-2 suppresses them, there is growing evidence suggesting that some of the NSAID/COXIB effects are COX-2 independent. For example, NSAIDs suppressed proliferation and soft agar colony formation of Ha-Ras- and/or SV40-COX-deficient transformed cells [138]. Exisulind, a sulindac metabolite lacking COX-blocking activity, inhibited tumor growth in mice [139]. Several mechanisms have been proposed to explain these COX-independent effects, such as inhibition of expression of the anti-apoptotic protein Bcl-X_L [140], suppressed activation of I- κ B kinase β and Nuclear Factor- κ B [141], downregulation of Protein Kinase C- β 1 expression and activity

[142], decreased expression of cyclin A, cyclin B1 and cdk-1, increased expression of p21^{WAF1} and p27^{KIP1} [143] and inhibition of PKB activation [76]. Ibuprofen mediated COX-2-independent reduction of hypoxia-inducible factors HIF-1 α and HIF-2 α in prostate cancer cells [144].

It should be emphasized that the doses of COXIBs used to achieve these COX-2 independent effects, however, were significantly higher (50-100 μ M) than the doses that can be safely reached in humans or experimental animal models (5-10 μ M). Molecular modeling of these compounds revealed that the structural requirements for these pro-apoptotic effects were different from those for COX-2 inhibition. These findings suggest the possibility of synthesizing new apoptosis-inducing compounds based on existing COX-2 inhibitors [145, 146].

CONCLUSIVE REMARKS

There is convincing evidence indicating that COX-2 plays an important role in promoting angiogenesis in benign, premalignant and malignant conditions. Inhibition of COX-2 represents an attractive approach for the prevention and treatment of angiogenesis-dependent diseases. Preclinical experiments have shown that the anti-tumor activity of NSAIDs and COXIBs is due, at least in part, to the suppression of tumor angiogenesis. Clinical trials for the evaluation of their potential therapeutic effects in human cancer are under way. Many questions, however, remain open and new ones have emerged. For instance it will be important to associate ongoing interventional trials with techniques allowing evaluation of the anti-angiogenic effects of these drugs, or to identify surrogate molecular markers to monitor the anti-vascular activity of COXIBs in patients. Recent advances in the field have also opened the road to new avenues of research, such as the development of EP receptor antagonists allowing selective targeting of angiogenic vessels, or the design of COXIBs-derived drugs that inhibit novel, non-COX-2 targets. Thus, after many years of successful preclinical and clinical research on NSAIDs and COXIBs and their widespread use as anti-inflammatory drugs in daily practice, time has come to go back to the laboratory again to further characterize the anti-angiogenic properties of this exciting class of drugs.

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ABBREVIATIONS

cAMP	=	Cyclic adenosine monophosphate
COX	=	Cyclooxygenase
EP	=	E-Prostane receptor
MAPK	=	Mitogen activated protein kinase
PG	=	Prostaglandin
PKA	=	Protein kinase A
TX	=	Thromboxane
VEGF	=	Vascular endothelial growth factor
VEGF-R2	=	VEGF-receptor-2

REFERENCES

- [1] Carmeliet, P. *Nat. Med.*, **2000**, *6*, 389.
- [2] Carmeliet, P. *Nat. Med.*, **2003**, *9*, 653.
- [3] Yancopoulos, G.D.; Davis, S.; Gale, N.W.; Rudge, J.S.; Wiegand, S.J.; Holash, J. *Nature*, **2000**, *407*, 242.
- [4] Folkman, J. *Nat. Med.*, **1995**, *1*, 27.
- [5] Folkman, J. *Semin. Oncol.*, **2002**, *29*, 15.
- [6] Gately, S. *Cancer Metastasis Rev.*, **2000**, *19*, 19.
- [7] Risau, W. *Nature*, **1997**, *386*, 671.
- [8] Jain, R.K. *Nat. Med.*, **2003**, *9*, 685.
- [9] Chang, Y.S.; di Tomaso, E.; McDonald, D.M.; Jones, R.; Jain, R.K.; Munn, L.L. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 14608.
- [10] Morikawa, S.; Baluk, P.; Kaidoh, T.; Haskell, A.; Jain, R.K.; McDonald, D.M. *Am. J. Pathol.*, **2002**, *160*, 985.
- [11] Eberhart, C.E.; Coffey, R.J.; Radhika, A.; Giardiello, F.M.; Ferrenbach, S.; DuBois, R.N. *Gastroenterology*, **1994**, *107*, 1183.
- [12] Cavallaro, U.; Christofori, G. *J. Neuro. Oncol.*, **2000**, *50*, 63.
