
MINI-REVIEW

Targeting Angiogenesis – a Novel Mode in Cancer Chemoprevention

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Abstract

Cancer prevention is fast emerging as a discipline with a promising potential. Chemoprevention has its rationale in the multistage process of carcinogenesis which provides an option for development of preventive approaches in the early, premalignant stages, before appearance of clinical symptoms. Evidence is mounting that the angiogenic switch may be an early event in carcinogenesis. Most chemopreventive agents currently under development probably act via multiple mechanisms. The chemopreventives used in clinical trials, such as nonsteroidal anti-inflammatories, tamoxifen and retinoids, have been shown to inhibit angiogenesis, the formation of new vessels from existing vasculature, which may contribute to their protective effect. Development and use, alone or in combination with other agents with other mechanisms of action, of specific antiangiogenic agents is likely to open new possibilities in cancer chemoprevention.

Key Words: angiogenesis - cancer prevention - eicosanoids - cyclooxygenases

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Introduction

Angiogenesis, the formation of new blood vessel networks to permit sustained growth, is essential to tumour growth, invasion and metastasis. Vascular proliferation is an important aspect of the tumourigenesis process (Folkman 1990). The intensity of angiogenesis, as assessed by counting the microvessels in neoplastic tissue, may act as a prognostic factor for many solid tumours such as early cancers of the lung and endometrium (Giatromanolaki et al., 1999; Chiba et al., 1999). Contrary to the conventional wisdom that angiogenesis is triggered when the tumour reaches a size of approximately 0.2-2 mm, i.e., relatively late in the process, recent evidence is mounting that angiogenic switch may be an early event in carcinogenesis (Hanahan et al., 1996; Hanahan and Folkman 1996), and may therefore provide a potential new avenue for cancer prevention. In rats, for instance, with chemically induced skin carcinogenesis, the angiogenic 'switch' is a very early event, occurring during focal hyperplasia (Bolontrade et al., 1998). Considerable progress has been made in the understanding of the regulation of new blood-vessel formation by protein growth factors and emerging delineation of the corresponding signal transduction pathways. Recently, it has been argued that

angiogenesis is not only relevant to preneoplastic stages of carcinogenesis (Carmeliet and Jain, 2000), but it could be an independent target of cancer chemopreventive and a surrogate biomarker for chemopreventive interventions (Sharma et al., 2001; Tosetti et al., 2002).

Angiogenesis has a Role at Early Stages of Carcinogenesis

The formation of new blood-vessels proceeds by mechanisms that primarily comprise vasculogenesis and angiogenesis. Vasculogenesis is mostly confined to early embryogenic development, and involves de novo differentiation of endothelial cells from mesodermal precursors (Patan 2000). Angiogenesis, on the other hand, is the process of recruitment of capillaries from preexisting blood vessels by sprouting or sometimes non-sprouting mechanism known as intussusception (Patan 1996). Angiogenesis is required for the supply of nutrients and oxygen to the cells and is therefore essential for cell survival. A vast array of cytokines, growth factors, growth factors receptors, adhesion factors, adhesion receptors, proteases, and protease inhibitors participate in multiple steps that occur during neovascularization. Obviously, each of these steps

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constitutes targets for designing prevention/intervention strategies directed against angiogenesis (Eatock et al., 2000). By far the most important growth factor, vascular endothelial growth factor or VEGF, is a specific mitogen and survival factor for endothelial cells (Ferrara and Alitalo, 1999). Loss of tumour suppressor gene (VHL), mutational inactivation of p53 gene, and the activation of nuclear factor- κ B play an important role in the upregulation of VEGF (Royds et al., 1998).

Another family of angiogenic factors specific for the vascular endothelial cells, named angiopoietins, and its endothelial cell-specific tyrosine kinase receptor (TIE-2), have been identified recently (Davis and Yancopoulos, 1999). Angiopoietin system appears to be crucial for the recruitment of pericytes and smooth muscle cells needed for the stabilization of the developing vasculature and thus maintaining vascular integrity (Papapetropoulos et al., 1999).

Angiogenesis in Human Cancers

Angiogenesis is seen in early neoplastic lesions associated with a variety of human cancers including cervical carcinoma, prostate cancer, skin cancer, and probably many other types of cancers. Histological markers of angiogenesis have been linked to dysplasia in humans. Carcinoma in situ (CIS) denotes severe dysplasia of epithelial tissue before invasion of the basement membrane (Boone et al., 1992). In the cervix, significant increases in microvessel density are seen as normal cells progress through cervical intraepithelial neoplasia stages I, II, and III to invasive squamous cell carcinoma (Dobbs et al., 1997). Similarly, increased angiogenesis has been noted in Barrett's oesophagus in comparison with normal tissue (Couvelard et al., 2000). Several chronic inflammatory conditions that are associated with development of tumours, including asbestos exposure, liver cirrhosis and cigarette smoking, have also been associated with angiogenesis (Bielefeldt-Ohmann et al., 1996; Shibata et al., 1998; Mayne et al., 1999).

