Molecular Pathways for Cancer Angioprevention

Adriana Albini,1 Douglas M. Noonan,2 and Nicoletta Ferrari3

Abstract

By analogy to the success of cardiovascular medicine in reducing mortality through preventive measures, cancer chemoprevention has the potential to significantly reduce incidence and mortality due to tumors. Angiogenesis is an event inhibited by most of the promising cancer chemoprevention compounds, a concept we termed “angioprevention.” Here, we review the signaling pathways that are targeted by diverse angioprevention compounds in endothelial cells. We highlight diverse mechanisms of action, implying that combination angioprevention approaches could further improve efficacy and be transferred to clinical practice.

Background

By the time most tumors are detected in the clinic, they have already undergone many of the molecular and microenvironmental alterations that lead to malignancy (1, 2). Thus, despite great strides made in therapy regimens, most treatments started at this time are destined to eventually fail. Prevention of tumor formation would be a powerful alternative. In fact, overall mortality due to cardiovascular disease, once by far the most prominent cause of death, has now been brought below that of cancer (3), a success largely due to prevention. Effective translation of this concept of prevention into cancer has the potential to save a vast number of lives and increase healthy life span (4). Cancer chemoprevention is defined as the use of agents to prevent transformation, or to slow, reverse, or inhibit the progression of carcinogenesis, with the aim of lowering the risk of developing significant and clinically invasive disease (5). Ideally, chemoprevention agents are devoid of toxicity and are well-tolerated over long-term use; however, this is in relationship to the relative risk of the individual: the greater the level of risk, the more the potential adverse effects may be acceptable.

We noted that a series of molecules proposed as chemopreventive agents all showed potent antiangiogenic properties when tested in vitro and in vivo angiogenesis models (6). This led us to hypothesize that antiangiogenesis is a primary action of efficacious chemoprevention molecules, a concept we named “angioprevention” (6). Most tumors require the capacity of inducing the formation of a new blood supply to overcome the physical limitations on the diffusion of nutrients and oxygen within the tumor, a critical step in progression known as the angiogenic switch (7). Inhibiting angiogenesis before it starts, that is, preventing the angiogenic switch, has the potential to block nascent tumors into clinically indolent hyperplastic foci, a condition noted in autopsies (see, for example, ref. 8). In this case, the target cells are not tumor cells themselves but those in the microenvironment associated with angiogenesis: endothelial and inflammatory cells. Substantial data from numerous laboratories now support the concept of angioprevention (2) and have begun to reveal the key signaling pathways targeted in host and cancer cells. Here, we review the signaling pathways involved in angioprevention in an attempt to propose effective, multiple-target strategies for the prevention and treatment of human cancers.

Master Targets of Angiopreventive Compounds

The “angiogenic switch” that controls tumor angiogenesis (7) actually seems to be the result of numerous switches, including substantial progressive alterations within the microenvironment. Angioprevention compounds often act through the interruption of autocrine or paracrine effects generated by the tumor or the microenvironment influencing specific endothelial cell molecules, targeting general pathways. Along these diverse pathways, several different, partially overlapping, categories of mechanisms leading to angioprevention could be discerned (Fig. 1), these include: (1) interference with transmembrane receptors, among these (a) blocking key tyrosine kinase receptors such as vascular endothelial growth factor (VEGF), fibroblast growth factor, platelet-derived growth factor, epidermal growth factor, hepatocyte growth factor, and the insulin-like growth factor-I axis; (b) altering the activity of serine/threonine kinases, in particular, members of the transforming growth factor-β (TGF-β) family, the ligands of which repress angiogenesis; and (c) G protein–coupled receptors like the chemokine receptors. (2) Blocking nonreceptor kinases and phospholipases; protein kinase C, phospholipase Cγ, diacylglycerol, and the phosphoinositide-3-kinase (PI3K)–Akt signaling axis which seems to be a critical common point of regulation (9), as well as mitogen-activated protein kinases. (3) Modulation of phosphatases and G protein regulators such as Ras and other GTPases. (4) Inhibition of intracellular hormone and nuclear receptors such as estrogen receptor, retinoic acid receptors, or retinoid X receptors. (5) Inhibition of prostaglandins,
particular, by blocking lipoxygenases and cyclooxygenases (COX), as is the case for nonsteroidal anti-inflammatory drugs (10). (6) Interference with transmembrane non–growth factor receptors including the integrins and CD44. (7) Modulation of the extracellular microenvironment, generally by altering cytokine profiles, or inhibition of matrix metalloproteinases, required for endothelial cell invasion, a common target of angioprevention agents (11). (8) Blocking nuclear factor-κB (NF-κB) and hypoxia-inducible factor 1α (HIF-1α), key pathways frequently regulated in concert with Akt, that stand out among the diverse transcription factors which operate to sense environmental clues that drive angiogenesis (12).