- [13] Ruegg, C.; Mariotti, A. *Cell Mol. Life Sci.*, **2003**, in press.

- [14] Conway, E.M.; Collen, D.; Carmeliet, P. *Cardiovasc. Res.*, **2001**, *49*, 507.
- [15] Vane, J.R.; Botting, R.M. *Inflamm. Res.*, **1998**, *47* Suppl. 2, S78.
- [16] Dubois, R.N.; Abramson, S.B.; Crofford, L.; Gupta, R.A.; Simon, L.S.; Van De Putte, L.B.; Lipsky, P.E. *FASEB J.*, **1998**, *12*, 1063.
- [17] Williams, C.S.; Mann, M.; DuBois, R.N. *Oncogene*, **1999**, *18*, 7908.
- [18] Tsuji, S.; Tsujii, M.; Kawano, S.; Hori, M. *J. Exp. Clin. Cancer Res.*, **2001**, *20*, 117.
- [19] Subbaramaiah, K.; Hart, J.C.; Norton, L.; Dannenberg, A.J. *J. Biol. Chem.*, **2000**, *275*, 14838.
- [20] Steinauer, K.K.; Gibbs, I.; Ning, S.; French, J.N.; Armstrong, J.; Knox, S.J. *Int. J. Radiat. Oncol. Biol. Phys.*, **2000**, *48*, 325.
- [21] Simmons, D.L.; Levy, D.B.; Yannoni, Y.; Erikson, R.L. *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 1178.
- [22] Vane, J.R.; Bakhle, Y.S.; Botting, R.M. *Annu. Rev. Pharmacol. Toxicol.*, **1998**, *38*, 97.
- [23] Subbaramaiah, K.; Dannenberg, A.J. *Trends Pharmacol. Sci.*, **2003**, *24*, 96.
- [24] Koki, A.T.; Masferrer, J.L. *Cancer Control*, **2002**, *9*, 28.
- [25] Koki, A.T.; Khan, N.K.; Woerner, B.M.; Seibert, K.; Harmon, J.L.; Dannenberg, A.J.; Soslow, R.A.; Masferrer, J.L. *Prost. Leukotr. Ess.*, **2002**, *66*, 13.
- [26] Masferrer, J.L.; Leahy, K.M.; Koki, A.T.; Zweifel, B.S.; Settle, S.L.; Woerner, B.M.; Edwards, D.A.; Flickinger, A.G.; Moore, R.J.; Seibert, K. *Cancer Res.*, **2000**, *60*, 1306.
- [27] Zweifel, B.S.; Davis, T.W.; Ornberg, R.L.; Masferrer, J.L. *Cancer Res.*, **2002**, *62*, 6706.
- [28] Li, S.; Miner, K.; Fannin, R.; Carl Barrett, J.; Davis, B.J. *Gynecol. Oncol.*, **2004**, *92*, 622.
- [29] Sales, K.J.; Katz, A.A.; Howard, B.; Soeters, R.P.; Millar, R.P.; Jabbour, H.N. *Cancer Res.*, **2002**, *62*, 424.
- [30] Gupta, R.A.; Tejada, L.V.; Tong, B.J.; Das, S.K.; Morrow, J.D.; Dey, S.K.; DuBois, R.N. *Cancer Res.*, **2003**, *63*, 906.
- [31] Hase, T.; Yoshimura, R.; Matsuyama, M.; Kawahito, Y.; Wada, S.; Tsuchida, K.; Sano, H.; Nakatani, T. *Eur. J. Cancer*, **2003**, *39*, 2043.
- [32] Takeda, H.; Sonoshita, M.; Oshima, H.; Sugihara, K.; Chulada, P.C.; Langenbach, R.; Oshima, M.; Taketo, M.M. *Cancer Res.*, **2003**, *63*, 4872.
- [33] Hasturk, S.; Kemp, B.; Kalapurakal, S.K.; Kurie, J.M.; Hong, W.K.; Lee, J.S. *Cancer*, **2002**, *94*, 1023.
- [34] Mungo, D.V.; Zhang, X.; O'Keefe, R.J.; Rosier, R.N.; Puzas, J.E.; Schwarz, E.M. *J. Orthop. Res.*, **2002**, *20*, 159.
- [35] Jang, T.J.; Jung, H.G.; Jung, K.H.; O, M.K. *Int. J. Exp. Pathol.*, **2002**, *83*, 173.
- [36] Chandrasekharan, N.V.; Dai, H.; Roos, K.L.; Evanson, N.K.; Tomsik, J.; Elton, T.S.; Simmons, D.L. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 13926.
