2 Chemoprevention of Colorectal Cancer

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Summary

This article emphasizes current understanding of the multistep process in colon carcinogenesis and discusses the promising strategies of targeting disruption of β-catenin-mediated signaling in colon epithelial cells. The 1,2-dimethylhydrazine (DMH)/azoxymethane (AOM) model of chemically induced colorectal cancer (CRC) and the murine multiple intestinal neoplasia (Min) model have provided useful information about the efficacy of available chemoprevention agents for CRC.

Clinical trials have determined that several classes of agents can reduce polyp incidence and, by extension, may defer the appearance of colon cancer. The most important ones are nonsteroidal anti-inflammatory drugs such as aspirin and selective cyclooxygenase-2 inhibitors such as celecoxib and calcium supplements. Preclinical and epidemiology evidence suggests that statins, eflornithine, ursodeoxycholic acid, selenium, folate, and estrogen may reduce polyps and prevent CRC. Neither increased fiber intake nor antioxidant supplements are associated with reduced polyp/cancer outcomes in carefully controlled clinical trials.

Patients who are predisposed to early onset of CRC may benefit from specific chemoprevention therapy: ulcerative colitis (5-aminosalicylic acid) and familial adenomatous polyposis (celecoxib or sulindac). Several promising agents are under study: curcumin, inulin derivatives, epidermal growth factor inhibitors, statins and nitric oxide-releasing nonsteroidal anti-inflammatory drugs.

Key Words: Chemoprevention; colon cancer; animal models; clinical trials.

1. INTRODUCTION

The colon is an ideal target organ in which to develop chemopreventive interventions for a number of reasons. First, it is estimated that colorectal cancers develop over a period of 10–20 yr, providing a large window of opportunity for

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therapeutic intervention. Second, the relative sequence of genetic events required for tumor formation has been investigated most extensively in the colon *(2,3)*. Third, the established histopathological progression of normal tissue to an intermediate adenoma and, ultimately, invasive cancer presents milestones with respect to where a particular lesion is in the carcinogenic sequence, both in the presence and absence of chemopreventive agent exposure *(4)*. Fourth, the adenomatous polyp serves as a preneoplastic marker of colorectal cancer risk, aiding in the identification of the subpopulation of individuals who would benefit most from chemopreventive therapy. Finally, unique insight can be gained from evaluating chemopreventive response in individuals known to carry germline mutations that predispose them to such familial colorectal syndromes as familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer.

Based on our current understanding of the multistep process of colorectal carcinogenesis, we can begin to identify potential points for intervention. Among the earliest known genetic alterations in colorectal cancer development are inactivating mutations in the tumor suppressor gene adenomatous polyposis coli (*APC*) and activating mutations of the *K-ras* oncogene. These alterations are associated with the formation of early and intermediate adenomas, respectively. Alteration of the transcription factors Smad2 and Smad4 appear to mediate the transition to late adenoma. Finally, mutations in the tumor suppressor gene *p53* pave the way for transformation of adenomas into malignant cancers. Additional alterations that occur early in colorectal tumorigenesis include genome-wide DNA hypomethylation and genomic instability, via defects in chromosome segregation or DNA replication fidelity.

Early changes within the colonic epithelium that persist throughout tumorigenesis represent ideal targets for chemopreventive intervention. Additional characteristics that make a potential molecular event an attractive target have been reviewed recently by Hawk and Levin *(5)*. These include (1) differential expression of the marker in neoplastic and normal tissue, (2) knowledge of its functional significance, (3) overexpression of the marker in the neoplastic state such that expression can be downregulated rather than replaced when absent, (4) pharmacologic accessibility, and (5) an established correlation between modulation of the marker and tumor reduction. Finally, appealing targets are typically characterized by overexpression or overactivity because molecular functions are more easily inhibited than replaced *(6)*.

