19 Angiogenesis and Cancer Prevention: A Vision

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Abstract

Angiogenesis is necessary for solid tumor growth and dissemination. In addition to angiogenesis, it has become increasingly clear that inflammation is a key component in cancer insurgence that can promote tumor angiogenesis. We noted that angiogenesis is a common and key target of most chemopreventive molecules, where they most likely suppress the angiogenic switch in premalignant tumors, a concept we termed angioprevention. We have shown that various molecules, such as flavonoids, antioxidants, and retinoids, act in the tumor microenvironment, inhibiting the recruitment and/or activation of endothelial cells and phagocytes of the innate immunity. N-acetyl-cysteine, and the green tea flavonoid epigallocatechin-3-gallate (EGCG) and the beer/hops-derived chalcone Xanthohumol all prevent angiogenesis in the Matrigel sponge angiogenic assay in vivo and inhibit the growth of the highly angiogenic Kaposi's sarcoma tumor cells (KS-Imm) in nude mice. The synthetic retinoid 4-hydroxyfenretinide (4HPR) also shows anti-angiogenic effects. We analyzed the regulation of gene expression they exert in primary human umbilical endothelial cells (HUVEC) in culture with functional genomics. Expression profiles obtained through Affymetrix GeneChip arrays identified overlapping sets of genes regulated by anti-oxidants. In contrast, the ROS-producing 4HPR induced members of the TGFβ-ligand superfamily, which, at least in part, explains its anti-angiogenic activity. NAC and the flavonoids all suppressed the IkB/NF-κB signaling pathway even in the presence of NF-κB stimulation by TNFα, and showed reduced expression of many NF-κB target genes. A selective apoptotic effect on transformed cells, but not on endothelial cells, of the anti-oxidants may be related to the reduced expression of the NF-κB-dependent survival factors Bcl2 and Birc5/surviving, which are selectively overexpressed in transformed cells by these factors. The repression of the NF-κB pathway suggests anti-inflammatory effects for the antioxidant compounds that may also represent an indirect role in angiogenesis inhibition. The green tea flavonoid EGCG does target inflammatory cells, mostly neutrophils, and inhibits inflammation-associated angiogenesis. The other angiopreventive molecules are turning out to be effective modulators of phagocyte recruitment and activation, further linking inflammation and vascularization to tumor onset and progression and providing a key target for cancer prevention.

Introduction

It is now well established that growth of a tumor to clinically relevant dimensions requires the ability to induce the formation of new blood vessels (Kerbel and Folkman 2002). Angiogenesis is a rate-limiting step in progression to tumor malignancy (Hanahan and Weinberg 2000), and not only do proliferating cells in nonangiogenic lesions have limited access to the blood and lymphatic systems, but the counterbalancing cell death that ensues because of insufficient oxygen and nutrients reduces the accumulation of cells that may have acquired additional genetic alterations favoring malignancy. Folkman introduced the concept that inhibition of angiogenesis could

be a strategy in cancer therapy in 1971 (Folkman 1971). Angiogenesis inhibition is now a clinical reality, the commercially available anti-VEGF antibody Avastin, or bevacizumab, significantly improves survival when combined with standard chemotherapy approaches (Ferrara and Kerbel 2005). Clearly this approach perturbs the VEGF-based tumor-endothelium loops that feed cancer angiogenesis. However, how this contributes to clinical benefit is currently under scrutiny. In addition to the original idea of suffocating tumors by cutting off the lifelines by eliminating new vessel formation, the concept that this therapeutic approach results in vascular normalization (Jain 2005) has been put forth by R. Jain. The vascular normalization hypothesis suggests that blocking the VEGF signal pathway results in less permeable, stabile vessels that paradoxically deliver the associated chemotherapeutics better. Perhaps both may be operational in the clinical setting. While the general goal in development of these drugs was to interrupt the tumor-endothelium crosstalk between tumor-derived VEGF and endothelial VEGF receptors, we may be doing much more than that. In addition to endothelial cells, we are starting to appreciate that other cells also express receptors for, and respond to, VEGF. These include the tumor cells themselves, which in some cases may rely on autocrine loops of VEGF (Carmeliet 2005), hematopoietic cells and leukocytes, and bone marrow-derived progenitors that appear to contribute to the endothelium, although the extent to which they do so is controversial, in newly forming vessels (Rafii et al. 2002).

In spite of the excitement surrounding the clinical success of these agents, the extent of improvement in survival with anti-angiogenics is as yet still only a few months rather than the long-term tumor suppression originally postulated. Furthermore, we must ask why, since we are targeting a normal cell, do tumors soon progress in a therapy-resistant manner? One possibility, among several (Carmeliet 2005), is the numerous roads that may be taken to induce vessel formation: targeting one molecular pathway, or even a few, may not be enough to combat the phenotypic plasticity of an established tumor.

