Angiogenesis Inhibition in the Prevention of Colorectal Cancer

Edited by William W. Li, M.D., and Randall E. Harris, M.D., Ph.D.

Cancer of the colon and rectum caused an estimated 49,920 deaths in the U.S. in 2009, with nearly 147,000 estimated new cases diagnosed. Adenomas, benign epithelial tumors of the large bowel, are the precursors of most colorectal cancers. Angiogenesis, the sprouting and growth of new blood vessels from existing ones, is critical for the development and metastasis of colorectal cancer (CRC), beginning at the stage of premalignant adenomatous polyposis.

From the Editor-in-Chief

Angiogenesis, the growth of new tumor blood vessels, is necessary for the development and spread of cancer. The early work of Dr. Judah Folkman showed that neovascularization is required for tumors to grow beyond 2-3 mm in size, a seminal finding that paved the way for new antiangiogenic cancer therapies aimed at disrupting angiogenesis signaling pathways. These therapies are primarily used in the advanced or metastatic disease settings.

Compelling data from a growing body of preclinical research demonstrate that angiogenesis occurs within pre-malignant lesions, much earlier than previously believed. In experiments with transgenic mice, Sung-Hee Chang and colleagues recently demonstrated that COX-2-derived prostaglandin E2 (PGE_2) induces angiogenesis at the earliest stage of tumor development, even before PGE_2-induced mammary gland hyperplasia. More importantly, administration of a COX-2 inhibitor at this very early disease stage suppressed both angiogenesis and tumor growth.

In a second study, Nina Korisisaari and colleagues showed that targeting VEGF (vascular endothelial growth factor) with an anti-VEGF monoclonal antibody both reduces tumor burden and prolongs survival in mice with benign adenomatous polyposis, a model for colorectal cancer precursor lesions in humans. These studies build upon preclinical and clinical data generated during the 1980s and 1990s supporting the concept of ‘angioprevention’—the prevention of disease by inhibiting angiogenesis.

There is now a compelling case for examining opportunities to suppress early tumors by inhibiting angiogenesis. Indeed, Judah Folkman argued strongly that tumors could be kept in a dormant microscopic state by interfering with their ability to become vascularized. In this issue of Targeting Tumor Angiogenesis we review the evidence supporting antiangiogenic chemoprevention of CRC, including the latest clinical data concerning NSAIDS, selective COX-2 inhibitors, and nutraceuticals. I am pleased to be joined by our newest faculty member, Randall E. Harris, M.D., Ph.D., Director of the Center of Molecular Epidemiology and Environmental Health at The Ohio State University Medical Center. Dr. Harris is a pre-eminent expert in the epidemiology of cancer prevention. He provides key insights into cancer prevention studies involving drugs that inhibit the initiation and development of CRC by suppressing angiogenesis and chronic inflammation.


William W. Li, M.D., President, The Angiogenesis Foundation

Randall E. Harris, M.D., Ph.D.

Antiangiogenesis, a treatment strategy that interferes with the formation of new tumor blood vessels, has been shown in laboratory studies to effectively suppress both polyp and CRC development. Clinically, antiangiogenic therapy improves outcomes in patients with advanced or metastatic colorectal cancer (mCRC) when used as an adjunct to cytotoxic chemotherapy.

In 2004, bevacizumab (Avastin®; BV), a monoclonal antibody against vascular endothelial growth factor (VEGF), was approved for front-line mCRC treatment in combination with IFL chemotherapy (irinotecan/bolus 5-FU/leucovorin), thus becoming the first specifically designed, clinically validated antiangiogenic cancer therapy. Subsequently, two randomized phase 3 trials established the efficacy of BV in combination with the more contemporary FOLFOX (5-FU/leucovorin/oxaliplatin) and XELOX (capecitabine and oxaliplatin) regimens in both front-line and second-line mCRC treatment settings. A number of antiangiogenic tyrosine kinase inhibitors (TKIs) are also in late stage clinical trials for advanced CRC. These orally administered agents disrupt angiogenic signaling at the intracellular level by binding competitively to the ATP binding sites on receptors for VEGF (VEGFR-1, -2, and -3), platelet-derived growth factor receptor (PDGFR), and stem cell factor receptor (c-KIT). Two of these small molecule drugs, sunitinib (Sutent®) and sorafenib (Nexavar®), are already approved for treating advanced cancers of the kidney, liver, and gastrointestinal stroma.