- [37] Marnett, L.J.; Rowlinson, S.W.; Goodwin, D.C.; Kalgutkar, A.S.; Lanzo, C.A. *J. Biol. Chem.*, **1999**, *274*, 22903.
- [38] DeWitt, D.L. *Mol. Pharmacol.*, **1999**, *55*, 625.
- [39] Dubois, R.W.; Melmed, G.Y.; Henning, J.M.; Laine, L. *Aliment. Pharmacol. Ther.*, **2004**, *19*, 197.
- [40] Wolfe, M.M. *Int. J. Clin. Pract. Suppl.*, **2003**, *32*.
- [41] Emery, P. *Am. J. Med.*, **2001**, *110*, 42S.
- [42] Buttgerit, F.; Burmester, G.R.; Simon, L.S. *Am. J. Med.*, **2001**, *110* Suppl. 3A, 13S.
- [43] Schnitzer, T.J. *Am. J. Med.*, **2001**, *110*, 46S.
- [44] Yang, T. *Acta Physiol. Scand.*, **2003**, *177*, 417.
- [45] Harris, R.C. Jr. *Am. J. Cardiol.*, **2002**, *89*, 10D.
- [46] Dannhardt, G.; Kiefer, W. *Eur. J. Med. Chem.*, **2001**, *36*, 109.
- [47] Mukherjee, D.; Nissen, S.E.; Topol, E.J. *JAMA*, **2001**, *286*, 954.
- [48] White, W.B.; Faich, G.; Whelton, A.; Maurath, C.; Ridge, N.J.; Verburg, K.M.; Geis, G.S.; Lefkowitz, J.B. *Amer. J. Cardiol.*, **2002**, *89*, 425.
- [49] Ruegg, C.; Dormond, O.; Mariotti, A. *Biochim. Biophys. Acta Rev. Cancer*, **2004**, *1654*, 51.
- [50] Majima, M.; Hayashi, I.; Muramatsu, M.; Katada, J.; Yamashina, S.; Katori, M. *Br. J. Pharmacol.*, **2000**, *130*, 641.
- [51] Majima, M.; Isono, M.; Ikeda, Y.; Hayashi, I.; Hatanaka, K.; Harada, Y.; Katsumata, O.; Yamashina, S.; Katori, M.; Yamamoto, S. *Jpn. J. Pharmacol.*, **1997**, *75*, 105.
- [52] Dormond, O.; Foletti, A.; Paroz, C.; Ruegg, C. *Nat. Med.*, **2001**, *7*, 1041.
- [53] Muller-Decker, K.; Neufang, G.; Berger, I.; Neumann, M.; Marks, F.; Furstenberger, G. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 12483.
- [54] Liu, C.H.; Chang, S.H.; Narko, K.; Trifan, O.C.; Wu, M.T.; Smith, E.; Haudenschild, C.; Lane, T.F.; Hla, T. *J. Biol. Chem.*, **2001**, *276*, 18563.
- [55] Chang, S.H.; Liu, C.H.; Conway, R.; Han, D.K.; Nithipatikom, K.; Trifan, O.C.; Lane, T.F.; Hla, T. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 591.
- [56] Ferrara, N.; Gerber, H.P.; Lecouter, J. *Nat. Med.*, **2003**, *9*, 669.
- [57] Ferrara, N. *Nat. Rev. Cancer*, **2002**, *2*, 795.
- [58] Tsujii, M.; Kawano, S.; Tsuji, S.; Sawaoka, H.; Hori, M.; DuBois, R.N. *Cell*, **1998**, *93*, 705.
- [59] Williams, C.S.; Tsujii, M.; Reese, J.; Dey, S.K.; DuBois, R.N. *J. Clin. Invest.*, **2000**, *105*, 1589.
- [60] Hoper, M.M.; Voelkel, N.F.; Bates, T.O.; Allard, J.D.; Horan, M.; Shepherd, D.; Tuder, R.M. *Am. J. Respir. Cell Mol. Biol.*, **1997**, *17*, 748.
- [61] Ben-Av, P.; Crofford, L.J.; Wilder, R.L.; Hla, T. *FEBS Lett.*, **1995**, *372*, 83.
- [62] Jones, M.K.; Wang, H.; Peskar, B.M.; Levin, E.; Itani, R.M.; Sarfeh, I.J.; Tarnawski, A.S. *Nat. Med.*, **1999**, *5*, 1418.
- [63] Tamura, M.; Sebastian, S.; Gurates, B.; Yang, S.; Fang, Z.; Bulun, S.E. *J. Clin. Endocrinol. Metab.*, **2002**, *87*, 3504.