One of the most promising strategies for early chemopreventive intervention in the colorectal carcinogenesis sequence is targeted disruption of β-catenin-mediated signaling. Low levels of cellular β-catenin are maintained in normal cells via the competitive binding of β-catenin to APC and E-cadherin, a calcium-dependent cell adhesion molecule thought to act as an "invasion suppressor." Complexing of β-catenin with the scaffold protein axin and the serine/threonine glycogen synthase kinase 3β (GSK3β) facilitates the phosphorylation of β-catenin by GSK3β

and targets its ubiquitin-mediated proteosomal degradation (Fig. 1). In contrast, inactivation of GSK3β by Wnt signals, mutational activation of β-catenin, and truncation of *APC* lead to the accumulation of β-catenin in the cytoplasm. Once stabilized, β-catenin is translocated to the nucleus where it cooperates with members of the TCF/LEF family of transcription factors *(7,8)* to activate the transcription of downstream target genes including *c-myc (9)*, cyclin D1 *(10)*, peroxisome proliferator-activated receptor (PPAR) delta *(11)*, multidrug resistance protein 1 *(12)*, cyclooxygenase 2 *(13)*, and immunoglobulin transcription factor-2 *(14)*. cDNA array analyses indicate that induction of dominant negative *TCF*-4 in colon carcinoma cells leads to the differential expression of more than 200 genes *(15)*. Based on these data, van de Wetering and colleagues *(15)* have coined the term "master switch" to describe the capability of the β-catenin/TCF-4 complex to effectively regulate the balance between proliferation and differentiation in both non-neoplastic and malignant intestinal epithelial cells.

Although our understanding of the colon carcinogenesis sequence is in general more advanced than that of many other cancer types, the establishment of an efficacious chemopreventive regimen has been severely hindered by the lack of accurate and sensitive biomarkers of chemopreventive response. Because tumor formation cannot be used as an endpoint for clinical investigation, it is essential that intermediate endpoints that can predict with high sensitivity and specificity the future progression and invasive potential of malignant cells be identified. The endpoints most frequently studied in current clinical colorectal chemoprevention trials are polyp number and polyp recurrence, often complemented by correlative measures of cell proliferation and apoptosis. Based upon the generic nature of these biochemical pathways, significant attention is currently focused on invaluable model systems where the molecular events associated with colorectal carcinogenesis and its inhibition can be interrogated extensively under controlled conditions.

2. ANIMAL MODELS OF COLORECTAL CARCINOGENESIS

One of the most widely used animal models for assessing the efficacy of chemopreventive agents against colorectal cancer is the 1,2-dimethylhydrazine (DMH)/azoxymethane (AOM) model of chemically induced colorectal cancer. DMH requires metabolic activation in vivo to AOM, which is then converted to the ultimate carcinogen methylazoxymethanol (MAM). In rats, AOM is injected i.p. once a week for 2 wk *(16)*. One week later, animals are randomized to control and experimental drug treatment groups. Aberrant crypt foci (ACF), a putative cancer precursor, can be identified 14 wk following AOM injection *(17)*. Gross colonic tumors (one per animal) are present 40 wk post-AOM. In mice, female CF1 mice are given MAM i.p. four times in 11 d (low dose) or eight times in 22 d (high dose). Colon tumors are observed within