At the other end of the disease spectrum, there is considerable interest in cancer chemoprevention—the long-term use of oral agents at safe and tolerable doses to delay, prevent, or even reverse the course of tumor progression from adenoma to carcinoma in the colon. The natural evolution of CRC from normal intestinal mucosa to adenoma to full-blown malignancy is a stepwise process that spans 10-20 years. Recent experimental studies have shown that angiogenesis is initiated much earlier in the continuum of tumorigenesis than previously thought, a finding that could lead to new opportunities for cancer prevention. This article reviews the major mechanisms of angiogenesis and tumorigenesis in CRC, with an emphasis on early stage disease and chemoprevention.

Angiogenesis and Colon Tumorigenesis

In the early stages of cancer development, premalignant cells must ‘switch’ to an angiogenic phenotype, at which time an increased expression of proangiogenic growth factors by abnormal cells and concurrent downregulation of endogenous angiogenesis inhibitory factors initiate neovascularization. The predominant mediator of tumor angiogenesis is vascular endothelial growth factor (VEGF), an endogenous cytokine that stimulates endothelial cells to proliferate and migrate from pre-existing vessels toward VEGF-expressing tumors cells to form new
capillary tubes and loops. VEGF production is driven primarily by hypoxia in the tumor microenvironment, but may also be stimulated independently by acquired genetic mutations, such as p53, a well-known marker of colon polypl dysplasia and invasiveness. Other growth factors implicated in angiogenesis and tumorogenesis in CRC include basic fibroblast growth factor (bFGF), transforming growth factor-α (TGF-α), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF). Additionally, colorectal tumors express reduced amounts of the angiogenesis inhibitor thrombospondin-1 (TSP-1). Deficiency of this protein correlates clinically with increased microvessel density, poor prognosis, and tumor recurrence in CRC.

After several epidemiological studies showed reduced cancer risk in individuals who regularly used aspirin or other NSAIDs that suppress cyclooxygenases-1 and -2 (COX-1 and COX-2), researchers began examining the role of these enzymes in the initiation of tumorgenesis and angiogenesis. COX-2 is overexpressed in a wide array of premalignant conditions and cancers, including approximately 70% of sporadic colorectal adenomas and 80-85% of CRC. Induction of the constitutive expression of the COX-2 gene during chronic inflammation is now recognized as a key event in the carcinogenic process. The COX pathway is one of three major metabolic pathways—along with the lipoperoxides (LOX) and cytochrome P450 pathways—for arachidonic acid (AA), a cell membrane fatty acid that is a key substrate in inflammation. Prostaglandin E2 (PGE2), one of the predominant products of AA metabolism, is an important mediator of chronic inflammation that has been linked to carcinogenesis. Expression of COX-2 and its metabolites, including PGE2, have been shown to promote cancer cell proliferation, tumor invasion, metastasis, recurrence (in CRC), angiogenesis, and immunosuppression.

Most recently, overexpression of COX-2 has been implicated in suppressing cancer cell apoptosis (programmed cell death) and promoting cellular immortality—two key mechanisms in carcinogenesis—by reducing the accumulation of AA within cells. High cellular expression of COX-2, one of the chief catalytic enzymes of AA, impedes apoptosis by increasing the metabolism of AA, aiding cancer cells’ survival. The anti-neoplastic effects of COX-2 inhibitors may therefore be attributable, in part, to increased apoptosis.

The role of COX-2 in inducing and maintaining angiogenesis is well established. COX-2 (but not COX-1) is overexpressed by tumor endothelial cells (but not by endothelial cells of normal vasculature). Overexpression of COX-2 in a study of transgenic mice induced the angiogenic switch in the mice’s mammary glands even before the induction of epithelial hyperplasia. Once initiated, angiogenesis was sustained through the entire progression from normal tissue to cancer. Moreover, exposure of tumor cells in vitro to PGE2 resulted in enhanced expression of known angiogenic stimulators, including VEGF, VEGFR-1, and angiopoietin-1 and -2 (Ang-1 and Ang-2). When researchers suppressed COX-2 using the selective COX-2 inhibitor celecoxib (Celebrex®), the tumor vessels exhibited microvessel collapse and diminished vessel density in association with increased tumor cell apoptosis. The production of VEGF in experimental cancer models occurs downstream of COX-2-mediated production of prostaglandins. Inhibition of either VEGF or COX-2, or both, in these experiments results in suppression of both angiogenesis and tumor growth. These findings refine our understanding about the importance of COX-2 in regulating the angiogenic switch and in sustaining angiogenesis though cancer progression.