- [64] Huh, Y.H.; Kim, S.H.; Kim, S.J.; Chun, J.S. *J. Biol. Chem.*, **2003**, *278*, 9691.
- [65] Pai, R.; Soreghan, B.; Szabo, I.L.; Pavelka, M.; Baatar, D.; Tarnawski, A.S. *Nat. Med.*, **2002**, *8*, 289.
- [66] Coleman, R.A.; Smith, W.L.; Narumiya, S. *Pharmacol. Rev.*, **1994**, *46*, 205.
- [67] Autiero, M.; Waltenberger, J.; Communi, D.; Kranz, A.; Moons, L.; Lambrechts, D.; Kroll, J.; Plaisance, S.; De Mol, M.; Bono, F.; Kliche, S.; Fellbrich, G.; Ballmer-Hofer, K.; Maglione, D.; Mayr-Beyrl, U.; Dewerchin, M.; Dombrowski, S.; Stanimirovic, D.; Van Hummelen, P.; Dehio, C.; Hicklin, D.J.; Persico, G.; Herbert, J.M.; Shibuya, M.; Collen, D.; Conway, E.M.; Carmeliet, P. *Nat. Med.*, **2003**, *9*, 936.
- [68] Li, M.; Wu, X.; Xu, X.C. *Int. J. Cancer*, **2001**, *93*, 218.
- [69] Sawaoka, H.; Kawano, S.; Tsuji, S.; Tsujii, M.; Gunawan, E.S.; Takei, Y.; Nagano, K.; Hori, M. *Am. J. Physiol.*, **1998**, *274*, G1061.
- [70] Ding, X.Z.; Tong, W.G.; Adrian, T.E. *Anticancer Res.*, **2000**, *20*, 2625.
- [71] McGinty, A.; Chang, Y.W.; Sorokin, A.; Bokemeyer, D.; Dunn, M.J. *J. Biol. Chem.*, **2000**, *275*, 12095.
- [72] Cao, Y.; Prescott, S.M. *Journal of Cellular Physiology*, **2002**, *190*, 279.
- [73] Sheng, H.; Shao, J.; Morrow, J.D.; Beauchamp, R.D.; DuBois, R.N. *Cancer Res.*, **1998**, *58*, 362.
- [74] Chang, Y.W.; Jakobi, R.; McGinty, A.; Foschi, M.; Dunn, M.J.; Sorokin, A. *Mol. Cell Biol.*, **2000**, *20*, 8571.
- [75] Brazil, D.P.; Hemmings, B.A. *Trends Biochem. Sci.*, **2001**, *26*, 657.
- [76] Hsu, A.L.; Ching, T.T.; Wang, D.S.; Song, X.; Rangnekar, V.M.; Chen, C.S. *J. Biol. Chem.*, **2000**, *275*, 11397.
- [77] Leahy, K.M.; Ornberg, R.L.; Wang, Y.; Zweifel, B.S.; Koki, A.T.; Masferrer, J.L. *Cancer Res.*, **2002**, *62*, 625.
- [78] Dimmeler, S.; Zeiher, A.M. *Cell Death Differ.*, **1999**, *6*, 964.
- [79] Cooke, J.P. *Atheroscler. Suppl.*, **2003**, *4*, 53.
- [80] Hynes, R.O. *Cell*, **1992**, *69*, 11.
- [81] Eliceiri, B.P.; Cheresch, D.A. *Curr. Opin. Cell Biol.*, **2001**, *13*, 563.
- [82] Brooks, P.C.; Clark, R.A.; Cheresch, D.A. *Science*, **1994**, *264*, 569.
- [83] Dormond, O.; Bezzi, M.; Mariotti, A.; Ruegg, C. *J. Biol. Chem.*, **2002**, *277*, 45838.
- [84] Soga, N.; Connolly, J.O.; Chellaiah, M.; Kawamura, J.; Hruska, K.A. *Cell Comm. Adhes.*, **2001**, *8*, 1.
- [85] Kioussis, W.B.; Hood, J.; Yang, S.; Gerritsen, M.E.; Cheresch, D.A.; Alderson, N.; Schwartz, M.A. *Circ. Res.*, **2002**, *90*, 697.
- [86] Salcedo, R.; Zhang, X.; Young, H.A.; Michael, N.; Wasserman, K.; Ma, W.H.; Martins-Green, M.; Murphy, W.J.; Oppenheim, J.J. *Blood*, **2003**, *102*, 1966.