Chemoprevention and Angiogenesis

The acquisition of the capacity to induce angiogenesis, the process necessary for tumor progression, is often a discreet step referred to as the angiogenic switch (Hanahan and Weinberg 2000). Intuitively, it is clear that if we can prevent the angiogenic switch, we should be able to prevent progression of hyperplastic foci, blocking these into a small, benign and clinically indolent state, effectively preventing cancer insurgence (Albini et al. 2005). Furthermore, if we assume that tumor progression depends on the increase in risk for malignant conversion as a function of tumor cell accumulation as suggested above, we could significantly reduce the risk for progression and malignancy.

Given that the principle of cancer chemoprevention is based on the use of agents that interfere with processes associated with malignant progression have limited collateral effects, anti-angiogenesis may be an effective strategy. In fact, while working with diverse chemoprevention agents, we observed that angiogenesis was both a common and key target of most chemopreventive molecules. We termed the concept that effective chemoprevention targets angiogenesis as "angioprevention" (Tosetti et al. 2002). As a corollary to this hypothesis, we would also suggest that many of the antiangiogenesis compounds developed for tumor therapy may be effective as cancer chemoprevention agents. The identification of more effective cancer prevention compounds will be enhanced by inclusion of antiangiogenesis as an endpoint for evaluation. Furthermore, we have begun searching for common pathways targeted by these molecules to identify the key molecular mechanisms and thus highly specific targets.

Molecular Mechanisms in Angioprevention

Our approach to analysis of the effects of angioprevention compounds on endothelial cells has been through employment of microarray analyses (Pfeffer et al. 2005). These studies have demonstrated that the flavonoids and antioxidant compounds all specifically target the NF-κB pathway in endothelial cells (Pfeffer et al. 2005).

This can be expanded to include the vast majority of the numerous potential cancer chemoprevention agents that have been studied by different laboratories; these have been shown interfere with pathways leading to NF-κB activation, and to repress AKT activation (Aggarwal and Shishodia 2004; Dorai and Aggarwal 2004; Pfeffer et al. 2005; Tosetti et al. 2002). The exceptions to this are the compounds devoid of antioxidant activity, such as the retinoid 4HPR (Ferrari et al. 2005) and possibly the steroid analogs. We have shown that repression of the NF-κB and Akt pathways produces downregulation of downstream elements such as p21, p53, and survivin (Dell'Eva et al., unpublished data), that in turn correlate with reduced endothelial cell activation, proliferation, migration, and even survival. Taken together, these data show that the AKT-NF-κB pathway lies at the core of angiogenesis as a common target for the angioprevention molecules. Given the central role of NF-κB in regulating inflammation (Karin 2005; Karin and Greten 2005), these data may also reflect an anti-inflammatory activity of these compounds. This appears to be the case.

Angiogenesis, Inflammation, and Angioprevention

Apart from the traditional and extensively studied tumor-endothelium axis in angiogenesis research, recent data indicate that a tumor–inflammation–endothelium exchange is of critical importance in cancer insurgence and progression (Balkwill and Mantovani 2001; Balkwill et al. 2005; Coussens and Werb 2002; Pollard 2004) and that it represents a potential therapeutic target (Albini et al. 2005; Benelli et al. 2006b; Brigati et al. 2002; Coussens and Werb 2001, 2002). As often observed in the molecular mechanisms mediating tumor cell invasion and metastatic dissemination, the tumor may ask host cells to orchestrate the angiogenic process as well. Recent data suggest that chronic inflammation is a driving force in angiogenesis associated with numerous pathologies, including tumor angiogenesis (Balkwill and Mantovani 2001). Currently, approximately 15% of the world's tumor burden can be ascribed to infectious agents (Coussens and Werb 2002). If we add clinically recognized chronic inflammation and subclinical chronic inflammation (Balkwill et al. 2005), the percentage of tumors associated with chronic inflammation rises further. These inflammatory components often appear to drive tumor angiogenesis. Inflammatory angiogenesis may be considered part of a normal homeostatic process occurring in conditions of tissue remodeling subsequent to injury. However, since the transformed tumor cells do not cease to proliferate, the injury cannot resolve; this is basically the Dvorak concept of tumors as wounds that never heal (Dvorak 2005). Innate inflammatory cells appear to often play a key role in assisting tumor growth, angiogenesis, and expansion as part of the tissue remodeling process (Benelli et al. 2003, 2006a, b).

