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Chemoprevention of colon cancer: Current status and future prospects

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Abstract

Colorectal cancer is an important public health problem in the western world. Although some progress has been made in the prevention and management of this disease, colon cancer still remains one of the most common types of epithelial malignancies in both genders and is essentially incurable when it reaches the most advanced stages. Given the substantial morbidity and mortality associated with colorectal malignancies and their treatment, cancer prevention in its many forms emerges as a very attractive approach. Colorectal cancer chemoprevention refers to the administration of natural or synthetic compounds to block, reverse, delay or prevent the development of invasive large bowel neoplasms. The ultimate goal of implementing a chemopreventive intervention in the general, or alternatively, in an at-risk population is to decrease the incidence rate of the specific cancer being targeted. This article reviews the present status of colorectal cancer chemoprevention. Current insights into the molecular and genetic models of human colorectal carcinogenesis, preclinical models for efficacy testing as well as into promising biomarkers for colorectal chemoprevention are provided. The developmental status of many promising agents is also discussed emphasizing the epidemiological evidence, preclinical information substantiating an anticarcinogenic effect, their postulated mechanism of action and the status of human clinical development. Our perspective of the future prospects in this scientific area is also provided and has been predicated primarily on the firm belief that the proper integration of advances in the biology of colon carcinogenesis, experimental therapeutics and clinical trial methodology will be critical for the success of this promising field.

I. Introduction

Colorectal cancer is an important public health problem in the western world. Although some progress has been made in the prevention and management of this disease, colon cancer still remains one of the most common types of epithelial malignancies in both genders and is essentially incurable when it reaches the most advanced stages [1]. Given the substantial morbidity and mortality associated with colorectal malignancies and their treatment, cancer prevention in its many forms emerges as a very attractive approach. Colorectal cancer may be prevented at different levels. Primary prevention leverages the knowledge and manipulation of risk factors that may be central to the initiation or development of the carcinogenic process in the large bowel epithelium. Operationally, it is usually conceptualized as the avoidance of hazardous environmental carcinogens [2]. Secondary prevention refers to the early identification and intervention in the transformation process in individual subjects prior to the actual transforming event [3]. Despite the attractiveness of a primary preventive approach, population based preventive approaches pose substantial challenges. Environmental risk factors for colorectal cancer remain poorly defined. Even if well understood, they may be so prevalent in the environment that their avoidance may not be feasible. As the carcinogenic process is likely the product of gene-environmental interactions, the environment is not the only variable of interest. Our current inability to manipulate the genetics of cancer risk substantially limits our ability to implement primary prevention at its highest level. A promising clinical interventive strategy is that of cancer chemoprevention [4].

Chemoprevention refers to the administration of natural or synthetic compounds to block, reverse, delay or prevent the development of invasive neoplasms. The ultimate goal of implementing a chemopreventive intervention in the general or alternatively in an at-risk population is to slow the onset of cancer or to decrease the incidence rate of the specific cancer being targeted [4].

Although chemoprevention is a relatively new clinical field, a substantial number of risk-reduction studies have been completed to date, with mixed results [5]. Most of these studies were conceptualized when epidemiological, preclinical (*in vitro* and chemical carcinogenesis), serum and tissue bank data existed only in very preliminary form. The unexpected results seen with two of the major micronutrient cancer prevention studies, the ATBC and CARET trials, underscore the importance of having a systematic process in place for the rational selection of the most promising agents to be tested in definitive risk-reduction clinical trials [6,7]. This process should include small-scale biomarker driven human clinical studies.

This article reviews the current status of colorectal cancer chemoprevention. Current insights into the molecular and genetic models of human colorectal carcinogenesis, preclinical models for efficacy testing as well as into promising biomarkers for colorectal chemoprevention are provided. The developmental status of many promising agents is also discussed emphasizing the epidemiological evidence, preclinical information substantiating an anticarcinogenic effect, their postulated mechanism of action and the status of human clinical development. Our perspective of the future prospects in this scientific area is also provided and has been predicated primarily on the firm belief that the proper integration of advances in the biology of colon carcinogenesis, experimental therapeutics and clinical trial methodology will be critical for the success of this promising field.

II. Molecular basis of colorectal cancer chemoprevention

Molecular genetics and biology have enhanced our understanding of the developmental processes that are involved in the genesis of colorectal neoplasms including 'sporadic' colorectal malignancies as well as those associated with inherited colon cancer syndromes. The opposite is also true in that the elucidation of hereditary genetic error provides us with a unique opportunity to improve our understanding of the carcinogenesis process. By defining the germ-line mutations associated with early transformation, we learn many of the events and pathways that are mutated, methylated, over or underexpressed in the carcinogenesis process in sporadic neoplasms. With a more detailed understanding of the molecular biological processes (both genetic and epigenetic) that are involved in colorectal cancer genesis, molecular genetic models of human carcinogenesis have emerged which provide a scientific framework that serves as the platform for further scientific inquiry in the field of chemoprevention.

Fearon and Vogelstein proposed a genetic model where colon cancer was depicted as a process that developed through the acquisition of a number of successive genetic changes in an orderly fashion. A number of histopathological changes would then occur in the colorectal epithelium as a phenotypic expression of these genetic abnormalities [8]. Once mechanistically understood, some of these events may become legitimate and feasible targets in the search for new chemopreventive agents. In addition, they may serve as useful markers of efficacy (surrogate endpoint biomarkers (SEB)) or of risk for selection of suitable human cohorts for clinical testing (high-risk populations). Fearon and Vogelstein's model is predicated on the notion that colorectal tumors occur as a consequence of mutational activation of oncogenes paired with mutational inactivation of tumor-suppressor genes. These mutations occur in the background of evolving chromosomal instability. Multiple mutations are to be required for the development of malignant tumors whereas fewer changes were necessary for premalignant lesions (adenomas) to occur. They also postulated that the total accumulation of changes was probably more important than the sequence in which they occurred [8].

A number of key biochemical events associated with colorectal carcinogenesis are presented here. This is not meant to be an exhaustive list, but a brief summary of the pivotal cellular changes associated with colorectal carcinogenesis known to date. These events might be overlapping, associated with different mutations or methylation sites.

Adenomatous polyposis coli silencing. In the process of sporadic colon carcinogenesis, the adenomatous polyposis coli (APC) gene must be silenced [9]. APC mutations occur in over 60–80% of adenomas and sporadic carcinomas, and have been identified as the germline genetic abnormality underlying the development of familial adenomatous polyposis [10,11]. In the

remaining 20–40% of sporadic colon cancers, the APC gene is silenced by methylations of promoter sequences [12,13]. The APC gene encodes for a 310 kDa protein which phosphorylates cellular β-catenin. The phosphorylation reaction marks β-catenin to ubiquinitation. Without this step, β-catenin accumulates in the cell and transduces key cellular growth and proliferation genes, including cyclin D and c-myc [14–17].

