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Combination Chemoprevention for Colon Cancer Targeting Polyamine Synthesis and Inflammation

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Abstract Increased polyamine synthesis and inflammation have long been associated with colon carcinogenesis in both preclinical models and in humans. Recent experimental studies suggest that polyamines may be mechanistically involved in colonic inflammatory processes. Genetic epidemiology results indicate that a single nucleotide polymorphism influencing the expression of a polyamine biosynthetic gene is associated with both risk of colon polyp occurrence and recurrence, and the response to aspirin as a polyp preventive agent. A prospective, randomized, placebo-controlled clinical trial of combination difluoromethylornithine, a selective inhibitor of polyamine synthesis, and sulindac, a nonsteroidal anti-inflammatory drug, found that the 3-year treatment was associated with a 70% reduction of recurrence of all adenomas, and over a 90% reduction of recurrence of advanced and/or multiple adenomas, without evidence of serious toxicities. This proof-of-principle trial indicates that targeting polyamine synthesis and inflammation can be an effective strategy for preventing the occurrence of the advanced and/or multiple adenomas that are most closely associated with the development of colon cancers in humans.

Background

Virchow speculated about the role of chronic inflammation in cancer in the 1860s; this topic has been more recently reviewed (1). A century later, Russell and Snyder (2) were among the first to document high levels of ornithine decarboxylase (ODC) enzyme activity in proliferating cells and tissues, including those derived from various tumor types. The role of ODC, the first enzyme in the synthesis of the ubiquitous polyamines that are involved in growth, development, and cancer has been reviewed elsewhere (3).

Evidence for the efficacy of targeting inflammation and polyamine synthesis for cancer chemoprevention began to accumulate over 30 years ago. Chemoprevention of cancer is a strategy that uses treatments during the stages of carcinogenesis before the development of invasive cancer (4). Sporn (5) was among the first to propose combinations of agents for cancer chemoprevention. The rationale for this proposal followed after the success of combination chemotherapy for certain types of cancer (6), and offered the prospect of reduced toxicities by lowering doses of individual agents. Representative studies targeting polyamine synthesis and inflammation for chemoprevention of colon and intestinal carcinogenesis evaluated

several rodent models treated with the selective ODC inhibitor *D,L*- α -difluoromethylornithine (DFMO) alone and in combination with several nonsteroidal anti-inflammatory drugs, including piroxicam (7), aspirin (8), celecoxib (9), and sulindac (10). These combinations have proved to be potent inhibitors of colon and intestinal polyps in all models and, in the case of carcinogen-treated rats, invasive colon cancers.

Risk factors for colon cancer include both genetic and intestinal luminal factors. One heritable genetic risk factor, which conveys risk of colon cancer, is the adenomatous polyposis coli tumor suppressor gene (11). Adenomatous polyposis coli and the *K-RAS* oncogene are two of the most commonly mutated genes found in human colon cancers (12). As depicted in Fig. 1A, adenomatous polyposis coli and *K-RAS* are both activators of polyamine synthesis, albeit by different mechanisms (3). Polyamines, which derive from the amino acid ornithine and its precursor arginine, are also intestinal luminal risk factors. Dietary polyamines enhance intestinal and colonic tumorigenesis (13, 14). Colonic luminal polyamines are also produced by enteric bacteria; both diet and enteric bacteria provide sources of potential tumor-promoting polyamines for colonic epithelial cells (see Fig. 1B).