Eicosanoids as Angiogenic Factors

The role of arachidonic acid derived prostanoids in the process of angiogenesis was proposed by Ben Ezra in 1978 (Ben Ezra, 1978), and later established directly by angiogenic bioassays (Ziche et al., 1982; Form and Auerbach, 1983). COX-2 is the inducible form of the enzyme that catalyzes prostanoid (prostaglandins and thromboxane) formation from arachidonic acid. Overexpression of COX-2 has been implicated in the cancers in the colon, rectum, stomach, oesophagus, lung, breast, and head and neck. COX-activity seems to confer survival advantage to transformed cells through the inhibition of apoptosis, enhanced cell proliferation, reduced cell-to-cell adhesion, and the induction of angiogenesis. The literature demonstrating a contribution of COX-2 in tumour angiogenesis has been recently reviewed (Gately, 2000). Both endothelium and smooth

muscle cell contain COX-2 or the isozyme COX-1; however, endothelial cells contain up to 20 times more of COX than smooth muscle cells (De Witt et al., 1983). Prostaglandin E₂, the formation of which is catalyzed by COX-2, is an angiogenic growth factor in vivo and also induces synthesis of angiogenic growth factors such as vascular endothelial growth factor (VEGF) in cultured non-malignant cells lines such as synovial cells and osteoblasts (Ben Av et al., 1995). Overexpression of COX-2 by colon cancer cells resulted in stimulation of vascular endothelial cell migration and formation of capillary like tubes in co-culture experiments.

As in the case of prostaglandins, the effect of products of lipoxygenase (LOX) activity on angiogenesis occurs in collaboration with the angiogenic protein growth factors. Matrix metalloproteinase-9 is a type IV collagenase that is expressed in microvascular endothelial cells, where it is inducible by TPA-mediated protein kinase C (PKC) activity (Hanemaaijer et al., 1993). The 12-hydroxyeicosatetraenoic acid (12-HETE), the 12-LOX product of arachidonic acid metabolism, which has been shown to promote tumour angiogenesis, is involved in the process is not known. In breast cancer cell lines, matrix metalloproteinase-9 (MMP-9) expression is induced by 12-HETE (Connolly et al., 1996).

Anti-angiogenic Agents in Cancer Chemoprevention

Angiogenesis as one of the early changes in the multistage carcinogenesis process qualifies as an important and specific target of chemoprevention. The development and clinical evaluation of pharmacologic and natural antiangiogenic agents as cancer chemopreventive drugs has currently received a heightened interest. 'Angioprevention' is expected to be highly tumour specific since almost 100% of the endothelial cells in the normal vasculature are in the state of quiescence (Hobson and Denekamp 1984). Furthermore, the normal endothelial cells of the tumour vasculature are genetically stable and thus commonly encountered problem of drug-resistance by the tumour cells can be avoided (Kerbel 1997), and they are easily accessible from the lumen of blood vessels.

The chemopreventive agents used in several prevention trials including retinoids, selenium, estrogen receptor modulators, protease inhibitors, tea flavonoids, and nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to inhibit angiogenesis (for references, see Tosetti et al., 2002). The antiangiogenic activity of these compounds may therefore contribute to their putative chemopreventive effect. The studies have, however, been few and many only of exploratory nature. For instance, while it is probable that suppressed neovascularization is an important component of the inhibitory activities of NSAIDs, including indomethacin (IARC, 1997), there is little published data on the antiangiogenic effects of inhibitors of eicosanoid synthesis in animal tumour models. One of them, by La

et al (1997), described reduced neovascularization in spontaneous mammary tumours that developed in the indomethacin-treated animals.

Suramin, one of the earliest antiangiogenic but more toxic agents to enter clinical trial, has been shown to exert multiple effects, including inhibition of vascular endothelial cell uPA expression and suppression of endothelial cell proliferation and migration. Flavonoids, which occur in a variety of plants, and are present in at biologically relevant concentrations in some human diets, have antiangiogenic properties: they block angiogenesis in in vitro assays, suppressing VEGF-induced invasion of microvascular endothelial cells by mechanisms that involve PKC (Fotsis et al., 1997). Epigallocatechin gallate, a flavonoid from tea, is a potent inhibitor of MMP-9 and MMP-2 activities (Tosetti et al., 2002).

Angiogenesis: a Biomarker and a Target for Prevention

Numerous experimental and clinical studies suggest that anti-angiogenic agents have promise as cancer chemopreventives. Angiogenesis is a prerequisite for tumour appearance, growth and metastasis. The association between cancer occurrence and angiogenesis, and ease of detection of this process in accessible tissues early in carcinogenesis, mean that angiogenesis may also be a biomarker of efficacy of the chemopreventive intervention (Sharma et al., 2001). For many of the well-known chemopreventive agents currently under intensive study, such as NSAIDs, aromatase inhibitors, and estrogen receptor modulators, the antiangiogenic activity may contribute to their cancer-preventive effect. In future, development and use of specific anti-angiogenic agents may open new avenues for cancer chemoprevention, alone or in combination with chemopreventive drugs acting through other mechanisms. However, a myriad of questions about safety, efficacy, optimal treatment regimen, and mechanisms of action of these anti-angiogenic agents need to be resolved before their clinical application is feasible among 'low-risk', healthy populations. The utility of angiogenesis as a chemoprevention target, and as a biomarker in chemoprevention trials would be enhanced by development of sensitive high-throughput technologies to measure the phenomenon in clinical settings. Currently most commonly used technique is to measure angiogenesis by microvessel density determination (by immunohistological staining) (Fox and Harris, 1997). In the future, molecular profiling of precancerous lesions by using expression microarrays and/or proteomics analysis will most likely identify a panel of markers suitable for developing preventive strategies based on anti-angiogenesis.

Anti-angiogenesis strategy alone may not be sufficient in preventing tumour growth and spread, and therefore it has been suggested that approaches combining different mechanisms may be more efficacious. A combination of

angiogenesis inhibitors together with antiestrogen drugs were reported to have enhanced effects to liposome-mediated angiostatin c-DNA alone in arresting tumour growth and metastasis in MMTV-neu mice (Sacco et al., 2002).

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