Cyclooxygenase-2 overexpression. The cyclooxygenases (prostaglandin H synthase) (EC 1.14.99.1), are two enzymes (Cox-1, Cox-2) that catalyze both oxidative and reductive reactions in the prostaglandin synthesis pathway [18–20]. Cox-1, a constitutive protein contains a 17 amino acid sequence that is not present in the inducible Cox-2 protein. Cox-2, an inducible protein, contains an 18 amino acid sequence near its carboxyl terminus that is not present in Cox-1. Cox-2 is induced by a wide range of mitogens, tumor promotors and cytokines [21]. The Cox-2 5′ regulatory sequence contains SP-1, NFκB, NF-IL6, AP-1, AP-2 sites, a serum response element, a *cis*-acting element confirming responsiveness to gonadotropic hormones, and an ATF/CRE site [21].

In normal human colon, Cox-1 is detectable by Western blot in all human subjects assessed [22,23] whereas Cox-2 appears to be variably present [22–24] Cox-2 gene expression is increased in human colon carcinomas when compared to normal mucosa while not detecting upregulation of Cox-1 [25]. Cox-2 expression is enhanced in cancers. Cox-2 is detected in cancer cells, inflammatory mononuclear cells, vascular endothelial cells and fibroblasts. Poorly differentiated tumors stain irregularly while well or moderately differentiated tumors stain more diffusely [22].

It is reasonable to postulate that increased Cox-2 expression plays a major role in overexpression of prostaglandins in malignant tissues from other anatomic sites. Both viral (v-Src) and oncogene (*Ras*) transformed mammary epithelial cells contain increased Cox-2 mRNA and protein compared to parental cells [26]. These data suggest that Cox-2 expression changes over the carcinogenesis process with early high expression and then reduced expression during or after transformation.

Genetic and phenotypic evidence suggests that Cox-2 overexpression is related to neoplastic transformation [27–29]. After transfection of a Cox-2 expression vector rat intestinal epithelial cells demonstrate increased adherence to matrigel extracellular matrix, blockade of apoptosis induced by butyrate, increased bcl-2 expression, loss of E-cadherin protein, and reduction of TGFβ2 receptors [30]. Apoptosis is blocked by Cox-2 expression [13]. That Cox-2 is expressed in noncolonic pretransformed and transformed cells suggests a more global role for Cox-2 in the carcinogenesis process.

Ras. Mutations of the *ras* oncogene have been well documented in colorectal cancer patients at the early and intermediate adenoma stages [8]. The farnesylation of Ras is critical for the appropriate localization of this protein in the cell membrane, a step that is essential for it to exert its function. A number of targeted approaches are being developed in an attempt to modulate the *ras*-signaling pathway. These include natural agents such as d-limonene and perillyl alcohol as well as targeted approaches to prevent the farnesylation of the Ras protein by the inhibition of the enzyme farnesyl-transferase.

Deleted in colon cancer. Another frequent genetic abnormality in colorectal cancer formation is the loss of 18q21. This chromosomal abnormality is usually seen at the later stages of adenoma formation. Two putative tumor-suppressor genes have been mapped to this region. Deleted in colorectal carcinogenesis (DCC) gene deletions are present in over 70% of colon carcinomas [31,32]. However, its function as a possible tumor-suppressor gene remains controversial.

Microsatellite instability. Late molecular events include abnormalities of 17p associated with loss of p53 function and other mutational events that result in enhanced genomic instability associated with a replication error positive (RER+) phenotype. Microsatellite instability (MSI) is the hallmark of hereditary nonpolyposis colorectal cancer syndrome (Lynch Syndrome). Central to its pathogenesis is a colorectal epithelium which is prone to errors in DNA repair due to mutations of mismatch repair genes. Genes associated with DNA repair include MSH2, MLH1, PMS2, PMS1 and MSH6 [33–35]. These genetic mutations are germline mutations and therefore are present early on in subjects affected by the nonpolyposis colorectal cancer syndrome. Somatic mutations of the MLH1 and MSH2 genes have been described in sporadic tumors and in these cases seem to develop late in tumorigenesis [36]. Recently, mutations affecting the type II TGF-β receptor (TGF-β RII) have been described in sporadic, hereditary nonpolyposis colorectal cancer

and ulcerative colitis-associated neoplasms. These mutations may result in MSI and are discussed in more detail in the following section [37,38].

Transforming growth factor beta II receptor mutations. Tumor growth factor beta (TGF-β) is a family of ligands that exert strong antiproliferative and pro-apoptotic effects in nontransformed human colon epithelium [39–43]. The TGF-β receptor is a heterodimeric receptor complex conformed by two subunits, RI and RII [40,44–50]. TGF-β signaling occurs through TGF-β binding to its receptor complex which results in phosphorylation of Smad2 and Smad3, followed by their association with Smad4, and subsequent translocation of this complex into the nucleus, ultimately resulting in the modulation of the expression of a number of genes [45,49–51]. The end result of this process is growth inhibition and promotion of programmed cell death. A large proportion of human cancers display functional resistance to TGF-β inhibitory effects. Approximately one-third of this resistance occurs as a consequence of mutations of the RII component of the TGF-β receptor. *In vitro* studies suggest that RII functions as a tumor suppressor. When wild type RII is reintroduced into an RII mutant colon cancer cell line by infection with an RII retrovirus, 90% of tumor colony formation is suppressed [51].

A large proportion of RII mutations occur within the RII coding region polyadenine repeat sequence (BAT-RII) [52,53]. Human colon cancers with altered base–base mismatch repair function, commonly display inactivation of RII tumor suppression function through genetic changes affecting BAT-RII [52,53]. Fifteen percent of sporadic human colon cancers display MSI as a consequence of loss of mismatch repair function [54,55]. This seems to occur through the silencing of mismatch repair genes via promoter hypermethylation. An alternative pathway of human colorectal carcinogenesis has therefore emerged in which silencing of mismatch repair function, through the hypermethylation of genes that participate in the DNA base–base mismatch repair system (such as hMLH1), promotes mutations in the BAT-RII segment effectively targeting the RII tumor suppressor for inactivation. These mutations seem to occur at the transition stage between adenoma and carcinoma [56]. Functional resistance to TGF-β has also been demonstrated in colon cancers without TGF-β RII mutations. These cases presumably have mutations on postreceptor elements of the TGF-β signaling pathway [56]. TGF-β signaling is therefore a crucial pathway in human colon carcinogenesis. Recent *in vitro* data suggests that hMLH1 gene expression can be reconstituted after treatment with the demethylating agent 5-azacytidine, suggesting that these kinds of agents may have some promise as colorectal chemopreventives [56].

III. Preclinical models of colorectal carcinogenesis

The development of well-characterized preclinical models of colorectal carcinogenesis has played a critical role in the process of screening and selection of agents deserving further clinical study. Systems have been developed to study the effects of putative chemopreventive agents on the modulation of relevant mechanistic pathways associated with carcinogenesis (*in vitro* systems) or to evaluate their overall effects in complex organisms that try to emulate environmental carcinogenesis (animal models of chemical carcinogenesis) or genetic predisposition syndromes (genetically modified rodent models). In this review we will focus our discussion on aberrant crypt foci (ACF) and on animal models of carcinogenesis.