Many potential angioprevention compounds seem to target multiple pathways, whereas others may be more specific (Fig. 1). Furthermore, some classes of compounds with similar actions can be identified, providing rough groups of most angioprevention compounds. Here, we focus on selected pathways frequently targeted by angioprevention compounds.

Tyrosine Kinase Inhibitors

Angiogenesis inhibition through the use of tyrosine kinase blockers, such as monoclonal antibodies, or inhibitors in the form of small molecules, is now a clinically established cancer therapy (13), a topic that has been extensively reviewed and will not be discussed in detail here. Some of the principal ligand-receptor couples that can be targeted for prevention of angiogenesis are VEGF and VEGF receptors (VEGFR1, VEGFR2, or VEGFR3), hepatocyte growth factor and c-met, insulin-like growth factor/insulin-like growth factor receptors, epidermal growth factor/epidermal growth factor receptors, platelet-derived growth factor/platelet-derived growth factors, angiopoietins and ties. The signals downstream of these “master” growth factor ligand–tyrosine kinase receptor switches generally belong to the PI3K, Akt, Erk1/2 axes which will be discussed below. Before moving the currently available antiangiogenesis compounds into the realm of cancer prevention, clinical trials

Fig. 1. The serine/threonine kinase Akt functions intracellularly as a cardinal point of converging upstream signaling pathways, which involve the stimulation of receptor tyrosine kinases (GFRs) such as insulin-like growth factor receptor 1, epidermal growth factor receptor, and VEGF receptor by their specific growth factors (GF), an assembly of membrane-localized complexes of receptors-PI3K and activation of Akt. The integration of these intracellular signals at the Akt level and its kinase activity regulates the phosphorylation of several downstream effectors such as mammalian target of rapamycin and NF-κB. These phosphorylation events, in turn, mediate the effects of Akt on cell growth, proliferation, protection from proapoptotic stimuli, and stimulation of neoangiogenesis. In this scenario, HIF-1α acts as a transcription switch for the regulation of oxygen homeostasis. NF-κB is normally under strict regulation by its sequestration in the cytoplasm: generation of reactive oxygen species (ROS) and activation of PI3K-Akt and MEK pathways, converge to activate NF-κB. The central role of Akt in the regulation of endothelial cell proliferation, survival, and angiogenesis/response to hypoxia provides several targets for angioprevention agents. Schematic representation of the functional role of ALK1 and ALK5 in TGF-β signaling in endothelial cells is also reported. The ratio of TGF-β signals via ALK1 versus ALK5 will determine whether TGF-β will have proangiogenic or antiangiogenic effects. Induction of COX-2 by NF-κB or met-linked pathways in endothelial as well as in other cells converts arachidonic acid (AcAc) to the prostaglandins prostaglandin E2 (PGE2) and prostaglandin I2 (PGI2), as well as thromboxane A2 (TXA2). Prostaglandins E2 seems to induce VEGF and met expression through a PKA-mediated pathway. Principal drug groups and examples: TKIs, monoclonal antibodies against growth factors (e.g., Avastin) or receptors (e.g., Herceptin); receptor tyrosine kinase inhibitors (e.g., Sorafenib, Sunitinib). FLVs, flavonoids and other phytochemicals active on AKT and NF-κB: epigallocatechin-3-gallate (EGCG), xanthohumol, silybin, deguelin, apigenin, curcumin, genistein, luteolin, capsaicin, evoivamide, ginseng extracts, and indole-3-carbinol. COXibs, NSAIDs: aspirin, celecoxib, rofecoxib. Raps, the mammalian target of rapamycin inhibitor rapamycin and its analogues. LY, the PI3K inhibitor LY294002. 4HPR, fenretinide. SLR, sulforaphane (as well as apigenin). AOXs, antioxidants, e.g., N-acetyl-cysteine. AA, other angioprevention agents such as thalidomide and its analogues, which have a broad range of activities.