The potential growth-promoting role of tumor-infiltrating macrophages has been well established (Pollard 2004), and it is suggested that these assume different phenotypes based on environmental stimuli (Balkwill et al. 2005), one M1 phenotype associated with tissue damage and tumor killing, another M2 phenotype associated with tissue salvage, remodeling and angiogenesis. Substantial data indicate that the M2 phenotype dominates in cancers (Balkwill et al. 2005). Mast cells and neutrophils also provide angiogenic stimuli necessary for tumor progression (Coussens and Werb 2001, 2002). Neutrophils mediate the vessel formation induced by the angiogenic CXC chemokines (Benelli et al. 2002; Scapini et al. 2004) and play a key role in tumor progression related to *ras* oncogene modulation of angiogenic CXC chemokine expression (Karin 2005; Sparmann and Bar-Sagi 2004).

Interestingly, neutrophils have been found to be a target for the angiogenesis inhibitor angiostatin (Benelli et al. 2002). We would extend this to suggest that inhibitors of angiogenesis will inhibit inflammation, and that anti-inflammatory agents will also repress angiogenesis. This has in part been suggested for classic COX inhibitors such as aspirin of specific COX2 inhibitors (Albini and Noonan 2005; Brown and DuBois 2005). These inflammation inhibitors have been shown to be effective in prevention of colon cancer (Brown and DuBois 2005), where the antiangiogenic activity has been postulated to play a role.

Toward Clinical Angioprevention

Development of successful angiogenesis-targeted therapies requires that we know the mechanisms of how tumor cells can induce the formation of new vessels. Knowledge of the diversity of the events occurring and examination of the mechanisms of molecules that interfere with these processes are providing critical insight into future directions for therapy. From the observations discussed here, it becomes clear that compounds that can repress tumor-endothelial cell and inflammation-induced angiogenesis will show promise in chemoprevention settings and perhaps in therapy (Fig. 1). We now need to focus on clinical evaluation of these concepts, potentially initially targeting high-risk groups.

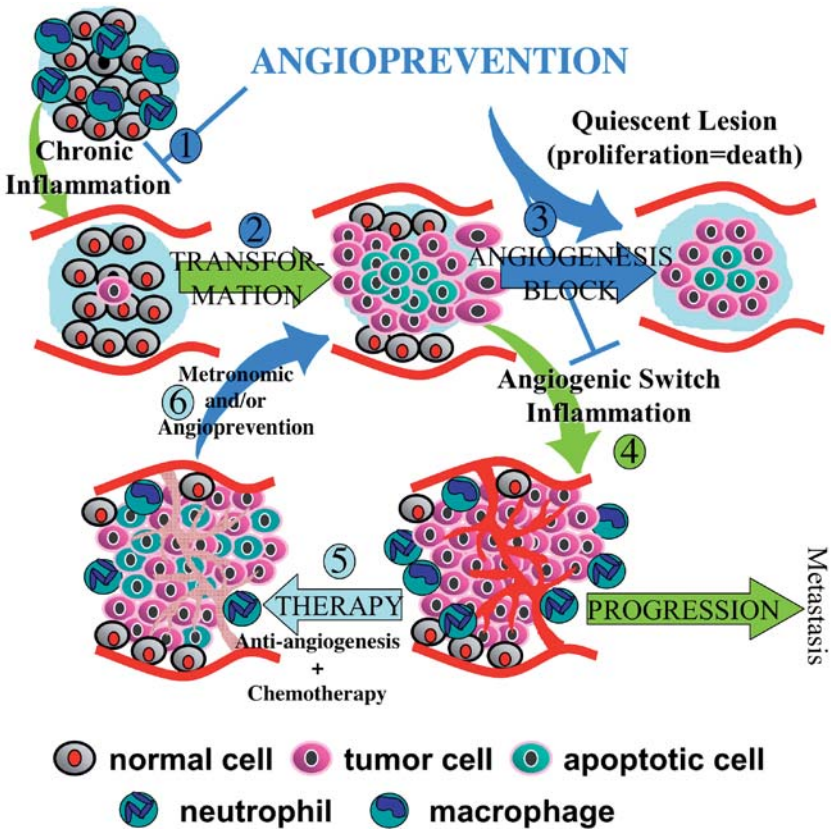

Fig. 1 Points in the carcinogenesis pathway where angioprevention may significantly delay cancer. (*1*) Chronic inflammation clearly predisposes to tumor development; the tight relationship between anti-inflammation and antiangiogenesis suggests that this may a common pathway, potentially reducing step (2) transformation. (*3*) Transformed foci of cells devoid of capacity to induce angiogenesis either directly or via inflammation are limited to small hyperplastic foci that are not clinically significant. Acquisition of angiogenic potential and/or inflammation, the angiogenic switch, results in tumor expansion and eventually clinical cancer. Angioprevention represses the angiogenic switch and favors quiescence, thus indirectly limits step (*4*) progression toward malignancy. Antiangiogenic VEGF blockade together with chemotherapy (*5*) reduce tumor burden, further antiangiogenic measures (6, metronomic therapy; angioprevention) may further favor maintenance of quiescence

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