Colonic bacteria provide sources of other luminal risk factors for colon cancer. These bacteria metabolize primary bile acids to secondary bile acids, which have been associated with colon cancers in humans (15) and are capable of promoting colon carcinogenesis in rodent models (16). Dietary administration of the secondary bile acid deoxycholate induces a colitis-like phenotype in mice (17). This bile acid-induced inflammatory response is suppressed by loss of nitric oxide synthase 2 alleles in genetically engineered mice (18), which imply a role for the nitric oxide synthase 2 substrate arginine (see Fig. 1B). Other studies have provided a direct linkage between dietary arginine and intestinal and colonic tumorigenesis. These studies indicate that loss of nitric oxide synthase 2 alleles (19), or treatment

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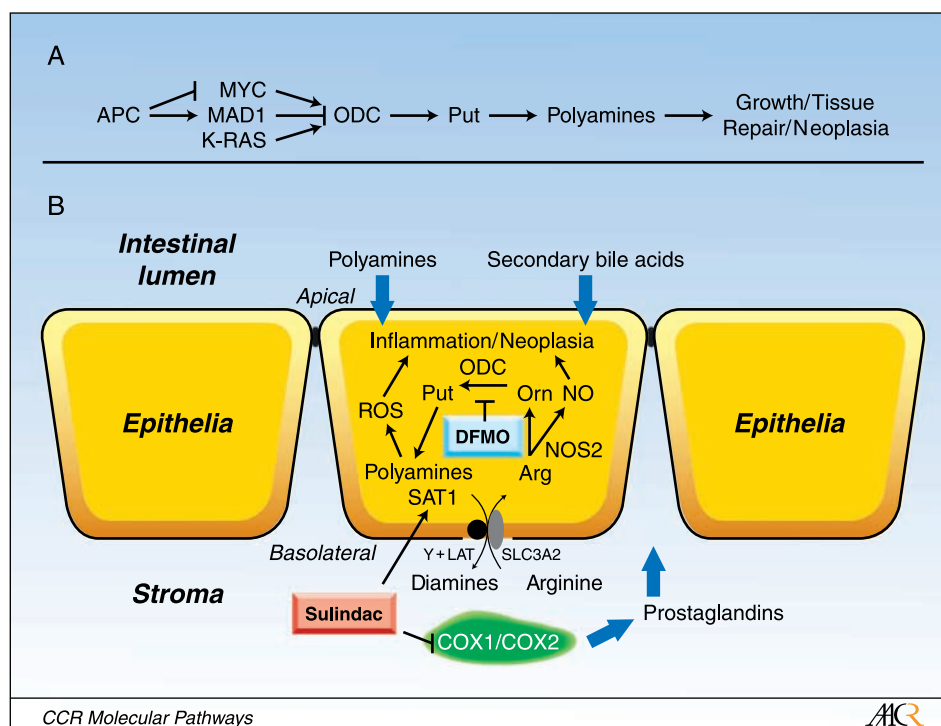


Fig. 1. *A*, ODC expression is regulated by a number of signaling pathways. The APC tumor suppressor gene influences the expression of the *MYC* oncogene and the *MYC* antagonist *MAD1* (34). These E-box transcription factors, in turn, bind to consensus elements, including two E-boxes flanking a SNP which has functional consequences for transcription factor binding, to regulate *ODC* transcription (27). ODC expression is also regulated by the *K-RAS* oncogene (35). ODC converts ornithine to the diamine putrescine. This diamine is the precursor for the longer chain amines spermidine and spermine. Genetic and pharmacologic evidence shows that this pathway is essential for normal growth, development, tissue repair, and neoplasia (3). *B*, the relationship between polyamine metabolism and inflammatory pathways in the colonic mucosa are depicted in this panel. Abbreviations include the following: reactive oxygen species (ROS), nitric oxide (NO), arginine (Arg), ornithine (Orn), putrescine (Put), cyclooxygenase (COX), nitric oxide synthase (NOS), ODC, and SAT1. Diamines, including putrescine, monoacetylspermidine, and diacetylspermidine, are exported by a common transporter (36), which was recently identified by us to include the solute carrier protein SLC3A2 (23). SLC3A2 partners with members of the Y+LAT family to form an arginine transporter. The roles of luminal factors, including polyamines and secondary bile acids, and stromal factors, such as prostaglandins, are shown. DFMO and sulindac lower polyamine contents in colonic epithelial cells by suppressing polyamine synthesis and activating polyamine catabolism and export. Sulindac also inhibits mucosal cyclooxygenases, which are predominately expressed in the colonic stroma before the formation of epithelial neoplasia.

with DFMO (20), can suppress arginine-induced intestinal carcinogenesis. Together with the studies of Bernstein et al. (17, 18), these latter studies suggest a linkage between polyamines and inflammation, and polyamines, inflammation, and colon cancer.