III.A. Aberrant crypt foci

The smallest recognizable histopathologic evidence of colorectal epithelial carcinogenesis is the ACF or microadenoma. ACFs are lesions consisting of large, thick crypts seen in methylene-blue stained specimens of colon, originally found in mice treated with azoxymethane (AOM) [57]. In humans, two types of ACF are observed after methylene blue staining of whole mount mucosa preparations: hyperplastic foci (hypercellullar but normal appearing cells) and dysplastic foci (contain dysplastic cells similar to those found in adenomas) [58–60]. Hyperplastic lesions commonly contain K-ras gene mutations, rarely contain APC mutations [61–63], and are unlikely to progress to neoplasia.

For several reasons, dysplastic lesions are thought to be precursors to adenomas. Numerous dysplastic ACFs are found in familial adenomatous polyposis patients [64]. Moreover, the occurrence patterns of ACF and their response to chemopreventive interventions parallel those of adenomas and carcinomas in rodent carcinogenesis models [57,65–67]. Dysplastic human ACFs contain APC mutations, cellular hyperproliferation to the top of the crypt, and disruption of p21/Ki67-hMSH2 crypt compartmentalization, all features common to adenomas [59,61,63]. Finally,

carcinoma *in situ* has been documented in human ACF [64]. Takayama et al. [62] reported that the prevalence of dysplastic ACFs in the rectosigmoid was 5.1%, 13.8% and 52.1% in normal subjects, those with prior adenomas, and those with prior carcinoma respectively. Siu et al. [68] reported dysplasia in 48% of rectosigmoid ACFs but do not describe the pathologic diagnoses associated with their samples. The data to date suggest that dysplastic ACFs may have a potential role as early pathologic surrogate endpoints for colorectal cellular transformation; however, the evidence is not conclusive. Rather, ACFs represent an excellent early pathologic surrogate to assess in rodent models and, potentially, in humans.

III.B. Animal models of chemical carcinogenesis

Animal models represent a pivotal step in the efficacy evaluation of chemopreventive agents. Chemical carcinogenesis rodent models provide reproducible development of tumors in the intestinal epithelium of rodents that have been exposed to a chemical compound which acts as an initiator or a combination of two chemicals that act as initiator–promoter pairs. These models have been most extensively used as pre-clinical *in vivo* platforms for efficacy testing of chemopreventive leads. Frequently used chemical carcinogens include dimethylhydrazine (DMH), AOM, methoxymethane (MAM) and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) [69,70]. Most of these compounds act through methylation of DNA nucleosides (primarily guanine) which eventually results in critical DNA mutations [71]. Dimethylhydrazine undergoes conversion to AOM and azoxymethanol which are subsequently conjugated with glucuronic acid and excreted in the bile. Bacterial hydrolysis occurs once the conjugated carcinogens reach the intestinal lumen but is not essential to their tumorigenic effect. A number of variables such as the dose, route and timing of carcinogen administration, the species of rodent being used and the age of the animal at the time of injection can be manipulated to provide the investigator with some control over critical features in the model. These features that can to some extent be controlled include latency period, development (or lack thereof) of extracolonic tumors (large bowel specificity) and tumor distribution [72–74]. Molecular characterization of some of these models is presently an area of active inquiry. AOM induces changes in the rodent intestinal mucosa that closely replicate, histologically, the adenoma-carcinoma sequence in humans [75]. Chemical carcinogens are also capable of inducing some of the molecular changes that are commonly seen during human carcinogenesis. APC mutations have been described in experimental colorectal carcinogenesis induced by AOM in F344 rats, but seem to occur less frequently in this model than in humans [76,77]. A substantial body of literature supports the presence of K-*ras* mutations in the AOM model [78–81]. Mutations of p-53, however, do not seem to be involved in the development of colon cancer induced by AOM in the rat [82,83]. Other pathways which seem to play important roles during both human and rat colon (chemical) carcinogenesis include the constitutive activation of the Wnt-signaling pathway, altered expression of cyclin D1 and cyclin-dependent kinase 4 (Cdk4) and others [84,85]. MSI has also been described in AOM-induced rodent cancers although seems to occur with much less frequency than in humans [86]. Chemically-induced carcinogenesis in rodents and human carcinogenesis are not identical. However, since AOM-induced rat colonic neoplasms are similar to human colonic tumors in their histological features and proliferation characteristics, and given some of the molecular parallels that exist between rodent and human carcinogenesis, these models are usually regarded as relevant tools for preclinical *in vivo* testing of new chemopreventives.

III.C. Genetic models

Single and multiple gene knock out and genetic mutational rodent models provide new and important insights into the carcinogenesis process of colonic epithelium. A mouse pedigree derived from treatment with *N*-ethyl-*N*-nitrosurea (ENU) causes a mutated APC resulting in a truncated protein similar to those found in humans (Min^+) [87]. The mutated gene displays full penetrance and is dominantly expressed [88]. Genes that act as modifiers of tumor number have also been demonstrated and designated as Modifiers of Min or 'Mom' genes [89]. Min^+ mice are increasingly being used as chemopreventive agent screening platforms. For example, Cox-2 inhibitors reduce but do not totally eliminate adenomas in these mice [90,91].

While highly attractive experimental and mechanistic models, genetically induced mutations in APC have not created an animal that is realistically close to human carcinogenesis. Although these models develop intestinal neoplasms with a histologic sequence similar to that observed in intestinal tumors of humans, the latency time for the development of these neoplasms if very short compared to human lifespans. Moreover,

the neoplasms occur primarily in the small intestine not in the colon and are submucosal, not polypoid. Adenomas and carcinomas in the Min^{+}mice do not display common mutational events found in human tumors such as *ras* and p53 mutations [92]. These findings suggest that APC mutations may be a common early event in human and genetically-modified rodent carcinogenesis; but subsequent mutational events play equally important roles in the phenotypic expression of carcinogenesis in sporadic human tumors. These differences require investigators to approach chemopreventive efficacy data from genetically modified rodent models with caution.

IV. Surrogate endpoint biomarkers for colon cancer chemoprevention

Since the process of colorectal carcinogenesis can take years to decades, assessment of clinical chemoprevention trials using cancer incidence as an endpoint would require extended follow-up periods and very large sample sizes. Given the large number of agents with chemopreventive potential currently under development, it is not feasible to use cancer incidence reduction or large polyp prevention clinical trials as a primary method of assessing preventive agent efficacy in humans. A solution to this problem involves the design and implementation of short duration, limited cohort human trials of potential chemopreventive agents that use surrogate markers of carcinogenesis, biological or morphologic events, in lieu of the actual cancer [93].

An ideal biomarker would demonstrate variability of expression across the different carcinogenic stages, detectable early in the carcinogenesis process, and linked to the occurrence of precancer or cancer. A chemopreventive agent can modulate this ideal biomarker and the degree of modulation reflects the efficacy of the agent. The modulation of the precancerous stage should result in predictable changes in the ultimate endpoint of interest: cancer incidence.