Clinical Evidence for CRC Chemoprevention

Aspirin

The concept that NSAIDs might inhibit CRC arose in the 1970s, when it was discovered that concentrations of PGE2 were higher in colorectal tumors than in surrounding normal colon mucosa. Since then, more than 200 randomized, controlled animal studies and dozens of epidemiological studies in humans have demonstrated a clear preventative effect of NSAIDs in the development of pre-cancerous adenomas and CRC. Long term (≥ 5 years) use of aspirin, a non-selective inhibitor of COX-1 and -2, has been shown to significantly reduce the risk of developing CRC. The Health Professionals Follow-up Study involving more than 47,000 male health professionals in the United States found that regular aspirin use (≥ 2 times per week) was associated with a 21% reduction in CRC risk compared with non-regular users. The cancer-lowering effect of aspirin in this study, however, required ≥ 6-10 years of therapy, with the greatest risk reduction observed at a cumulative dose of > 14 standard (325 mg) tablets per week (RR, 0.30). By contrast, the aspirin dose typically used for cardioprevention or reducing adenoma recurrence is 81 mg/day. In the Cancer Prevention Study II Nutrition Cohort, which examined the long-term use of aspirin (325 mg/day) in 69,810 men and 76,303 women, daily aspirin for > 5 years was associated with a 32% lower risk of developing CRC.

As a chemopreventive agent for polyposis and CRC, the dose of aspirin appears to be important. The Women’s Health Study—a randomized trial of aspirin for the primary prevention of cancer and cardiovascular events—failed to demonstrate a reduction in CRC risk after 10 years of treatment. A secondary analysis in the Physicians’ Health Study also found no evidence of a reduction in CRC among men after 5 years of aspirin therapy. Both of these studies, however, used relatively low aspirin doses (100-325 mg every other day). In the Health Professionals Follow-up Study, low aspirin doses also had no effect on CRC risk (RR, 0.94). Rather, progressively greater risk reductions did not become apparent until the aspirin dose exceeded 6 standard tablets per week. Other clinical studies have confirmed a strong dose-dependent relationship between aspirin and CRC risk reduction, and preclinical research indicates that higher aspirin doses are required to maximize suppression of both COX-2 and non-COX-2 pathways of angiogenesis and tumorgenesis. Taken as a whole, the available data suggest that 10-20 years of aspirin therapy at doses ≥ 325 mg/day are required to realize a significant risk reduction in primary CRC.

For people with a documented history of adenomas or CRC, however, much shorter durations of aspirin therapy have shown substantial activity for preventing recurrence of these conditions. In results from two randomized, placebo-controlled trials, aspirin at doses ranging from 81-325 mg/day significantly reduced the incidence of adenoma recurrence compared with placebo in patients with a prior history or either adenoma or CRC. In the first study, 635 patients with a history of colon or rectal cancer who were at low risk for recurrence were randomized to receive either placebo or aspirin 325 mg/day. At a median follow-up of just over 1 year, 27% of patients in the placebo-treated group had ≥ 1 recurrent adenoma compared with 17% in the aspirin-treated group (P=0.004). The average number of adenomas that developed was also significantly lower in the aspirin cohort (P=0.003). The second study randomized 1,121 patients with a recent history of adenomas to receive either placebo or daily aspirin at 81 mg or 325 mg. For reasons that are not clear, only the 81 mg aspirin-treated group showed a significant relative reduction (19%) in the overall risk of adenomas at approximately 3 years, with a 41% risk reduction for advanced adenomas. By contrast, the 325 mg group had only a non-significant 4% risk reduction.