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Research.
are currently focusing on the use of these drugs in preventing relapse, metastases, or in adjuvant therapy due to the relatively high costs and partial toxicities of the compounds that, to date, shifts the risk-benefit balance away from use in primary prevention. However, as less expensive, better tolerated compounds become available, chemoprevention through tyrosine kinase inhibition will be feasible.

**The PI3K-Akt/NF-κB/HIF-1 Axis**

The PI3K-Akt pathway is activated by a variety of stimuli in endothelial cells and regulates multiple steps in angiogenesis (Fig. 1), including endothelial cell survival, migration, and invasion (14). The role of this pathway in vessel formation is underscored by the observation that gene targeting of Akt1 modifies angiogenesis in murine models (15). Under hypoxic conditions, or under stimulation by growth factors such as epidermal growth factor, fibroblast growth factor, insulin-like growth factor, tumor necrosis factor-α, platelet-derived growth factor, or serum in some cell types, HIF-1α is either stabilized or overexpressed through the PI3K-Akt pathway and their downstream targets: mammalian target of rapamycin and mitogen-activated protein kinases-ERK (MEKK) pathways (16, 17), as well as having other effects on tumor cells (18). This activation leads to nuclear translocation, dimerization with HIF-1β, and transactivation of angiogenesis promoter genes such as VEGF (19). Additional implication of the PI3K-AKT pathway in the HIF-1 response comes from the observation that loss of the tumor suppressor gene PTEN, a negative regulator of Akt phosphorylation and activation, increases HIF-1-mediated gene expression (20).

Under nonhypoxic conditions, the von Hippel-Lindau tumor suppressor protein targets HIF-1α for rapid ubiquitination and degradation. von Hippel-Lindau–independent pathways for degradation of HIF-1α involve the tumor suppressor p53 or the heat shock protein 90 (Hsp-90). Indeed, up-regulation of p53 leads to ubiquitination and degradation of HIF-1α (21), whereas loss of p53 in tumor cells enhances HIF-1α levels (22). Hsp-90 inhibitors such as geldanamycin promote the loss of HIF-1α even in cell lines lacking von Hippel-Lindau protein (23).

NF-κB is activated by the PI3K-AKT and MEKK pathways, as well as by changes in redox potential due to reactive oxygen species generation (24, 25). NF-κB activation promotes a process that moves NF-κB from the cytoplasm into the nucleus to bind promoters of genes that regulate cell proliferation, survival, and angiogenesis such as the chemokine IL-8 (CXCL8). A number of studies have also suggested a role for redox-dependent processes in the control of HIF-1 because treatment with diamide or hydrogen peroxide results in loss of DNA binding activity (26). Although transfection of the small redox protein thioredoxin-1 increases HIF-1α protein and activity and VEGF expression (27), lipoic acid treatment, which strongly increases thioredoxin reductase 1 expression, has antiangiogenic activities both in vitro and in vivo (28).

Targeting PI3K-Akt and NF-κB pathways are effective mechanisms to control endothelial cell growth and to inhibit angiogenesis, as shown by the similar effects obtained with the natural compounds silibinin (29), xanthohumol (30), and deguelin (31). Akt and NF-κB activation in tumor cells is associated with downstream regulation of genes controlling cell proliferation, survival, and apoptosis. In this context, Akt has been shown to influence the expression and cellular localization and functions of p21, which induces cell growth arrest by inhibiting the functions of cyclin-dependent kinases in the nucleus (32). The PI3K-Akt pathway can also influence cell growth by delaying the onset of p53-induced apoptosis (33) as Akt, via IKK, induces nuclear translocation of NF-κB and targets p53 for degradation by the proteasome (34). PI3K-Akt inhibitors up-regulate p53 and block tumor-induced angiogenesis by acting on the endothelial compartment, as well as the tumor cells (35).

The angiogenic response also entails the activation of the endothelial cell survival machinery to preserve the integrity of newly formed vessels. Activation of Akt and NF-κB was associated with up-regulation of the apoptosis inhibitor survivin in endothelial cells (31), as seen in many tumors (36). Survivin plays an important role in cell cycle control, protects endothelial cells from death-inducing stimuli (37) and its up-regulation, by preserving the microtubule network, has been proposed as a mechanism of endothelial cell drug “resistance” (38). As the human survivin gene is negatively regulated by wild-type p53 at both the mRNA and protein levels (39), the decreased NF-κB and Akt activities associated with exposure to angioprevention agents would induce p53 protein, whereas decreasing survivin. In conclusion, the PI3K-Akt pathway functions intracellularly as a cardinal nodal point converging upstream signaling pathways from receptor tyrosine kinases to regulate several downstream effectors of the angiogenic process.