Very few if any of the biomarkers currently available or in use meet all or most of these criteria [94]. Many promising SEBs for colorectal cancer chemoprevention are currently being evaluated [95] (Table 1). A surrogate marker may be associated with a specific phase of carcinogenesis and/or it may be mechanistically linked to the mode of action of the agent being evaluated. SEB currently under evaluation include pathologic markers (adenomatous polyps), functional cellular biomarkers (proliferation, apoptosis and differentiation), biochemical markers (enzymatic function and their metabolites), and molecular as well as genetic markers.

Biomarkers must be validated prior to use in chemoprevention clinical trials. Validation procedures are step wise and consist of the following:

Stage 1 – Preliminary clinical biomarker characterization: In order to interpret future data and to appropriately design subsequent validation trials, clinical characterization of a biomarker is necessary: within-day and between-day variability, variability within subjects and among subjects as well as gender differences.

Stage 2 – Cross sectional case-control screen with model construction: This is a case-control sample. Biomarkers are measured from subjects with and without neoplasia. The data are analyzed in a linear model for predictive efficacy. A logistic regression model may be used to create a clinical prediction rule.

Stage 3 – Observational longitudinal study: This study focuses on temporal associations and prediction of future clinical events. The design follows up high-risk people for a clinical endpoint using baseline biomarker measurement as well as serial measurements of biomarkers over time. Changes in biomarkers are associated with the clinical event. The data are analyzed using linear models and predictive efficacy.

Stage 4 – Long-term randomized intervention: This design collects data on a relevant clinical endpoint in a more prolonged prospective randomized clinical trial of a therapeutic intervention. Such a study addresses the questions (a) Is the biomarker a surrogate endpoint? (b) Can the biomarker be used to evaluate a specific therapy? (c) To what extent can the effect of the treatment be explained by the effect on the biomarker?

IV.A. Pathologic biomarkers

These group of biomarkers refer to histological lesions that are amenable to modulation by a chemopreventive agent. Adenomatous polyps have received substantial attention as pathologic biomarkers of colorectal cancer. Results from the National Polyp Study suggest that the majority of invasive colorectal adenocarcinomas pass through the adenoma stage prior to transformation and invasion. Adenoma resection is associated with a 70% decrease in the incidence of infiltrating colorectal neoplasms [96]. Furthermore, the relatively high recurrence rate of these lesions in a short period of time after their removal (up to 50% in 2 years), makes them useful and practical endpoints to be used in efficient and short biomarker-driven clinical studies [97]. Given the

Table 1. Possible biomarkers for use in early human colorectal chemoprevention studies

Class	Biomarker
Pathologic	Aberrant crypt foci
	Adenomas
Cellular	
Proliferation	[^{3}H]thymidine
	BrdUrd
	PCNA
	Ki67
Apoptosis	Cell morphology
	DNA ladder
	Flow cytometry-based techniques
	TUNEL
Differentiation	Mucin antigens
	Cytokeratines
	Blood group antigens
Biochemical	
Polyunsaturated acid metabolism	Prostaglandins (PGE_2)
	Cyclooxygenases (COX-1 and COX-2)
	Lipooxygenases (5-, 8-, 12-, 15-LOX)
Polyamine metabolism	Polyamines (spermine, spermidine, putrescine), ODC
Phase II enzymes	Glutathione-S-transferase
Molecular Markers	Cyclin D1
	NFκB system (NFκB proteins, IκB proteins)
Genetic Markers	Ras (gene and protein)
	APC (gene and truncated protein)
	18q21 loss (DCC, DPC4/Smad4)
	TGF-β receptor II
	p-53
	DNA methylation
	Microsatellite instability

National Polyp Study data, it is not surprising the many observers consider prevention of adenoma recurrence as sufficient evidence of chemopreventive efficacy to permit drug labeling and marketing [98]. Such a position remains controversial, with many believing that invasive adenocarcinomas of the large bowel may occur without a polypoid dysplastic stage. The recent interest in flat adenomas with dysplasia supports the position that flat dysplastic lesions commonly occur in the colon [99–103]. The lesions may predict for more aggressive behavior than standard polypoid lesions, are commonly missed on routine endoscopies in North America, and may represent a form of dysplasia in the colonic epithelium without the classical polypoid adenoma.

IV.B. Biochemical biomarkers

These SEBs refer to biochemical events in the target cell, which may be modulated by a chemopreventive compound. Usually, they are closely related to the mechanism of action of the agent under study. A possible strategy for their initial evaluation may involve looking at how well their modulation correlates with favorable effects on a pathologic or on a cellular marker. Good examples of these kinds of biomarkers include the measurement of biochemical events associated with COX activity, lipooxygenase (LOX) activity and polyamine metabolism [104–107]. They may be useful as pharmacodynamic endpoints in Phase I chemoprevention studies. In principle, dose selection in early human chemoprevention studies should not be guided only by safety considerations, but also the lowest 'bioefficacious' dose should be sought whenever possible because chemopreventive interventions may be used for life in populations that are for the most part healthy [108].

IV.C. Cellular markers

IV.C.1. Markers of proliferation and differentiation

A number of assays have been developed to measure cellular proliferation (e.g. labeling indices using tritiated thymidine, bromodeoxyuridine [BrdU], proliferation cell nuclear antigen [PCNA], Ki67) [95,109].

Interventions that reduce proliferation have been considered to retard or halt the process of cancer genesis. However, this assumption may not necessarily be true. Cellular differentiation is evaluated through the expression (or lack thereof) of markers that reflect proper epithelial maturation. Propensity towards an undifferentiated phenotype is characteristic of neoplastic processes. Molecules that are expressed in a fully differentiated epithelial cell may then be used as a marker of the pro-differentiating effects of an intervention. For example, *Dilidros biflorus agglutinin* (DBA) which labels mucin in goblet cells has been used as a marker of differentiation. Undifferentiated phenotypes are associated with failure of oligosaccharide synthesis and a decrease in DBA staining [110]. Reduced staining has been documented in colon adenomas and cancers as well as some familial colon cancer syndromes [111].

IV.C.2. Markers of apoptosis

Normal colonic epithelial homeostasis requires a proper balance between cell proliferation and programmed cell death [112]. Failure of apoptotic function is characteristic of neoplastic phenotypes and may play a role in tumor progression [113]. A number of biochemical and genetic changes have been associated with inhibition of apoptosis. An example relevant to chemoprevention of colorectal malignancy involves the association between COX-2 overexpression and failure of apoptosis [114]. Furthermore, modulation of COX-2 enzymatic activity may result in reconstitution of apoptotic capability. Quantitation of apoptotic endpoints in colonic epithelium has been difficult. Many investigators including ourselves have not been able to detect fragmented DNA *in situ* characteristic of the apoptotic endpoints as measured by TUNEL immunohistochemistry. TUNEL positive cells account for less than 1% of all rat colonic epithelial cells in the normal colonic crypt, a surprise finding considering that the apoptotic program is activated as colonic crypt cells senesce at the top of the crypt [115]. Other methods of detected apoptotic activity in human colonic crypts (flow cytometry, caspase induction, Bcl-2/Bax ratio) have not been sufficiently explored to be useful as reproducible biomarkers of apoptosis in the human colonic crypt. Apoptosis indices using TUNEL have proven useful biomarkers in human adenomatous polyps [116]. Apoptotic endpoints as biomarkers for chemopreventive activity in morphologically normal human colonic epithelium, while of interest and potential importance, remain unvalidated.