Most recently, findings were published on the effects of aspirin use in 1,279 patients—840 women from the Nurses’ Health Study and 439 men from the Health Professionals Follow-up Study—with pathologically confirmed stage I-III (non-metastatic) CRC. After a median follow-up of 11.8 years, researchers documented a 29% reduction in CRC-specific mortality and a 21% reduction in overall mortality among regular aspirin users (325 mg two or more times per week) compared with non-users. For the 719 patients who initiated aspirin therapy only after a diagnosis of CRC, the benefits were even more pronounced: CRC-specific mortality and overall mortality were reduced by 47% and 32%, respectively. The survival benefit was independent of age, sex, cancer site (colon vs. rectum), and disease stage, and held for both patients who received standard adjuvant CRC chemotherapy and those who did not.
Aspirin and other NSAIDs
Folate
Calcium

Aspirin and other NSAIDs

Estrogen Aspirin and other NSAIDs

Normal epithelium
Hyperproliferative epithelium Aberrant cryptic foci
Small adenoma
Large adenoma
Colon carcinoma

COX-2 overexpression
APC mutation
K-ras mutation
p53 mutation
Loss of 18q

Notably, only patients whose tumors were positive for COX-2 expression had improved survival, a finding that supports both the biological underpinnings of COX-2 suppression for prevention of CRC or its recurrence, and the potential utility of COX-2 expression status as a predictive biomarker for CRC. In addition, CRC patients who used aspirin before their cancer diagnosis did not have an anti-tumor benefit from continuing aspirin therapy post-diagnosis, suggesting that these patients’ tumors had already developed resistance to COX-2 suppression.

**Selective COX-2 Inhibitors**

Selective COX-2 inhibitors were developed to provide a safer non-ulcerogenic alternative to non-selective NSAIDS for patients who required long-term treatment for chronic pain. The first cancer prevention study using a selective COX-2 inhibitor was a randomized, placebo-controlled trial of celecoxib, a drug known to inhibit angiogenesis, in 83 individuals with familial adenomatous polyposis (FAP), an inherited condition associated with an excessive rate of invasive CRC if left untreated. After just 6 months of treatment with celecoxib (400 mg twice daily), FAP patients had a significant 28% reduction in the average number of colorectal polyps, and a 31% reduction in polyp burden (the sum of polyp diameters) compared with placebo.

Based on this initial FAP study, three major chemopreventive trials were initiated using selective COX-2 inhibitors: the Adenoma Prevention with Celecoxib (APC) trial, the Prevention of Sporadic Adenomatous Polyps (PreSAP) trial, and the Adenomatous Polyp Prevention on Vioxx® (APPROVe) trial. In the APC trial, 2,035 patients at high risk for adenomas were randomized to receive either placebo or celecoxib (200 mg or 400 mg) twice daily. After 3 years of surveillance, adenomas were reduced by 33% and 45%, respectively, for patients receiving 200 mg or 400 mg of celecoxib. A 5-year follow-up colonoscopy was conducted in 639 patients participating in an extension of the APC trial. Due to the early discontinuation of treatment in the study, however, the median duration of celecoxib exposure was just over 3 years, with an even distribution among treatment groups.

At 5 years, there was a 13.7% reduction for the celecoxib 200 mg arm and a 12.1% reduction for the 400 mg arm relative to placebo. The cumulative incidence of advanced adenomas over the 5 years was 21.3% in the placebo arm compared with 12.5% and 15.8% in the celecoxib 200 mg and 400 mg arms, respectively. In the PreSAP trial, celecoxib 400 mg once daily was associated with a 36% reduction in the risk of developing adenomas through 3 years. The third trial, APPROVe, examined the efficacy of rofecoxib (Vioxx®) 25 mg once daily for prevention of adenomas. Over 3 years there was a 24% reduction in adenoma development in the rofecoxib arm relative to placebo.

**Calcium and Vitamin D**

Preclinical, clinical, and epidemiologic studies have suggested a chemopreventive role for calcium and vitamin D supplements for adenomas and CRC. Calcitriol, the active form of vitamin D, has been established to inhibit angiogenesis. Supplemental calcium of 2-3 g daily was shown to moderately reduce the risk of colon adenoma recurrence in two randomized clinical trials, and high dietary intake of calcium plus vitamin D has been associated with a reduced risk of CRC and adenoma in epidemiologic studies in women. A recent meta-analysis of five studies that examined an association between levels of serum 25-hydroxyvitamin D (25(OH)D), the main circulating form of vitamin D, and CRC risk found that a serum 25(OH)D level ≥ 33 ng/mL was associated with a 50% lower incidence of CRC than a level < 12 ng/mL. Vitamin D has been shown to be non-toxic at levels of 2000 IU/day, and possibly at much higher levels. Therefore, daily vitamin D intake of 1000-2000 IU—dosages necessary to achieve median serum 25(OH)D levels of 33-46 ng/mL—could substantially reduce incidence rates of CRC with minimal risk of toxicity. The largest randomized study to date concerning vitamin D and cancer risk was the Women’s Health Initiative Trial involving 36,282 postmenopausal women over age