- [87] Daniel, T.O.; Liu, H.; Morrow, J.D.; Crews, B.C.; Marnett, L.J. *Cancer Res.*, **1999**, *59*, 4574.
- [88] Ashton, A.W.; Yokota, R.; John, G.; Zhao, S.; Suadicani, S.O.; Spray, D.C.; Ware, J.A. *J. Biol. Chem.*, **1999**, *274*, 35562.
- [89] Baron, J.A.; Sandler, R.S. *Annu. Rev. Med.*, **2000**, *51*, 511.
- [90] Giovannucci, E. *Biomed. Pharmacother.*, **1999**, *53*, 303.
- [91] Giardiello, F.M.; Hamilton, S.R.; Krush, A.J.; Piantadosi, S.; Hyland, L.M.; Celano, P.; Booker, S.V.; Robinson, C.R.; Offerhaus, G.J. *N. Engl. J. Med.*, **1993**, *328*, 1313.
- [92] Steinbach, G.; Lynch, P.M.; Phillips, R.K.; Wallace, M.H.; Hawk, E.; Gordon, G.B.; Wakabayashi, N.; Saunders, B.; Shen, Y.; Fujimura, T.; Su, L.K.; Levin, B. *N. Engl. J. Med.*, **2000**, *342*, 1946.
- [93] Phillips, R.K.; Wallace, M.H.; Lynch, P.M.; Hawk, E.; Gordon, G.B.; Saunders, B.P.; Wakabayashi, N.; Shen, Y.; Zimmerman, S.; Godio, L.; Rodrigues-Bigas, M.; Su, L.K.; Sherman, J.; Kelloff, G.; Levin, B.; Steinbach, G.; Group, F.A.P.S. *Gut*, **2002**, *50*, 857.
- [94] Soslow, R.A.; Dannenberg, A.J.; Rush, D.; Woerner, B.M.; Khan, K.N.; Masferrer, J.; Koki, A.T. *Cancer*, **2000**, *89*, 2637.
- [95] Ruegg, C.; Meuwly, J.Y.; Driscoll, R.; Werfelli, P.; Zaman, K.; Stupp, R. *Curr. Mol. Med.*, **2003**, *3*, 673.
- [96] Bergers, G.; Javaherian, K.; Lo, K.M.; Folkman, J.; Hanahan, D. *Science*, **1999**, *284*, 808.
- [97] Xu, X.C. *Anticancer Drugs*, **2002**, *13*, 127.
- [98] Stratton, M.S.; Alberts, D.S. *Oncology (Huntington)*, **2002**, *16*, 37.
- [99] Reddy, B.S.; Rao, C.V. *J. Environ. Pathol. Toxicol. Oncol.*, **2002**, *21*, 155.
- [100] Koch, A.E. *Ann. Rheum. Dis.*, **2003**, *62*, ii60.
- [101] Walsh, D.A.; Haywood, L. *Curr. Opin. Investig. Drugs*, **2001**, *2*, 1054.
- [102] Yin, G.; Liu, W.; An, P.; Li, P.; Ding, L.; Planelles, V.; Schwarz, E.M.; Min, W. *Mol. Ther.*, **2002**, *5*, 547.
- [103] Wilder, R.L. *Ann. Rheum. Dis.*, **2002**, *61* Suppl. 2, ii96.
- [104] Storgard, C.M.; Stupack, D.G.; Jonczyk, A.; Goodman, S.L.; Fox, R.L.; Cheresch, D.A. *J. Clin. Invest.*, **1999**, *103*, 47.

- [105] Siegle, I.; Klein, T.; Backman, J.T.; Saal, J.G.; Nusing, R.M.; Fritz, P. *Arthritis Rheum.*, **1998**, *41*, 122.
- [106] Woods, J.M.; Mogollon, A.; Amin, M.A.; Martinez, R.J.; Koch, A.E. *Exp. Mol. Pathol.*, **2003**, *74*, 282.
- [107] Roman, C.D.; Choy, H.; Nanne, L.; Riordan, C.; Parman, K.; Johnson, D.; Beauchamp, R.D. *J. Surg. Res.*, **2002**, *105*, 43.
- [108] Lange-Asschenfeldt, B.; Velasco, P.; Streit, M.; Hawighorst, T.; Pike, S.E.; Tosato, G.; Detmar, M. *J. Invest. Dermatol.*, **2001**, *117*, 1036.
- [109] Altorki, N.K.; Keresztes, R.S.; Port, J.L.; Libby, D.M.; Korst, R.J.; Flieder, D.B.; Ferrara, C.A.; Yankelevitz, D.F.; Subbaramaiah, K.; Pasmantier, M.W.; Dannenberg, A.J. *J. Clin. Oncol.*, **2003**, *21*, 2645.