IV.D. Molecular markers

Critical components of signal transduction pathways may theoretically be used as surrogate markers in chemoprevention if linked in some form to epithelial carcinogenesis [95]. Cell cycle regulators such as cyclin D proteins are a good example. Rodent epithelial cells engineered to overexpress COX-2 display increased levels of cyclin D1 and delayed G1 progression. Cyclin D1 overexpression may increase the number of cells in S-phase. Treatment with nonsteroidal antiinflammatory drugs (NSAIDs) may promote cell cycle suppression through the modulation of COX-2 expression. Thus, cyclin D1 is a reasonable candidate for further validation as a surrogate marker [117]. The pathways associated with prostanoid metabolism and signaling are probably the ones that have received the most attention in colorectal cancer chemoprevention. As our understanding of the relationship between the metabolism of polyunsutared fatty acids and carcinogenesis has improved, a number of new molecular targets have emerged [118,119]. A good example relates to prostaglandin E_2 (PGE_2) signaling. PGE_2 has been implicated in epithelial carcinogenesis. We now know that there are at least four different membrane prostanoid receptors that mediate PGE_2 signaling: EP1, EP2, EP3 and EP4 [119]. Knockout mice deficient in each of these receptors have now been developed and are helping to improve our understanding of downstream events to COX function that may more specifically relate to cancer genesis. EP1-deficient mice exposed to the carcinogen AOM demonstrated increased resistance to the development of ACFs, an early step in rodent colorectal carcinogenesis [120]. These protective effects suggest that signaling through EP1 is critical to the permissive effects of PG on colorectal carcinogenesis. Molecules involved with EP1 signaling or EP1 itself may then become surrogate markers of the anticarcinogenic effects of agents that target prostanoid metabolism as well as more specific targets for the development of novel chemopreventive approaches. Furthermore, we now know that the protective effects of NSAIDs may not only relate to inhibition of COX but to the modulation of LOX. LOX enzymes metabolize arachidonic acid into diverse and multifunctional metabolites. Some of these pathways seem to be procarcinogenic (5-, 8- and 12-LOX) and others seem to display protective effects against carcinogenesis (15-LOX-1 and 15-LOX-2). This new information suggests that when LOX activity is being modulated with a chemopreventive intent, close attention needs

to be paid to the balance between procarcinogenic and anticarcinogenic LOX effects [118]. Thus, as signaling pathways involved with carcinogenesis are better known and their critical molecular components identified, our opportunity to develop novel, more specific and better biomarkers will also increase.

IV.E. Genetic biomarkers

As our understanding of the genetic changes associated with human colon tumorigenesis increases, so does our opportunity to develop novel targeted interventions. These therapeutic approaches would be directed towards the modulation of critical genetically determined molecular abnormalities. Assays can then be established and validated to measure some of these pathways, which may serve as surrogate markers of therapeutic bioactivity. Furthermore, genetic abnormalities associated with the initiation or progression of colorectal carcinogenesis involves the development and expansion of premalignant clones that may be defined and quantified through the detection of specific mutations. The ability of chemoprevention agents of inducing clearance of abnormal clones thus identified can then be used a surrogate marker of bioactivity. Using PCR-based methods, it is now possible to qualify and quantify critical mutations not only in tissue but also in exfoliated cells present in body fluids such as urine, respiratory secretions and feces. These measurements would have clinical value only if they correlate with favorable changes in the actual epithelium at risk. Preclinical *in vivo* data generated using rodent models of chemical carcinogenesis suggest that these approaches may have some promise. For instance, difluoromethylornithine (DFMO) a chemopreventive agent thought to work through the modulation of the enzyme ornithine decarboxylase (ODC) can block the expression of K-ras [121]. NSAIDs have been shown to decrease the expression of p53 and K-ras on preclinical models of carcinogenesis [122]. These changes presumably occur through the elimination of premalignant (or malignant) clones.

An increasing body of literature suggests that mutations and other structural changes of DNA such as deletions or amplifications offer only a partial view into the events that modify gene expression. The methylation of gene promoters has been shown to have a critical impact on gene transduction. In fact, DNA methylation is now recognized as one of the most common abnormalities in human neoplasms [123]. CpG dinucleotides have substantial biological properties and are excellent substracts for DNA methyltransferase [124]. Approximately 70% of cytosines (in CpG dinucleotides) present in human DNA undergo methylation. CpG islands (CPI) are formed by the enrichment of segments of DNA with a large number of CpG dinucleotides (0.5–2 kB long). CPIs are found in the 5′ region of a large number of genes [124]. Young individuals display CPIs that are for the most part unmethylated [124]. CpG islands in promoter regions need to be in an unmethylated state in order to allow the involved genes to remain in an actively transcribed (or transcription-ready) state [125]. There is ample evidence that support hypermethylation of CPIs as a common mechanism causing inhibition of gene expression [126]. The mechanism by which hypermethylation impairs gene transcription is not totally clear. It has been postulated that hypermethylation may interfere with binding of transcription complexes to specific regulatory segments of DNA. This is possible as many transcription factors have GC-rich binding sites as well as CPIs in their DNA recognition elements [127]. An alternative mechanism suggests that methylation may induce changes in the nucleosome core that result in interference with gene transcription. Histone hyperacetylation plays an important facilitating role in DNA transcription. Hypermethylation may induce changes in the composition of DNA in terms of content of hyperacetylated histones affecting gene transcription and functionally inducing gene silencing [128]. Hypermethylation seems to play an important role in the inactivation of a number of tumor suppressor genes involved in colorectal carcinogenesis. Inactivation of $p16^{INK4a}$, *hMLH1* and *APC* genes by hypermethylation has been described in colonic cancers [129–131]. The proportion of hypermethylation of the APC gene in premalignant as well malignant lesions of the colon is the same (approx. 18%) [131]. Ample experimental data supporting hypermethylation as an important mechanism of inactivation of other relevant tumor suppressor genes in colorectal cancer has been reviewed elsewhere [132]. Hypermethylation is a crucial component of carcinogenesis. This component may be therapeutically targeted and exploited as biomarkers of carcinogenesis progression [133].

V. Promising agents for colorectal cancer chemoprevention

Here, we review some of the most promising dietary and pharmacological compounds currently under

evaluation for the chemoprevention of colorectal malignancies (Table 2). This is not meant as a comprehensive evaluation of potential chemopreventive agents. Rather, we have selected the more promising agents and approaches for review.