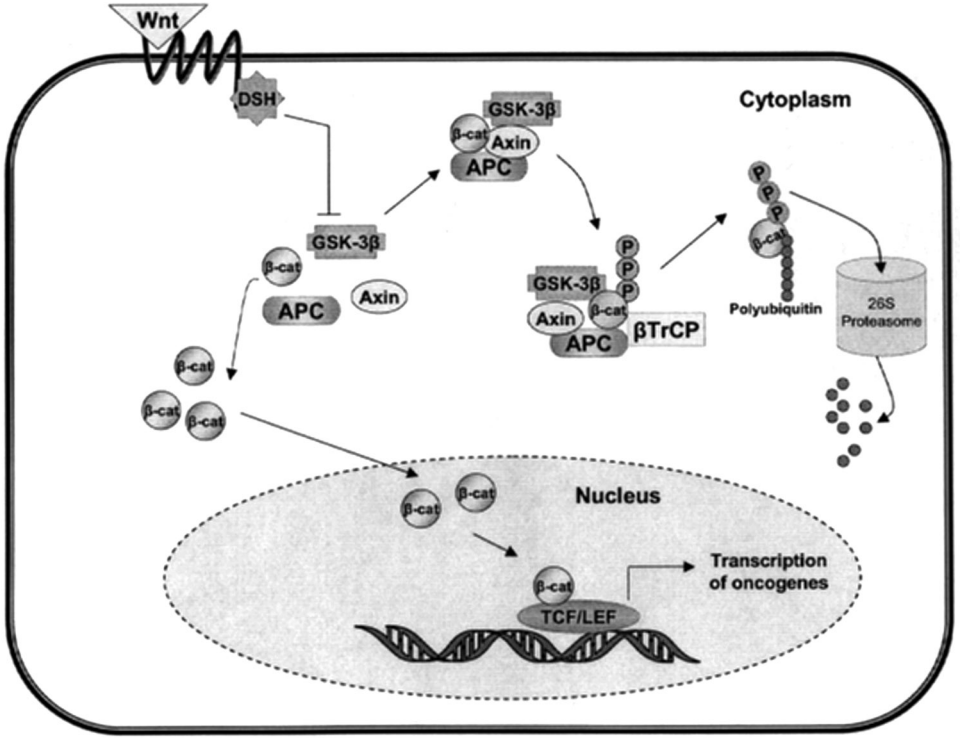

Fig. 1. Role of APC/β-catenin Signaling in Colorectal Carcinogenesis. In the absence of Wnt signals, low levels of cellular β-catenin are maintained via interaction of APC with β-catenin, glycogen synthase kinase-3β (GSK3β) and Axin to facilitate the phosphorylation of β-catenin by GSK3β and lead to the targeted degradation of β-catenin. In contrast, inactivation of GSK3β by Wnt signals, mutational activation of β-catenin and truncation of *APC* lead to the accumulation of β-catenin in the cytoplasm. Once stabilized, β-catenin is translocated to the nucleus where it cooperates with members of the TCF/LEF family of transcription factors to activate the transcription of downstream oncogenes.

38 wk after dosing *(18)*. There are several advantages to using the DMH/AOM model of colon carcinogenesis for chemoprevention studies. First, experimentation to date indicates that the promotional and protective effects of experimental diets can be discriminated in this model *(19,20)*. Second, the evolution of colon tumors in the DMH/AOM model is similar to that in humans, including the progression of ACF to adenomas (often polyps), and ultimately carcinomas. Third, the histopathological features of DMH/AOM-induced colon tumors are similar to those of human tumors. Finally, 30–60% of DMH/AOM-induced colon tumors possess *K-ras* mutations as seen in human colon tumors. The pitfalls of using the DMH/AOM model system to study colorectal carcinogenesis include the fact that both DMH and AOM are carcinogens to which humans are not exposed either environmentally or in their diet. Furthermore, unlike human colon tumors, DMH/AOM-induced tumors seldom exhibit mutations in either *Apc* (approx 8%) or *p53*. However, nuclear localization of β-catenin is observed in AOM-induced colon tumors owing to mutations of β-catenin.

Another animal model used frequently for evaluating the efficacy of chemopreventive agents is the murine model of multiple intestinal neoplasia (*Min*). Conventional *Min* mice carry a germline mutation in the *Apc* gene, which results in a premature translational stop codon at amino acid 850 *(21)*. Since their discovery, *Min* mice have been used widely for chemoprevention studies for several reasons. First, *Min* mice spontaneously develop intestinal tumors at 60–90 d of age. Second, COX-2 and iNOS play an important role in intestinal tumorigenesis in this model as in humans. When the genes for either COX-2 or iNOS were deleted in *Min* mice, few intestinal tumors were observed *(22–24)*. Third, similar to humans, a reduction in DNA methyltransferase activity in *Min* mice suppresses polyp formation *(22,23)*. As with the DMH/AOM model, the *Min* mouse model carries some disadvantages for chemopreventive analyses. The relevance of this model to the study of human colorectal cancer remains uncertain because of the predominance of small intestinal lesions and few, if any, colorectal tumors, as well as no invasive colorectal carcinomas. In addition, mutations in *K-ras* and *p53* are not detected in intestinal tumors from *Min* mice *(25,26)*.

Currently, more than 400 studies have been performed using either the DMH/AOM or *Min* mouse model to assess the chemopreventive activity of synthetic or naturally occurring agents or diets against colorectal cancer (reviewed in *27,28*) Based on these studies, Corpet and colleagues have created a comprehensive database of agents and diets that have been tested to date and have ranked these agents based on their ability to inhibit colorectal cancer (http://www.inra.fr/reseau-nacre/sci-memb/corpet/indexan.html). Agents that afford strong protection against intestinal tumorigenesis in the DMH/AOM rat and/or *Min* mouse models include piroxicam, sulindac, celecoxib, difluoromethylornithine, polyethylene glycol, thiosulfonate, protease inhibitor, sphingomyelin, epidermal growth factor receptor kinase inhibitor, resveratrol, fish oil, curcumin, and calcium.