This linkage may be more than simply an association. Microarray analysis of human colon cancer-derived cells identified the spermidine/spermine acetyltransferase (SAT1) as a target of the nonsteroidal anti-inflammatory drug sulindac (21). Sulindac and other nonsteroidal anti-inflammatory drug act by distinct transcriptional mechanisms to induce SAT1 and promote the export of diamines and acetylpolyamines (the products of SAT1) in both human cell and mouse models (14, 22). Acetylation and export work in concert with inhibition of polyamine synthesis to lower cell and tissue polyamine contents. A diamine and acetylpolyamine exporter has recently been identified as a component of the solute carrier and arginine transporter containing SLC3A2 and Y+LAT subunits. SAT1 physically associates with SLC3A2 (Fig. 1B; ref. 23). Because this complex works as an arginine/diamine (or acetylpolyamine) antiporter, increased production of the diamine putrescine as a consequence of increased ODC activity would enhance, whereas decreased production of putrescine would suppress, the activity of the SLC3A2/Y+LAT transporter. In certain cases, increased polyamine synthesis may be anti-inflammatory by channeling arginine metabolism away from

nitric oxide production (24). In this model, treatment with DFMO exacerbated the colitis, presumably by increasing nitric oxide levels. It is unknown whether DFMO would have a similar effect on inflammation-associated carcinogenesis in this model. However, these data suggest that DFMO combinations with nitric oxide inhibitors might be useful in this context. We have obtained evidence for channeling of polyamine metabolism with the unexpected finding that ODC, SAT1, and SLC3A2 are physically associated in colon-derived cells (23).

Polyamines contribute to inflammatory responses by mechanisms in addition to those affecting tissue arginine levels. Polyamines are oxidized by several amine oxidases to produce reactive oxygen species and aldehydes (Fig. 1B; ref. 25). Polyamines can also influence the expression of the proinflammatory gene cyclooxygenase 2 by a posttranscriptional mechanism (26).

Clinical-Translational Advances

The hypothesis that increased arginine might be associated with colon carcinogenesis in humans was evaluated in a genetic and dietary epidemiology study of patients in a cancer registry (9). Increased survival in colon cancer patients with a family history of this disease was associated with low consumption of red meat, which was used as a surrogate for arginine consumption.

The hypothesis that polyamines, inflammation, and carcinogenesis are linked is strengthened by several genetic epidemiology studies. Our group first reported that a single nucleotide polymorphism (SNP) in the *ODC* promoter, which displayed functional consequences for E-Box activator (e.g., *MYC*) and repressor (e.g., *MAD1*) binding (see Fig. 1A), was associated with risk of recurrence of colon polyps in a clinical cancer prevention trial (27). Six hundred eighty-eight individuals in the Wheat Bran Fiber prevention trial were genotyped for the *ODC* G316A SNP. The *ODC* 316AA genotype was associated with approximately a 50% reduction in risk of polyp recurrence, compared with those individuals with the *ODC* 316GG genotype (odds ratio, 0.48). In reported aspirin users, the *ODC* 316AA genotype was associated with a 90% reduction in risk of polyp recurrence, compared with nonaspirin use reporters with the *ODC* 316GG genotype. Hubner and coworkers (28) genotyped 546 participants for the *ODC* G316A SNP in the United Kingdom Colorectal Adenoma (CRA) Prevention trial of aspirin for CRA recurrence prevention. They found a similar reduction in risk of adenoma recurrence in people with the *ODC* 316AA genotype, compared with those with the *ODC* 316GG genotype (relative risk of 0.43). The risk of polyp recurrence in this trial was further decreased in the *ODC* 316AA group, compared with the *ODC* 316GG group, by aspirin use. A third group provided independent corroboration of an association between the *ODC* G316A SNP and aspirin use. Barry and coworkers (29) genotyped participants in a prospective, randomized study of aspirin for prevention of CRA conducted by the Polyp Prevention Study Group. The *ODC* G316A SNP was not an independent prognostic factor for adenoma recurrence but was a statistically significant predictor of response to aspirin for prevention of CRA recurrence in this study.