V.A. Nonsteroidal antiinflammatory drugs

Epidemiology. In the majority of multiple population-based case-controlled studies, aspirin has repeatedly been found to confer a 50% reduction in risk of occurrence of colorectal cancer [134–137]. The largest study consisted of 662,424 North American adults evaluated over a 7-year period (1982–88). On multivariate analyses, a significant relationship between the use of low doses of aspirin and decreased risk of colorectal cancer was demonstrated [135,136]. While not all studies have demonstrated a connection between aspirin intake and colorectal cancer risk reduction [138], there is strong evidence for an association between aspirin use and colorectal risk reduction.

Animal models. Colonic tumorigenesis induced by 1,2-dimethylhydrazine (1,2-DMH) or its metabolites methylazoxymethanol and AOM can be suppressed by treatment of rats with NSAIDs, the most work has been completed using indomethacin and piroxicam [139–142], but many other NSAIDs including sulindac, ibuprofen and ketoprofen have been shown to be chemoprotective in both tumor endpoint and the shorter term ACF assay studies [143,144]. Aspirin at 40% and 80% of maximum tolerated dose administered in the diet of AOM treated male F-344 rats caused significant suppression of colonic mucosal PGE_2 levels and was associated with inhibition in the incidence and number of these tumors [145]. These drugs act, at least in part, during the promotion phase of carcinogenesis, in that most of the chemopreventive activity is retained if piroxicam is started as late as 14 weeks after carcinogen administration [143]. Selective cyclooxygenase-2 inhibitors, are potent colonic chemopreventive agents in rodent models [146,147]. The rodent model data provide a strong and consistent body of information supporting the study of NSAIDs as colorectal carcinoma chemopreventives.

Human studies. Many human studies using a variety of NSAIDs have been conducted to date. Patients with familial adenomatous polyposis have been an obvious at-risk group which has been targeted in some of these studies. Three of these studies were small, randomized clinical trials in which sulindac was administered. Altogether, a total of 45 FAP patients were treated on these studies, and the administration of

Table 2. Agents under study or with developmental potential for colorectal cancer chemoprevention

Class	Sample Agents	Proposed mechanism of action
Non-selective non-steroidal anti-inflammatories	Aspirin, sulindac, piroxicam	Inhibition of COX enzymes, suppression of prostaglandin synthesis (PGE_2), modulation of LOX, non-COX-related mechanisms, pro-apoptotic mechanisms
Selective non-steroidal antiinflammatories (COX-2 inhibitors)	Celecoxib, rofecoxib	Inhibition of prostanoid metabolism through the selective inhibition of COX-2
Micronutrients	Calcium and vitamin D	Binding of bile and fatty acids, induction of cellular differentiation
	Vitamin C	Blocking agent (neutralization of carcinogens), antioxidant
	Vitamin E	Antioxidant
	Organoseleniums (e.g. P-methoxybenzyl selenocyanide)	Inhibition of carcinogen activation, decrease in DNA binding of carcinogens, antioxidative properties, others
	Monoterpenoids (e.g. d-limonene, perillyl alcohol)	Ras inhibition
	Curcuminoids	COX-inhibition, LOX-modulation, antioxidation, anti-angiogenesis, modulation of NFκB
	Folate	DNA synthesis, DNA methylation
Polyamine inhibitors	Difluoromethylornithine (DFMO)	Affects the production of polyamine through the inhibition of ornithine decarboxylase
Hormones	Estrogens	Decrease production of secondary bile acids, inhibition of insulin-like growth factors
Complex dietary interventions	Fiber (especially wheat bran)	Carcinogen binding, bile-acid binding, promotion of stool bulk, decrease conversion of primary into secondary bile acids
	Berries (black raspberries)	Multimechanism

sulindac resulted in a statistically significant decrease in the number of polyps [148–150]. A fourth placebo-controlled randomized clinical study was reported by Steinbach and coworkers, in which a COX-2 inhibitor (celecoxib) was tested at two different dosing schedules (100 mg twice daily and 400 mg twice daily). Subjects were treated for a total of 6 months. Treatment with the lower dose resulted in a decrease in the number of polyps by 12% from baseline and did not reach statistical significance. Celecoxib at the highest dose was able to reduce the number of polyps by 28% from baseline. This reduction was statistically significant ($P = 0.003$) [151]. Baron et al. have recently reported that aspirin reduced sporadic adenoma recurrence at a dose of 81 mg daily. This preliminary report remains to be published at the time of this publication.

These provocative data suggest that NSAIDs are likely to play some role in prevention of colorectal cancer. However, at this time, the magnitude of the beneficial effect and the risks of chronic intake of NSAIDs over a long period of time do not seem sufficient to endorse NSAIDs as a colorectal cancer chemopreventive in individuals at risk for sporadic cancers. While COX-2 inhibition has demonstrated chemopreventive activity in human subjects with familial adenomatous polyposis, the effect is modest and does not replace frequent screening. Moreover, new cases of colorectal cancer have been reported in FAP patients undergoing chemopreventive treatment with NSAIDs [152]. The beneficial effects of NSAIDs and COX-2 inhibitors on polyp formation are transient and disappear soon after drug withdrawal [153]. This suggests that chemopreventive approaches that modulate COX may need to be maintained for life in order to be efficacious in this patient population.

V.B. Dietary multimechanistic compounds

Ample epidemiologic information supports the notion that diets rich in fruits and vegetables are protective against a variety of cancers, including colorectal neoplasms [154]. Based on this information, a number of investigators have attempted to identify the specific compounds in diet that concede protection. Interpretation of observational epidemiologic studies is difficult, as micronutrients and other dietary components are consumed in the form of more complex dietary elements (whole fruits or vegetables), which contain a variety of other substances which could conceivably display chemopreventive properties. Nutritional science has made some progress in the development of improved tools for the estimation of specific micronutrient ingestion, derived from complex dietary information. These tools have allowed the study of protective associations between specific dietary components and cancer occurrence. Unfortunately, substantial methodological problems still remain and further refinements of these methods are still needed [155]. The epidemiologic data derived from patterns of dietary consumption, should be regarded as hypothesis generating, and must be subjected to proper scrutiny in well-designed preclinical and clinical experiments. A large number of specific compounds have been identified through epidemiological techniques as possible colorectal cancer chemopreventives. Many of these compounds have been subjected to stringent preclinical and clinical evalution, and some of them will be presented in this review.