3. CLINICAL TRIALS FOR THE CHEMOPREVENTION OF COLORECTAL CANCER

Once promising chemopreventive agents have been identified through observational and in vivo efficacy studies or have exhibited evidence of in vitro molecular targeting *(6)*, these agents are then subjected to examination in clinical trials. The long-term goals for the clinical chemoprevention of colorectal cancer include at least one of the following: (1) An additive clinical benefit when combined with colonoscopic colorectal polyp screening/surveillance; (2) an alternative to current colonoscopic screening/surveillance guidelines; (3) improvement in the overall risk profile for the development of serious adverse events or death linked to colonoscopy or polypectomy; and (4) an improvement in colorectal cancer rates in individuals who do not or are unable to comply with standard screening and surveillance recommendations *(6,29)*.

A significant amount of knowledge has been gleaned from the colorectal cancer chemoprevention trials that have been performed to date, data that has proven invaluable in establishing the guidelines for future colon trials. In general, 25 to 35% reduction in adenoma formation represents a reasonable minimum threshold of effect. Positive trials should yield supportive data, including an increase in the number of adenoma-free patients and/or reductions in adenoma size and histopathologic grade. It is essential that trials be well controlled, have adequate compliance, and be 3 to 6 yr in duration so that issues of safety and tumor recurrence upon agent withdrawal can be addressed.

4. CHEMOPREVENTIVE AGENTS TESTED IN HUMANS FOR THEIR EFFICACY AGAINST COLORECTAL CANCER

4.1. Nonsteroidal Anti-Inflammatory Agents

Nonsteroidal anti-inflammatory agents (NSAIDS) are among the most well described chemopreventive agents. Cyclooxygenase (COX)-1 and -2 catalyze the conversion of arachadonic acid to the intermediate PGG2 and then to PGH2. PGH2 is then metabolized to thromboxane and other prostaglandins, which affect various physiologic functions. Whereas COX-1 produces constitutive prostanoids used for normal tissue functions such as platelet aggregation and gastric mucosal protection, COX-2 is inducible, with expression increasing during inflammation and neoplasia *(30)*.

Aspirin, like most nonselective NSAIDs, works to competitively inhibit the active binding site on both COX-1 and COX-2. Side effects of NSAIDs, including renal, gastrointestinal, and antiplatelet effects, are attributed to inhibition of COX-1, whereas their anti-inflammatory activity is a result of their ability to inhibit COX-2 *(31,32)*. Cancers of most organs, including colon, bladder,

breast, liver, and lung express increased levels of COX-2 as compared to the non-neoplastic adjacent tissue, making the COX-2 gene an important target in the study of carcinogenesis *(30)*.

The molecular basis for the activity of NSAIDs in the prevention and treatment of cancer is thought to be pleiotropic. COX-2 overexpression has been shown to increase factors associated with angiogenesis, a mechanism that can be blocked by selective COX-2 inhibitors and some nonselective COX inhibitors. COX-2 overexpression also inhibits apoptosis, a condition that may be reversed by NSAIDs. Animal studies have shown that chemical inhibition or elimination of COX activity results in decreased or slower tumor formation *(30)*.

Four randomized colorectal cancer chemoprevention studies have been published that compare the effect of aspirin administration vs placebo. In the Physicians' Health Study of 22,071 healthy men, aspirin intake did not provide protection against colorectal cancer. *(33)*. However, three smaller studies of aspirin vs placebo in patients with previous adenomas have reported protective effects on subsequent adenoma formation *(34–36)*. Interestingly, the study by Baron et al. *(35)* found that the adenoma prevention occurred in patients taking 81 mg of aspirin, rather than 325 mg. The cause and implication of this inverse dose response is unclear.

4.2. COX-2-Selective Inhibitors

Despite promising results of studies involving nonselective COX inhibitors, concerns about the gastrointestinal and antiplatelet effects of NSAIDs have limited their use as chemopreventive agents. Selective COX-2 inhibitors such as celecoxib and rofecoxib were initially thought to be promising chemopreventive agents based on their favorable gastrointestinal toxicity profile.