- [110] Kerbel, R.; Folkman, J. *Nat. Rev. Cancer*, **2002**, *2*, 727.
- [111] Folkman, J.; Browder, T.; Palmblad, J. *Thromb. Haemost.*, **2001**, *86*, 23.
- [112] Anderson, H.; Price, P.; Blomley, M.; Leach, M.O.; Workman, P.; The Cancer Research Campaign, P.K.P.D.T.A.C. *Br. J. Cancer*, **2001**, *85*, 1085.
- [113] Folkman, J.; Beckner, K. *Acad. Radiol.*, **2000**, *7*, 783.
- [114] Padhani, A.R. *J. Magn. Reson. Imaging*, **2002**, *16*, 407.
- [115] Hawighorst, H.; Weikel, W.; Knapstein, P.G.; Knopp, M.V.; Zuna, I.; Schonberg, S.O.; Vaupel, P.; van Kaick, G. *Clin. Cancer Res.*, **1998**, *4*, 2305.
- [116] George, M.L.; Dzik-Jurasz, A.S.; Padhani, A.R.; Brown, G.; Tait, D.M.; Eccles, S.A.; Swift, R.I. *Br. J. Surg.*, **2001**, *88*, 1628.
- [117] Cuenod, C.A.; Leconte, I.; Siaue, N.; Frouin, F.; Dromain, C.; Clement, O.; Frija, G. *Acad. Radiol.*, **2002**, *9*, S205.
- [118] Tateishi, U.; Nishihara, H.; Watanabe, S.; Morikawa, T.; Abe, K.; Miyasaka, K. *J. Computer Assist. Tomography*, **2001**, *25*, 23.
- [119] Blankenbreg, F.G.; Eckelman, W.C.; Strauss, H.W.; Welch, M.J.; Alavi, A.; Anderson, C.; Bacharach, S.; Blasberg, R.G.; Graham, M.M.; Weber, W. *Acad. Radiol.*, **2000**, *7*, 851.
- [120] Denis, F.; Bougnoux, P.; de Poncheville, L.; Prat, M.; Catroux, R.; Tranquart, F. *Ultrasound Med. Biol.*, **2002**, *28*, 431.
- [121] Yang, W.T.; Tse, G.M.; Lam, P.K.; Metreweli, C.; Chang, J. *J. Ultrasound Med.*, **2002**, *21*, 1227.
- [122] Ferrara, K.W.; Merritt, C.R.; Burns, P.N.; Foster, F.S.; Mattrey, R.F.; Wickline, S.A. *Acad. Radiol.*, **2000**, *7*, 824.
- [123] Fleischer, A.C. *J. Ultrasound Med.*, **2000**, *19*, 55.
- [124] Ogura, O.; Takebayashi, Y.; Sameshima, T.; Maeda, S.; Yamada, K.; Hata, K.; Akiba, S.; Aikou, T. *Dis. Colon Rectum.*, **2001**, *44*, 538.
- [125] Lin, Y.; Weisdorf, D.J.; Solovey, A.; Hebbel, R.P. *J. Clin. Invest.*, **2000**, *105*, 71.
- [126] Janssens, D.; Michiels, C.; Guillaume, G.; Cuisinier, B.; Louagie, Y.; Remacle, J. *J. Cardiovasc. Pharm.*, **1999**, *33*, 7.
- [127] Dignat-George, F.; Blann, A.; Sampol, J. *Blood*, **2000**, *95*, 728.
- [128] Mancuso, P.; Burlini, A.; Pruner, G.; Goldhirsch, A.; Martinelli, G.; Bertolini, F. *Blood*, **2001**, *97*, 3658.
- [129] Lyden, D.; Hattori, K.; Dias, S.; Costa, C.; Blaikie, P.; Butros, L.; Chadburn, A.; Heissig, B.; Marks, W.; Witte, L.; Wu, Y.; Hicklin, D.; Zhu, Z.; Hackett, N.R.; Crystal, R.G.; Moore, M.A.; Hajar, K.A.; Manova, K.; Benezra, R.; Rafii, S. *Nat. Med.*, **2001**, *7*, 1194.
- [130] Rafii, S.; Lyden, D.; Benezra, R.; Hattori, K.; Heissig, B. *Nat. Rev. Cancer*, **2002**, *2*, 826.
- [131] Chang, E.; Boyd, A.; Nelson, C.C.; Crowley, D.; Law, T.; Keough, K.M.; Folkman, J.; Ezekowitz, R.A.; Castle, V.P. *J. Pediatr. Hematol. Oncol.*, **1997**, *19*, 237.