**TGF-β Family Members**

TGF-β is also an important regulator of cell proliferation and is one of several cytokines that regulate angiogenesis. Although first identified and named for its ability to stimulate the proliferation and transformation of mesenchymal cells, TGF-β potently inhibits endothelial and hematopoietic cell proliferation. The growth-inhibitory effects of TGF-β are mediated by Smad-dependent and Smad-independent pathways (40), and by preventing progression through the cell cycle by inducing expression of the cyclin kinase inhibitors p15, p21, and p27 (41–43). Conversely, the PI3K-Akt pathway protects against TGF-β–induced apoptosis by inhibiting a step downstream of Smad but upstream of caspase-3 (44). TGF-β can function either as a proangiogenic or antiangiogenic factor in vitro, in that it can activate two distinct pathways: the classic Smad-dependent signaling through TβRII and TβRI (also known as ALK-5) to activate Smads 2 and 3, or through TβRII and ALK-1 to activate Smads 1, 5, and 8, which are usually activated by the TGF-β superfamily members, the bone morphogenetic proteins. These two pathways have opposing effects on endothelial cell proliferation and migration. The balance of signaling regulates endothelial cell biology through the activation (increased cell proliferation and migration) and maturation (decreased cell proliferation and migration) phases of angiogenesis (45, 46). Endoglin (a type III receptor in the TGF-β family) is a likely candidate to regulate the balance of TGF-β signaling through these pathways in endothelial cells, by inhibiting the ALK-5 pathway (47), while activating the ALK-1 pathway (48). Interestingly, the chemopreventive molecule fenretinide showed strong antiangiogenic activities both in vitro.
and in *in vivo* through up-regulation in endothelial cells of two members of the bone morphogenetic proteins: BMP2 and GDF15 (49, 50). Presumably, synthetic triterpenoids, which have recently been shown to be highly potent agents for chemoprevention, will share common pathways (51, 52).

**COX-2**

Inhibitors of COX (NSAIDs) and COX-2 inhibitors, in particular, have been found to be effective chemoprevention agents in preclinical and clinical studies (53–55). Suppression of COX-2 is a critical target for the control of tumors associated with chronic inflammation. Increasing data on the association between expression of COX-2 with VEGF in tumors (56) and on COX-2 as a downstream effector of c-MET (57, 58), now support its role in angiogenesis. COX-2 seems to be a potent stimulator of VEGF production in diverse tissues (Fig. 1) that is downstream of inflammatory signals activated by NF-κB, possibly by helping surrounding stromal cells provide VEGF (56). Indeed, studies suggesting that COX-2 plays a significant role in colon polyp formation found that COX-2 expression occurred in stromal cells, rather than in the epithelium (59).

Interestingly, COX-2 inhibition in preclinical studies has recently been associated with a reduction in the formation of vascular channels by tumor cells that lack endothelial cells (60). The generic COX inhibitor aspirin has been found to be effective in preventing colon cancer in clinical studies (54), leading to the application of more specific COX-2 inhibitors as a cancer prevention approach. Two studies have shown that the COX-2 inhibitor Celebrex effectively prevents colon cancer, although both studies observed increased risk for cardiovascular events that brought into question the prevention advantage (61–63). Interestingly, recent studies on female cohorts making chronic use of NSAIDs for other indications found a striking reduction in risk for breast cancer (55).