The Adenomatous Polyp Prevention on Vioxx (APPROVe) trial randomized 2586 patients with a history of adenomatous polyps to either Vioxx (rofecoxib) (25 mg daily) or placebo in order to determine if rofecoxib would reduce the risk of recurrent neoplastic polyps. However, the study was closed prematurely when the investigators found an increased risk of thrombotic events associated with long-term use of rofecoxib (response rate [RR] 1.92, 95% confidence interval [CI] 1.19–3.11) *(37)*. Based on these data, the Merck Pharmaceutical Company subsequently withdrew rofecoxib from the market.

The results of the APPROVe study prompted a review of the adverse events recorded in a similar study, the Adenoma Prevention With Celecoxib (APC) trial. In this study, 2035 patients with a personal history of previous neoplastic polyps were randomized to receive either celecoxib (200 mg or 400 mg twice daily) or placebo. A dose-dependent increase in cardiovascular disease, myocardial infarction, stroke, or heart failure was observed among the patients who took celecoxib. The attributable risk for death from cardiovascular disease compared to placebo in the 200 mg twice daily group: 2.3 (95% CI 0.9–5.5); 400 mg twice daily group:

3.4 (95% CI 1.4–7.8) *(38)*. As a result, the APC study was also terminated early at the recommendation of the external data safety monitoring board.

The results of these large randomized studies suggest that the increased cardiovascular risk associated with the use of rofecoxib and celecoxib is likely an effect specific to this chemical class of agents, thus limiting the application of these drugs in the chemopreventive setting for average- to moderate-risk individuals.

4.3. Statins

Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase and are widely used in the management of hyperlipidemia. Clinical trials evaluating the relationship between statins and cardiovascular disease have produced conflicting data on the drugs' effects on incidence of cancer. Results from the Molecular Epidemiology of Colorectal Cancer study, a population-based case control study in Northern Israel of 1953 patients with colorectal cancer and 2015 controls, indicate that at least 5 yr of statin use reduces the relative risk of colorectal cancer (odds ratio [OR] 0.5, 95% CI 0.4 to 0.63) *(39)*. This study supports further investigation into the appropriate dose, optimal length of treatment, and most active type of statin. The potential benefit of using this class of compounds, as well as their well-tolerated toxicity profile, make these agents particularly attractive for chemoprevention. However, placebo-controlled trials may be difficult to conduct as the number of patients who are prescribed these medications for treatment for cardiovascular disease continues to increase *(40)*.

4.4. Eflornithine

D,L-alpha-difluoromethylornithine (DFMO or eflornithine) irreversibly inhibits ornithine decarboxylase (ODC), which is the first and rate-limiting step in polyamine synthesis. ODC and polyamines are both elevated in colorectal cancer and adenomatous polyps *(41)*. Earlier studies of eflornithine at high doses (3 gm/[$m^2 \cdot$ d]) did not demonstrate significant clinical benefit, and its administration was also limited by high rates of gastrointestinal and hematological toxicity as well as ototoxicity. However, interest in this drug as a chemopreventive agent prompted phase I studies to determine the lowest effective dose of eflornithine that would still inhibit ODC *(42)*. One study randomized patients with a history of resected colon polyps to three dose levels of eflornithine and found that a dose of 0.2 g/m^2/d suppressed polyamine levels in rectal mucosa at doses with minimal side effects *(43)*. A review by Meyskens et al. *(44)* proposed that future colorectal cancer chemoprevention trials with eflornithine use doses of 0.2–0.4 g/m^2. However, because eflornithine does not completely suppress tumorigenesis, there is ongoing interest in combining it with other agents such as NSAIDs *(44)*.

4.5. Ursodeoxycholic Acid (UDCA)

UDCA is indicated for the treatment of gallstones and primary biliary cirrhosis. A recently published phase III study randomized 1285 patients with personal histories of adenoma removal to either UDCA or placebo for 3 yr or until the time of follow-up colonoscopy. Although the odds ratio of the rate of adenoma recurrence was not statistically significant, a significant reduction in high-grade adenomas was observed in the treatment arm. (adjusted OR 0.61, 95% CI 0.39–0.96) *(45)*. Because high-grade lesions often progress to cancer, the potential therapeutic benefit, along with its favorable side-effect profile, make this an intriguing agent for future chemoprevention research.