---

**Cardiotoxicity with Selective COX-2 Inhibitors — What is the Evidence?**

The three major preventative COX-2 studies, PreSAP, APC, and APPROVe, were halted early after interim analyses from the APPROVe and APC trials showed an increased incidence of thrombotic cardiovascular events in patients receiving COX-2 inhibitors compared with placebo-treated patients. For rofecoxib, the increased risk of cardiovascular complications (myocardial infarction and stroke) was observed in patients who took the drug for > 18 months. A subsequent meta-analysis also noted an increased risk of thrombotic cardiotoxicities in patients who took daily rofecoxib at doses exceeding 25 mg. Patients in the rofecoxib treatment arm in APPROVe also experienced a significant and unexpected increase in the incidence of upper gastrointestinal complications, which were not seen with celecoxib. Based largely on cardiotoxicity concerns, the manufacturer of rofecoxib voluntarily withdrew the drug from the market.

The cardiovascular safety issues surrounding celecoxib are less established and more controversial. An examination of cardiovascular events in the APC trial found a small dose-dependent increase in the risk of cardiovascular events for patients in the celecoxib arms compared with placebo. This observation, however, contradicted a number of earlier studies with celecoxib that showed no such effect. Specifically, a composite endpoint of death from cardiovascular causes, MI, stroke or heart failure was reached in 7 of 769 patients (1.0%) in the placebo arm, as compared with 16 of 685 patients (2.3%) in the celecoxib 200 mg twice daily arm, and in 23 of 671 patients (3.4%) in the high-dose celecoxib cohort receiving 400 mg twice daily (800 mg/day). There was also a small, non-significant increase in the number of venous thromboembolic events in the celecoxib arms. These findings, along with the rofecoxib safety concerns, prompted the early discontinuation of the celecoxib treatment arms in the APC trial. In the PreSAP trial, a lower daily celecoxib dose (400 mg) was used, with no apparent increase in cardiovascular risk.

A recent examination of the literature raises questions about whether cardiovascular concerns with celecoxib are valid. In a meta-analysis of 72 studies (39 short-term randomized trials, 26 observational studies, and 7 long-term randomized trials), regular use of celecoxib at doses < 400 mg/day was not associated with an increased risk of thrombotic cardiovascular events (composite relative risk = 0.98, 95% CI: 0.88-1.10). Furthermore, a recent study suggests that cardiotoxicities associated with rofecoxib may be attributable to unique chemical and metabolic properties. Specifically, rofecoxib was shown to increase the susceptibility of human LDL and cell membrane lipids to oxidative modification, a hallmark feature of atherosclerosis that has not been observed with other chemically distinct (sulfonamide) COX-2 inhibitors, such as celecoxib, under the same conditions. These results cast doubt on the broad assumptions within the medical community that all selective COX-2 inhibitors increase the risk of cardiovascular events.

---

**Celebrex at 400 mg or less daily: RR of CVD in Epidemiologic Studies**

[Graph showing relative risk estimates from 73 epidemiologic studies combined with 97% CI, No change in risk]
and activity of COX-2 in patients with recently resected sporadic adenomatous polyps. Selenium, a trace element that possesses antiangiogenic activity, has also shown evidence in some epidemiological studies of a protective effect for CRC. A pooled analysis from three randomized studies found an inverse association between blood selenium levels and risk for colorectal adenomas, such that individuals whose blood selenium values were in the highest quartile (median, 150 ng/mL) were at significantly lower risk for developing new adenomas than those in the lowest quartile (odds ratio, 0.66). A randomized, placebo-controlled phase 3 trial is underway to evaluate the efficacy of selenium for preventing the recurrence of adenomatous colorectal polyps.

Clearly, a significant number of naturally occurring substances found in everyday foods have shown considerable potential for reducing the risk of developing cancer and/or its recurrence. Advanced technologies that can isolate, synthesize, and test the antiangiogenic and antitumorigenic potency of these compounds may lead to the development of powerful new tools for chemoprevention.

**References**