**Clinical-Translational Advances: Strategies for Clinical Angioprevention**

Therapeutic strategies specifically targeting Akt have been the subject of intense research efforts; however, the very same pleiotropy of Akt signaling and the large number of downstream outputs raises the question of whether such targeting can be achieved within a safe therapeutic window sparing the normal cells of cancer patients (see refs. 64, 65). It is notable, however, that suppression of Akt signaling, both upstream and downstream of Akt itself, provides a wide range and a graded selectivity of targets for therapeutic strategies. Acceptable profiles and side effects with objective responses have been achieved in some models with inhibitors of upstream Akt activators, such as the monoclonal antibody Trastuzumab to HER-2/neu, or the small molecule inhibitors of the epidermal growth factor receptor Iressa, Tarceva, and many others, the anti-VEGF antibodies bevacizumab, or the VEGF receptor tyrosine kinase inhibitors PK787 and SU-6668. However, although clinical experience is often sufficient to declare therapeutic effectiveness, dependence on Akt is not directly shown for these compounds. The kinase domain of Akt itself is an obvious goal of therapeutic targeting, and many natural compounds (mostly phytochemicals and flavonoids from different sources) have shown the inhibition of Akt activation, associated with decreased NF-κB activity, and modulation of growth/apoptosis-related proteins such as p21, p53, and survivin as described above. Epigallocatechin-3-gallate, xanthohumol, silybinin, deguelin, apiigenin, curcumin, genistein, luteolin, capsaicin, evodiamine, ginseng extracts, indole-3-carbinol, zerumbone, and 1-acetoxychavicol acetate are a few of these natural angiopreventive agents, some of which have already entered clinical trials as single agents or in combination therapy (29–31, 66–70). A 1-year proof-of-principle study by Bettuzzi et al. (71) showed that green tea catechins were very effective in preventing conversion to prostate cancer in patients with high-grade prostate intraepithelial neoplasia, a process partly depending on AKT activation (72), in agreement with experimental observations (73). Most of these compounds also interfere with insulin-like growth factor-I signaling, which strongly activates AKT, further enhancing chemopreventive properties (74). Other synthetic compounds of different origin, such as the PI3K inhibitor LY294002 (75), thalidomide (76), and its analogue lenalidomide, the Hsp-90–binding antibiotic geldanamycin (77), mammalian target of rapamycin inhibitors like rapamycin and its analogues (78), the COX-2 inhibitors, have all shown mechanisms of action in part resembling those of the “natural compounds” listed above, and many have entered phase I to IV clinical trials showing angiogenesis inhibition–associated effects. A detailed list of ongoing clinical trials making use of antiangiogenesis compounds is available online.4

**Multiple Pathways: A Rational for “Combination Chemoprevention”**

The pathways depicted in Fig. 1 suggest an important advancement that must be made: development of chemoprevention based on combinations of drugs with diverse molecular targets, as suggested by Sporn and Liby (5). Specific cancer histologies that may be targeted with these angioprevention approaches are those having biomarkers that provide a ready read-out from early stages of hyperplasia to cancer. One example is prostate cancer, in which increases in PSA levels often predict eventual progression. Clearly, several pathologies associated with chronic inflammatory disease fall into this category, and further support the concept of combining, for example, anti-COX NSAIDs and anti-inflammatory NF-κB–repressing flavonoids (2). An accurate biomarker for angiogenesis is still lacking, which would help in the application of angioprevention.

**Concluding Remarks**

Higher levels of activated Akt, NF-κB, and HIF-1α in neoplastic cells are associated with an increased risk for cancer development. All these molecular pathways are also crucial regulators of the angiogenic process, a rate-limiting step in cancer progression and a rational target for tailoring cancer chemoprevention regimens. Signaling pathways in endothelial cells are key targets of “angiopreventive compounds.” If we prevent angiogenesis, we avoid the angiogenic switch. We can do this in several ways, as exemplified by the COX-2 inhibitors,

4 http://www.cancer.gov/clinicaltrials/developments/anti-angio-table
which are effective angiopreventive compounds, although they can show undesirable side effects that should be compensated through protection from off-target toxicity. Low doses and combination approaches could resolve these issues. Well-tolerated synthetic drugs such as the retinoid 4HPR, which was shown to be effective in premenopausal women (79), or known dietary compounds that interfere with endothelial signaling pathways, provide clues for developing agents that can be used for extended time periods with little toxicity. In any case, it is reasonable to raise the question as to whether Akt inhibition, for example, will affect not only tumor cells but also normal tissues. In the case of clinical trials of putative Akt inhibitors, the pharmacologic suppression of Akt activity may not necessarily be required to be either complete or permanent. As observed for the angiopreventive agents N-acetyl-cysteine, epigallocatechin-3-gallate, and dequelin, these molecules spare normal cells: this phenomenon seems to be related to the “oncogene addiction” paradigm, in which transformed cells show increased sensitivity to agents interrupting signal transduction, as compared with normal cells (80).

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