4.6. Calcium

Calcium is thought to reduce the risk of colorectal cancer by binding bile acids in the lumen of the bowel and inhibiting their proliferative and carcinogenic effects. A systematic meta-analysis of three randomized trials that evaluated the use of calcium in patients with previous adenomas has been performed. Of the 1279 patients who completed these three trials, a significant reduction in risk of recurrent adenomas was observed in the treatment groups (RR 0.8, CI 0.68–0.93) *(46)*. The largest of these three studies evaluated 930 patients with previous adenomas following treatment with either 3 g of calcium carbonate or placebo daily. Compared to the placebo group, the patients in the treatment group had an adjusted risk ratio for recurrent adenomas of 0.85 (95% CI 0.74–0.98) *(47)*. Given the low cost and low risk associated with calcium supplementation, as well as the potential benefit of osteoporosis prevention, some experts advocate its use as an adjunct to surveillance colonoscopies in patients with previous adenomas *(48)*.

4.7. Selenium

Selenium is a trace element that occurs in meats and grains. Its potential anticancer mechanisms include the induction of apoptosis, protection from oxidative DNA damage, and increased immune system function *(49)*. In a secondary analysis of a randomized study of selenium vs placebo in the prevention of nonmelanoma skin cancer, the treatment group was found to have a statistically significant decrease in colorectal cancer *(50)*, though these differences did not persist after additional years of follow-up *(51)*. A pooled analysis of the results of three randomized studies (the Wheat Bran Fiber Trial, the Polyp Prevention Trial, and the Polyp Prevention Study) revealed an inverse association between higher blood selenium concentration and recurrent adenomas in patients with previous adenomas. This analysis found that the patients with the highest selenium levels had statistically significantly lower odds of developing recurrent adenomas compared to those with the lowest levels *(49)*. These results support further investigation of the use of selenium as a chemopreventive agent.

4.8. Folate

Because folate is necessary for DNA synthesis, it has been hypothesized that a folic acid deficiency may lead to cancer. An inverse relationship between folate and the risk of colon cancer was found in the Nurses Health Study, particularly in women with a first-degree relative with disease *(52,53)*. An analysis of participants in the Wheat Brand Fiber trial also revealed a lower incidence of adenomatous polyp recurrence in patients with a higher self-reported folate intake (OR 0.61, 95% CI 0.42–0.89) and plasma folate concentrations (0.66, 95% CI 0.46–0.97) *(54)*. Folate is under investigation in several ongoing and recently completed studies.

4.9. Estrogen

The chemopreventive activity of postmenopausal hormone replacement therapy (HRT) against colorectal cancer risk is thought to be caused in part by the effect of estrogen on bile acids, estrogen receptors within the intestinal epithelium, and insulin and insulin-like growth factor I *(55)*. The Women's Health Initiative (WHI) found that HRT increased the risk of invasive breast cancer, cardiovascular disease, stroke, and pulmonary embolism in this group of 16,608 healthy postmenopausal women. The analysis also showed a decrease in the risk of colorectal cancer (HR 0.63, 95% CI 0.43–0.92) in the treatment group *(56)*. A separate analysis of the colorectal cancer data from the WHI found that the 43 invasive cancers in the treatment group and 72 cancers in the placebo group (HR 0.56, 95% CI 0.38–0.81) were of similar grade and shared histological characteristics in common. However, a greater number of cases with positive lymph nodes and metastatic disease were present in the treatment group *(55)*. Therefore, although there is a decreased overall risk of colorectal cancer with the use of HRT, it should not be used as a chemopreventive agent, given its risk–benefit profile.

5. AGENTS LACKING CHEMOPREVENTIVE ACTIVITY AGAINST COLORECTAL CANCER

5.1. Fiber

Two large randomized studies failed to find a benefit of a high fiber diet in reducing recurrent polyp formation. The Phoenix Colon Cancer Prevention Physicians' Network randomized 1429 patients with previously resected adenomas to dietary supplementation with either high (>13.5 g/d) or low (2 g/d) wheat bran fiber. No difference in recurrent adenoma formation was observed at a median follow-up time of 34 and 36 mo, respectively *(57)*. In the Polyp Prevention Trial 2079 patients with previous adenomatous polyps were randomized to intensive dietary counseling with a low-fat, high fiber (18 g of dietary fiber/1000 kcal) diet plus fruits and vegetables or observation. Again, there was no difference in the rate of recurrent adenoma

formation, or the number of large or advanced adenomas detected by endoscopy *(58)*. At this point, there is insufficient data to support fiber as a chemopreventive agent.