V.B.1. Calcium

Epidemiology. Epidemiological data regarding the association between calcium ingestion and colorectal cancer (or adenoma) risk have varied. A number of case-control and cohort studies have suggested that increase intake of calcium and of vitamin D may be associated with a protective effect. However, other studies have not clearly supported this association. The Iowa Women's Health Study investigated whether a high intake of calcium, vitamin D or dairy products protected against colon cancer. In this report, data obtained from a prospective cohort study of 35,216 Iowa women over 55 years of age who had completed a dietary questionnaire in 1986, was analyzed. The association between dietary calcium, vitamin D and the incidence of colorectal cancer was studied. The relative risks (RR) observed for the highest quintile of intake as compared with the lowest were 0.52 (95% confidence interval (CI) 0.33–0.82) for calcium and 0.54 (95% CI 0.35–0.84) for vitamin D. However, when incorporated into a multivariate model the trends lost statistical significance and the RR were attenuated [156]. Tseng et al. evaluated the association between a number of micronutrients (folate, calcium, iron and antioxidant vitamin) and the risk of colorectal neoplasia. In this study, a reduced risk of adenomas was confined to men in the highest calcium quartile [157]. The associations between fermented dairy products, calcium intake and risk of colorectal cancer were evaluated in a population with a wide variation in intake of dairy product that participated in the Netherlands Cohort Study of diet and cancer, through a case-control study that analyzed data obtained from 215 incident cases of colon cancer

and 111 of rectal cancer, after 3.3 years of follow-up. In crude and multivariate models, total dietary calcium intake (highest vs. lowest quintile, RR 0.92, 95% CI 0.64–1.34) and calcium from fermented dairy products (RR 1.14, 95% CI 0.77–1.68) were not significantly associated with colorectal cancer risk [158]. The association between colorectal adenomas and diet were evaluated in subjects participating in the Nottingham fecal occult blood screening program. In this study, a diet history was obtained from 147 patients with colorectal adenomas and 153 age and sex matched fecal occult blood (FOB)-negative and 176 FOB-positive controls. No association was seen between calcium intake and adenoma protection [159]. Two additional case-control studies evaluated the association between regular usage of supplementation with vitamin A, C, D and E, or with calcium or multivitamins and the risk of developing colorectal adenomas (new or recurrent). No protective effect was documented for any of the studied variables [160]. Bergsma-Kadijk et al. [161] provided a quantitative summary of a large number of epidemiological studies (24 articles reporting 43 measures of RR) addressing the hypothesis of colorectal neoplasia protection by dietary calcium. The weighted mean using a random effects model revealed a RR of 0.89 (95% CI 0.79–1.01). Results obtained from different studies showed substantial heterogeneity with the 'true' RRs falling between 0.50 and 1.60. Summary RRs for cohort and case-control studies were 0.90 and 0.88, respectively. Summary RRs for adenomas and carcinomas were also reported, and calculated at 1.13 (95% CI 0.91–1.39) and 0.86 (95% CI 0.74–0.98), respectively. Overall the epidemiological data on calcium intake is quite heterogeneous, suggesting a very modest beneficial effect at best.

Mechanism and preclinical data. A number of mechanisms have been postulated in order to explain the putative chemopreventive effect of calcium in the gut. Calcium may act through the binding of bile and fatty acids in the intestinal lumen and decreasing exposure of the large bowel epithelium to these substances. Bile and fatty acids may display toxic effects to the colonic epithelium which may result in primary increases in cell proliferation or alternatively in compensatory hyperproliferation secondary to repair responses [162]. In either case, increased proliferation may ultimately lead to carcinogenesis of the colonic epithelium. Calcium may also act through the induction of terminal differentiation of colonic epithelial cells [163]. In preclinical *in vivo* studies using rodent models of chemical carcinogenesis (promoted by high fat diets), calcium administration was associated with a protective effect against colorectal carcinogenesis (decrease proliferation and decrease tumor formation) [164].

Human studies. Some but not all the clinical studies have demonstrated an anticarcinogenic effect as a result of calcium supplementation. Lipkin et al. studied the frequency and distribution of proliferating epithelial cells lining the colonic crypts in 10 subjects at high risk for familial colon cancer. The patterns of cell proliferation were evaluated before and after supplementation of their usual diets with 1.25 g of calcium as calcium carbonate. After 2 to 3 months of calcium supplementation, the investigators found a substantial reduction in epithelial proliferation indicating a beneficial effect [165]. The results of three clinical trials were reported by Wargovich et al. These small studies evaluated the effect of calcium supplementation (calcium carbonate) on rectal epithelial proliferation in subjects with history of sporadic adenomas. In 6 participants, 3-month supplementation with 1500 mg of calcium carbonate failed to suppress proliferation in normal appearing mucosa (thymidine labeling). A daily dose of 2000 mg was, however, associated with a substantial reduction after 30 days. A small randomized placebo-controlled study of calcium was then conducted and revealed a marked suppression of rectal proliferation with calcium administration [166]. A randomized double-blinded, placebo-controlled study of supplemental calcium was conducted in familes with hereditary nonpolyposis colorectal cancer. A total of 30 subjects participated in the study. Study participants received 1.5 g of calcium carbonate or placebo three times a day for a total of 12 weeks. No substantial differences were seen in epithelial cell proliferation determined by labeling index of whole crypts and crypt compartments by 5-bromo-2′-deoxyuridine [167]. Thirty-one patients with familial polyposis, after subtotal colectomy, were randomized to placebo or to supplementation with 1200 mg of calcium daily. The intervention was given for 9 months, and the patients underwent evaluations that included food questionnaires, measurement of fecal pH as well as calcium and bile acid content. Rectal biopsies were also obtained and assessed for epithelial proliferation using thymidine labeling. Fecal pH, weight and bile acid levels were similar before intervention and remained unchanged. Fecal calcium levels were similar before the intervention in both groups but progressively increased in

the calcium group. No substantial differences were observed in rectal epithelial proliferation between the two groups [168]. A randomized placebo-controlled study reported no effect of calcium supplementation on rectal mucosal proliferation in patients with previous large-bowel adenomas [169]. The effects of calcium supplementation on bile acid content have been evaluated clinically. Two different studies documented beneficial calcium-induced changes in bile acid composition, which were characterized by a decrease in total as well as secondary bile acids levels in feces [170,171]. A recently completed randomized placebo-controlled phase III study has been reported by Baron et al. In this study, 930 patients with a history of colorectal adenomas were randomized to receive 3 g of calcium carbonate (1200 mg of elemental calcium) or placebo [172]. A moderate (20%) but statistically significant reduction in the incidence of new adenomas was seen in the cohort receiving calcium supplementation. Overall, the beneficial effects of calcium on colorectal carcinogenesis seem to be modest.

V.B.2. Fiber

Epidemiology. Fiber is another dietary component that has received substantial attention as a possible chemopreventive agent against colon cancer. Ecological studies reported in the 1970s suggested that colorectal cancer incidence in selected populations may relate to the composition of their diet, with diets containing high fiber content being protective [173,174]. The epidemiology of fiber and colon cancer protection is extensive and has been reviewed elsewhere [175].

Mechanisms. It was postulated that dietary fiber may exert protective effects through a number of mechanisms. Increased fiber content may result in the dilution of potential dietary carcinogens through the promotion of increase fecal bulk [174]. Fiber may also bind bile acids, which may be carcinogenic, and may also indirectly affect bile acid conversion in the large bowel from primary to secondary bile acids by inducing a more acidic environment due to its increased bacterial fermentation in the gut [176,177]. Furthermore, increased delivery of the fermentable component of fiber (also known as 'resistant starch') to the large bowel lumen, results in the increase production of short-chain fatty acids through its fermentation by bacteria present in the gut [178]. Short-chain fatty acids such as butyrate may have anticarcinogenic effects in the colorectal epithelium [179].