- [132] Berglund, A.; Molin, D.; Larsson, A.; Einarsson, R.; Glimelius, B. *Ann. Oncol.*, **2002**, *13*, 1430.
- [133] Kuroi, K.; Toi, M. *Int. J. Biol. Markers*, **2001**, *16*, 5.
- [134] Kumar, H.; Heer, K.; Greenman, J.; Kerin, M.J.; Monson, J.R. *Anticancer Res.*, **2002**, *22*, 1877.
- [135] Byrne, G.J.; Ghellal, A.; Iddon, J.; Blann, A.D.; Venizelos, V.; Kumar, S.; Howell, A.; Bundred, N.J. *Journal of the Natl. Cancer Inst.*, **2000**, *92*, 1329.
- [136] Amano, H.; Hayashi, I.; Endo, H.; Kitasato, H.; Yamashina, S.; Maruyama, T.; Kobayashi, M.; Satoh, K.; Narita, M.; Sugimoto, Y.; Murata, T.; Yoshimura, H.; Narumiya, S.; Majima, M. *J. Exp. Med.*, **2003**, *197*, 221.
- [137] Narumiya, S.; Sugimoto, Y.; Ushikubi, F. *Physiol. Rev.*, **1999**, *79*, 1193.
- [138] Zhang, X.; Morham, S.G.; Langenbach, R.; Young, D.A. *J. Exp. Med.*, **1999**, *190*, 451.
- [139] Skopinska-Rozewska, E.; Piazza, G.A.; Sommer, E.; Pamukcu, R.; Barcz, E.; Filewska, M.; Kupis, W.; Caban, R.; Rudzinski, P.; Bogdan, J.; Mlekodaj, S.; Sikorska, E. *Int. J. Tissue React.*, **1998**, *20*, 85.
- [140] Zhang, L.; Yu, J.; Park, B.H.; Kinzler, K.W.; Vogelstein, B. *Science*, **2000**, *290*, 989.
- [141] Yamamoto, Y.; Yin, M.J.; Lin, K.M.; Gaynor, R.B. *J. Biol. Chem.*, **1999**, *274*, 27307.
- [142] Jiang, X.H.; Lam, S.K.; Lin, M.C.; Jiang, S.H.; Kung, H.F.; Slosberg, E.D.; Soh, J.W.; Weinstein, I.B.; Wong, B.C. *Oncogene*, **2002**, *21*, 6113.
- [143] Grosch, S.; Tegeder, I.; Niederberger, E.; Brautigam, L.; Geisslinger, G. *FASEB J.*, **2001**, *15*, 2742.
- [144] Palayoor, S.T.; Tofilon, P.J.; Coleman, C.N. *Clin. Cancer Res.*, **2003**, *9*, 3150.
- [145] Zhu, J.; Song, X.; Lin, H.P.; Young, D.C.; Yan, S.; Marquez, V.E.; Chen, C.S. *J. Natl. Cancer Inst.*, **2002**, *94*, 1745.
- [146] Rosenbaum, C.; Baumhof, P.; Mazitschek, R.; Muller, O.; Giannis, A.; Waldmann, H. *Angew Chem. Int. Ed. Engl.*, **2004**, *43*, 224.
- [147] Yoshida, S.; Amano, H.; Hayashi, I.; Kitasato, H.; Kamata, M.; Inukai, M.; Yoshimura, H.; Majima, M. *Lab. Invest.*, **2003**, *83*, 1385.
- [148] Pearce, H.R.; Kalia, N.; Bardhan, K.D.; Brown, N.J. *J. Gastroenterol. Hepatol.*, **2003**, *18*, 1180.
- [149] Shtivelband, M.I.; Juneja, H.S.; Lee, S.; Wu, K.K. *J. Thromb. Haemost.*, **2003**, *1*, 2225.
- [150] Fierro, I.M.; Kutok, J.L.; Serhan, C.N. *J. Pharmacol. Exp. Ther.*, **2002**, *300*, 385.
- [151] Sawaoka, H.; Tsuji, S.; Tsujii, M.; Gunawan, E.S.; Sasaki, Y.; Kawano, S.; Hori, M. *Lab. Invest.*, **1999**, *79*, 1469.
- [152] Li, G.; Yang, T.; Yan, J. *Biochem. Biophys. Res. Commun.*, **2002**, *299*, 886.
- [153] Rozic, J.G.; Chakraborty, C.; Lala, P.K. *Int. J. Cancer*, **2001**, *93*, 497.