5.2. Antioxidants

Antioxidant vitamins, such as vitamin C (ascorbic acid), vitamin E (tocopherols), and β-carotene are thought to prevent cancer by neutralizing free radicals, resulting in reduced oxidative damage, as well as stimulation of the immune system to inhibit tumorigenesis *(6)*.

The Polyp Prevention Study used a two-by-two factorial design to randomize 864 patients with previously resected adenomas to placebo, β-carotene alone, vitamins C and E, or β-carotene and vitamins C and E. The incidence of adenomas in patients receiving either β-carotene or vitamins C and E was comparable to that of the placebo control group *(59)*. The Alpha-Tocopherol and Beta-Carotene Cancer Prevention (ATBC) study randomized 50- to 69-yr-old Finnish male cigarette smokers ($N = 29{,}133$) to β-carotene, α-tocopherol, both agents, or placebo. Colorectal cancer incidence in the α-tocopherol arm and in the β-carotene arm were not significantly different *(60)*.

6. CHEMOPREVENTIVE AGENTS FOR PATIENTS AT HIGH RISK OF COLORECTAL CANCER

6.1. 5-Aminosalcylic Acid (5-ASA)

Patients with ulcerative colitis (UC) have an increased risk of colorectal cancer—2% at 10 yr, 8% by 20 yr, and 18% by 30 yr *(61)*. 5-ASA is a derivative of aspirin and is commonly used to treatUC. It has been proposed as a potential chemopreventive agent in this high-risk patient population. A systematic review and meta-analysis of cohort and case control studies examining the relationship between 5-ASA and dysplasia or cancer in 1932 patients with UC showed a protective effect of treatment against cancer (OR 0.51, 95% CI 0.37–0.69), cancer and dysplasia (OR 0.51, 95% CI 0.38–0.69), but not dysplasia alone (OR 1.18, 95% CI 0.41–3.43) *(62)*. Unfortunately, a confirmatory randomized double-blind placebo-controlled study would be difficult to conduct in this population given the ethical difficulties in withholding 5-ASA in one arm, as well as the large sample size and long time frame required to conduct this trial. Because it is unlikely that such a trial will take place, some experts recommend that given the drug's safety and available evidence, it is reasonable to adopt this agent as an adjunct to secondary prevention of surveillance colonoscopy *(63)*.

6.2. Sulindac

Patients with FAP develop innumerable adenomas and virtually all will develop colon cancer in the absence of surgery. In a small randomized double-blind placebo-controlled study of patients with FAP who had not undergone

prior colectomy or had subtotal colectomy, sulindac was found to be effective in reducing both the size and number of polyps when administered for 9 mo. However, 3 mo after the discontinuation of therapy, patients treated with sulindac experienced an increase in both the size and number of polyps *(64)*. In a nonrandomized study, patients with FAP who had total colectomy with ileorectal anastomosis were treated with sulindac to prevent cancer within the rectal stump. The authors concluded that long-term treatment with sulindac was effective in reducing the size and number of polyps in the retained rectal segment *(65)*.

6.3. Celecoxib

In a study of 77 patients with FAP who had previous colectomy, cases were randomized to receive 100 mg celecoxib, 400 mg celecoxib, or placebo twice daily for 6 mo. The patients who were treated with high-dose celecoxib (400 mg twice daily) experienced a significant regression in the number of colorectal polyps *(66)*. Based on these data, the drug is approved by the FDA as an adjunct to endoscopic surveillance in patients with FAP.