A model for the interaction between the *ODC* SNP and nonsteroidal anti-inflammatory drug action in colon carcinogenesis (3), which includes both cyclooxygenase-dependent and cyclooxygenase-independent actions of nonsteroidal anti-inflammatory drug, is depicted in Fig. 1B.

This model has been tested over the past decade in a prospective, randomized placebo-controlled trial of combination DFMO and sulindac for prevention of recurrence in patients with prior CRA (30). Entry criteria for this trial included removal of a CRA within 1 year of study entry. Patients with genetic risk of colon cancer, such as individuals with familial adenomatous polyposis and other polyposis syndromes, were excluded. Participants with current or prior colon or other cancers were also excluded. Participants received the combination of DFMO (two 250-mg pills daily) and sulindac (one 150 mg pill daily), or placebo pills, for 3 years. Primary end points included adenoma recurrence and toxicity assessment. Treatment with DFMO and sulindac was associated with a 70% reduction in total polyps, and over a 90% reduction in both advanced adenomas and in patients with multiple recurrent adenomas, at the end of 3 years. The only statistically

significant toxicity noted in this trial was a hearing loss of uncertain clinical significance, which seems to be limited to a small subset of participants (31).

The clinical trial of combination DFMO and sulindac serves as an important proof of the principle that targeting polyamine synthesis and inflammation is an effective method for prevention of recurrent colon polyps. The challenge facing us and other workers in this field is to bring this advance into clinical practice for the management of patients with high risk for colon and, potentially, other cancers. It is clear that this drug combination has some toxicities, and initial applications should be limited to those individuals where a clear positive benefit to risk ratio can be established, such as people with high risk of developing colon cancer. High-risk groups would include those with genetic risk factors, prior advanced and/or multiple colon adenomas, and prior colon cancer. Future clinical trials in all three of these risk groups are in the planning stages.

Drug availability is another serious challenge. Both DFMO and sulindac are old drugs, and DFMO has not been commercially available for some time. We have recently established a company to produce DFMO for future clinical cancer prevention and treatment trials in humans.

The time to completion of clinical trials that might support an approval by the Food and Drug Administration for a cancer prevention indication is a very serious problem that must be solved. Most epithelial cancers have long natural histories, with years often separating the development of invasive cancers after appearance of precancerous intraepithelial neoplasia (32). As evidenced by the approval of celecoxib for treatment of patients with the genetic syndrome familial adenomatous polyposis, which confers risk of colon cancer, the Food and Drug Administration has not accepted reduction in number of colon polyps as an indication of clinical benefit in this patient population. In the approval of celecoxib for familial adenomatous polyposis, the Food and Drug Administration expressed concerns regarding both clinical benefit and long term safety of the drug.

Cancer researchers should consider the example followed by investigators working to prevent deaths due to cardiovascular disease. It has been estimated that the dramatic decrease in deaths due to heart disease over the past 30 years are roughly equally divided between efforts to reduce risk factors, including use of chemoprevention methods, and mechanism-based therapies (33). Public and private sector interests involving academic research, professional medical and commercial pharmaceutical groups, aided by patient advocacy groups, need to work with the Food and Drug Administration to identify paths to implement cancer chemoprevention strategies into the standard practice of managing patients with high risk of colon and other cancers.

Disclosure of Potential Conflicts of Interest

The authors have an ownership interest in Cancer Prevention Pharmaceuticals, LLC.

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