- [154] Ghosh, A.K.; Hirasawa, N.; Niki, H.; Ohuchi, K. *J. Pharmacol. Exp. Ther.*, **2000**, *295*, 802.
- [155] Milas, L.; Hunter, N.; Furuta, Y.; Nishiguchi, I.; Runkel, S. *Int. J. Radiat. Biol.*, **1991**, *60*, 65.
- [156] Yamada, M.; Kawai, M.; Kawai, Y.; Mashima, Y. *Curr. Eye Res.*, **1999**, *19*, 300.
- [157] Amano, H.; Haysahi, I.; Yoshida, S.; Yoshimura, H.; Majima, M. *Hum. Cell*, **2002**, *15*, 13.
- [158] Pai, R.; Szabo, I.L.; Kawanaka, H.; Soreghan, B.A.; Jones, M.K.; Tarnawski, A.S. *Mol. Cell Biol. Res. Commun.*, **2000**, *4*, 111.
- [159] Farrell, C.L.; Megyesi, J.; Del Maestro, R.F. *J. Neurosurg.*, **1988**, *68*, 925.
- [160] Szabo, I.L.; Pai, R.; Soreghan, B.; Jones, M.K.; Baatar, D.; Kawanaka, H.; Tarnawski, A.S. *J. Physiol. Paris*, **2001**, *95*, 379.
- [161] Seed, M.P.; Brown, J.R.; Freemantle, C.N.; Papworth, J.L.; Colville-Nash, P.R.; Willis, D.; Somerville, K.W.; Asculai, S.; Willoughby, D.A. *Cancer Res.*, **1997**, *57*, 1625.
- [162] Seed, M.P.; Freemantle, C.N.; Alam, C.A.; Colville-Nash, P.R.; Brown, J.R.; Papworth, J.L.; Somerville, K.W.; Willoughby, D.A. *Adv. Exp. Med. Biol.*, **1997**, *433*, 339.
- [163] Freemantle, C.; Alam, C.A.; Brown, J.R.; Seed, M.P.; Willoughby, D.A. *Int. J. Tissue React.*, **1995**, *17*, 157.
- [164] Shen, W.Y.; Constable, I.J.; Chelva, E.; Rakoczy, P.E. *Graefes Arch. Clin. Exp. Ophthalmol.*, **2000**, *238*, 273.
- [165] Mohammed, S.I.; Craig, B.A.; Mutsaers, A.J.; Glickman, N.W.; Snyder, P.W.; deGortari, A.E.; Schlittler, D.L.; Coffman, K.T.; Bonney, P.L.; Knapp, D.W. *Mol. Cancer Ther.*, **2003**, *2*, 183.
- [166] Mohammed, S.I.; Bennett, P.F.; Craig, B.A.; Glickman, N.W.; Mutsaers, A.J.; Snyder, P.W.; Widmer, W.R.; DeGortari, A.E.; Bonney, P.L.; Knapp, D.W. *Cancer Res.*, **2002**, *62*, 356.
- [167] Liu, X.H.; Kirschenbaum, A.; Yao, S.; Stearns, M.E.; Holland, J.F.; Claffey, K.; Levine, A.C. *Clin. Exp. Metastasis*, **1999**, *17*, 687.
- [168] Liu, X.H.; Kirschenbaum, A.; Yao, S.; Lee, R.; Holland, J.F.; Levine, A.C. *J. Urol.*, **2000**, *164*, 820.
- [169] Sengupta, S.; Sellers, L.A.; Cindrova, T.; Skepper, J.; Gherardi, E.; Sasisekharan, R.; Fan, T.P. *Cancer Res.*, **2003**, *63*, 8351.
- [170] Guo, J.S.; Cho, C.H.; Lam, L.; E.S.; Choy, H.T.; Wang, J.Y.; Leung Koo, M.W. *Toxicol. Appl. Pharmacol.*, **2002**, *183*, 41.
- [171] Wilkinson-Berka, J.L.; Alousis, N.S.; Kelly, D.J.; Gilbert, R.E. *Invest. Ophthalmol. Vis. Sci.*, **2003**, *44*, 974.
- [172] Emanuelli, C.; Zacheo, A.; Minasi, A.; Chao, J.; Chao, L.; Salis, M.B.; Stacca, T.; Straino, S.; Capogrossi, M.C.; Madeddu, P. *Arterioscler. Thromb. Vasc. Biol.*, **2000**, *20*, 2379.
- [173] Tamarat, R.; Silvestre, J.S.; Durie, M.; Levy, B.I. *Lab. Invest.*, **2002**, *82*, 747.