7. NEW CHEMOPREVENTIVE AGENTS UNDER INVESTIGATION

7.1. Curcumin

Curcumin (diferuloyl-methane) is a low molecular weight polyphenol that is a major component of the yellow spice tumeric. It is thought to prevent cancer by suppressing COX-2 *(67)*, as well as glutathione S-transferase activity *(68)*. In a Phase I study, it was found to induce regressions of premalignant lesions of the skin, bladder, stomach, cervix, and oral mucosa with an acceptable toxicity profile *(69)*. One pilot study in humans found that oral curcumin was rapidly degraded to metabolites that exhibit less COX-2 inhibitory potential. Interestingly, dose-dependent reduction in COX-2 activity and prostaglandin E2 levels was reported *(67)*. Another study of patients with colorectal cancer showed that although peripheral blood levels may be low, high levels of curcumin glucuronide and curcumin sulfate are found in the rectal mucosa. However, this study did not show an increase in COX-2 levels *(70)*.

7.2. Inulin Derivatives

Inulin stimulates the growth of gut *Bifidobacterium*, which is thought to decrease intestinal genotoxins. When mice were treated with the inulin-like oligofructoses, *Lactobacillus* LGG and *Bifidobacterium* BB12 alone (probiotic), a combination of the two (symbiotic), or control, the prebiotic and symbiotic combinations reduced both DNA damage of the colonic mucosa as well as cancer incidence *(71)*.

Raftilose Synergy-1, an oral compound containing oligofructose and polyfructose chains, was studied in the recently completed Symbiotics and Cancer Prevention in Humans (SYNCAN) trial. This study was a double-blind, randomized, placebo-controlled trial of 80 patients with personal histories of either resected colon cancer or resected adenomatous polyps. Subjects were treated with a 12-wk course of a food supplement containing Synergy-1 or placebo. End points included biomarkers within the colonic mucosal and fecal water as well as immunological and inflammatory response markers. Preliminary results show that subjects in the treatment group had decreased DNA damage and cell proliferation *(72)*. Synergy-1 is currently being studied in a multi-center Phase II study in the United States using ACF as an end point.

7.3. Epidermal Growth Factor Receptor Inhibitors

Inhibitors of epidermal growth factor receptor tyrosine kinase (EGFR-TK) are thought to be chemoprotective through their effects on transforming growth factor-α, which increases as neoplasms progress from adenomas to *in situ* disease to invasive cancer *(73)*. Nearly half of the Min mice treated with a combination of sulindac and EKI-569, a newly developed EGFR inhibitor, showed reduced polyp formation *(73,74)*. When the Min mice were crossed with mice with significantly reduced levels of EGFR (the EGFRWA model), intestinal polyps were reduced by 90% as compared to those with the wild-type allele *(75)*. Both findings support the role of EGFR in intestinal polyp formation and carcinogenesis *(76)*.

7.4. Nitric Oxide-Releasing NSAIDs

Nitric oxide-releasing NSAIDs (NO-NSAIDs) are a class of anti-inflammatory agents that contain an NSAID molecule linked to a nitric oxide-donating group. Preliminary studies in animals and healthy humans suggest that these drugs may have less gastrointestinal toxicity than their traditional counterparts. In vitro studies indicate that NO-ASA, NO-sulindac, and NO-ibuprofen reduce colon cancer cell growth more effectively than their corresponding NSAIDs, making these potential chemopreventive agents worthy of further study *(77)*.

7.5. Future Directions in Colorectal Cancer Prevention Research

The therapeutic dilemmas surrounding the use of COX-2-selective inhibitors highlight several of the challenges in chemoprevention research. Potential agents must have both chemopreventive effects and acceptable risk–benefit profiles for the target population. As scientists continue to dissect both the molecular pathways altered during the colon carcinogenesis sequence and the mechanisms of action of potential chemopreventive agents using animal models, it is anticipated that it will be possible to combine agents at doses that are low enough to limit

toxicity while still preserving or enhancing their antitumor activity. Progress in the establishment of a clinical regimen for the chemoprevention of colorectal cancer continues to be hindered by the lack of validated biomarkers of cancer risk and chemopreventive response. These markers are essential to select a high-risk population who will benefit most from clinical intervention and to determine if use of a test agent confers significant protection from cancer, respectively. A number of strategies are currently being implemented in clinical trials to expedite the discovery of an efficacious regimen for the prevention of colon cancer. These include the use of ACF and other early neoplastic features (i.e., mutations in fecal DNA) as primary end points, "nesting" secondary prevention and toxicity end points within therapeutic trials, and establishing a cooperative network of leading gastroenterologists who are dedicated to the conduct of large, cost-effective clinical chemoprevention trials.

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