Human studies. Although a number of case-control studies and at least two meta-analyses have suggested a protective effect for dietary fiber, most of the presently available cohort and randomized studies do not support this hypothesis [180]. A study conducted by the Phoenix Colon Cancer Prevention Physicians' Network accrued 1429 patients with a history of resected adenomas and randomized them to two different levels of daily wheat bran supplementation (2.5 g vs. 13.5 g) [181]. No differences in adenoma recurrence rates were found between the two groups. The second study, the Polyp Prevention Trial, enrolled a total of 2079 participants with a history of colorectal adenomas [182]. The subjects were randomized to an active intervention (dietary counseling coupled with a low-fat, high-fiber diet) or to a control group (no counseling plus usual diet). Again, no differences were observed in adenoma recurrence between the two groups at 1 and at 4 years after the initiation of the intervention. Currently, the available prospective and randomized data do not support the hypothesis of colorectal cancer chemoprevention by fiber.

V.B.3. Folate

Epidemiology. Folate is an essential element in the human diet that plays important biological roles. Folates participate in the regeneration of methionine and are involved in the production of purines and pyrimidines, which are required for the synthesis of DNA. Inadequate intake of folate may result in abnormalities in DNA synthesis and/or repair as well as in changes in DNA methylation, all of which may predispose to colorectal carcinogenesis. The epidemiologic evidence suggests an association between inadequate intake of folic acid and colorectal cancer occurrence. The relationship between folate status and colorectal cancer was evaluated in a case-control study nested within the Alpha-Tocopherol Beta Carotene Study cohort of male smokers 50–69 years of age. Serum folate was measured in 144 incident cases (colorectal cancer) and in 276 age- and clinic-matched controls. The time of blood collection was the same between the groups. Baseline dietary folate information was available from a food questionnaire for 92% of these men. No statistically significant association was seen between serum folate levels and colorectal cancer. The results of dietary intake of folate proved protective. The odds ratios were as follows: 0.40 (95% CI 0.20–1.31), 0.16 (95% CI 0.13–0.88) and 0.51 (95% CI 0.20–1.31) for the second, third and fourth quartiles of energy

adjusted folate intake, respectively, when compared to the first [183]. The relationship between vitamin supplement used and colon cancer was assessed in another case-control study. Two hundred and fifty-one men and 193 women who had been diagnosed with colon cancer were identified through the Surveillance, Epidemiology and End Results (SEER) cancer registry, and were used as cases. Frequency, duration and dose per day of supplement use was assessed during the 10 years prior to cancer diagnosis, ending 2 years before the cancer was found. The average daily intake of supplemental folate was associated with reduced colon cancer. Other supplements also were associated with protective effects in this study [184]. Additional epidemiologic studies have also suggested a protective effect either singly or in association with other factors such as alcohol [185,186]. Smaller explorative case-control studies have also found protective effects in cohorts of patients with inflammatory bowel disease. A case control study was conducted in a cohort of patients with ulcerative colitis at the University of Chicago. This study was designed to evaluate the effects of folate supplementation on the risk of dysplasia or cancer in ulcerative colitis patients. Data was collected from records obtained from 99 patients with diagnoses of pancolitis for periods longer than 7 years. Thirty-five patients with neoplasia were compared with 64 patients in whom dysplasia was never found to assess the effect of folate supplementation on the rate of neoplastic development. A protective association (62% lower incidence) was seen with folate supplementation [RR 0.38 (95% CI 0.12–1.20)] [187]. A second case-control study published by the same group was consistent with those findings as it demonstrated an increased risk for dysplasia or cancer in association with depressed RBC folate [odds ratio 0.82 (95% CI 0.68–0.99)] [188]. Thus, the epidemiology of folate use and colon cancer risk supports a protective association. It remains unclear, however, whether folic acid intake above that required to prevent deficiency is associated with additional protection.

Mechanisms. Folic acid and its metabolites 5,10-methylenetetrahydrofolate and 5-methylhydrofolate are critical to proper DNA synthesis. 5,10-methylenetetrahydrofolate is converted to 5-methyltetrahydrofolate by the enzyme methylenetetrahydrofolate reductase (MTHFR). 5-methyltetrahydrofolate then serves as a methyl donor during the conversion of homocystein into methionine by methionine synthase (MS). MTHFR and MS polymorphisms have been described which may be associated with protection against colon cancer in patients with adequate ingestion of folate [189,190]. Methionine is a precursor of S-adenosyl-methionine, which acts as a major donor of methyl groups *in vivo*. Decrease production of S-adenosyl-methionine may result in abnormal DNA methylation (hypomethylation) which may contribute to carcinogenesis [191,192].

Human studies. Giovannucci evaluated the association between high alcohol plus methyl deficient diets (low folate and low methionine) and colon cancer risk. They analyzed dietary data obtained from a cohort of 47,931 male health professionals. After 6 years of follow-up in this large cohort, 205 new cases of colon cancer were diagnosed and their dietary data was used for purposes of this study. Current alcohol intake was associated with increased risk [RR 2.07 (95% CI 1.29–3.32)] for >2 drinks *versus* less or equal than 0.25 drinks per day. Folate and methionine intakes were weakly associated with increased risk. However, striking associations were seen for combinations of high alcohol ingestion and low intakes of methionine and folate. The RR for total colon cancer was 3.30 (95% CI 1.58–6.88). These findings suggeted that important interactions existed between alcohol, methionine and folate [193]. Data obtained from another large prospective study, the Nurses' Health Study, suggested that supplementation with high daily doses of folate are protective against colorectal cancer. In this report, higher energy-adjusted folate intake was related to a lower risk of colorectal cancer with a RR of 0.69 (95% CI 0.52–0.93). Risk reduction became statistically significant only after 15 years of use [RR 0.25 (95% CI 0.13–0.51)], suggesting that long consumption times may be required to see an effect and also, that the chemopreventive effect of folate may take place early on during the carcinogenesis process [194]. A few small biomarker-driven studies have also been completed. Kim et al. evaluated the effects of folate supplementation on genomic DNA methylation and DNA strand breaks in exons 5–8 of the p53 gene of the colonic mucosa of 20 patients with adenomas that were randomized to receive folate (5 mg/day) or placebo for 1 year after polypectomy. Biomarker measurements occurred at baseline, 6 months and after 1 year. Folate levels in serum, red blood cells (RBC) and colonic mucosa were also measured. Folate supplementation was associated with increases in serum, RBC and

colonic mucosal folate concentrations ($P < 0.002$). More rapid changes (increase) in methylation were seen in the group supplemented with folate as well as a decrease in the extent of p53 strand breaks. This 'positive' changes were realized at 6 months in the intervention group, although improvement was also seen in the placebo group, but only after a year [195]. Overall these data suggest a possible protective effect for folate in populations at increased risk for colon neoplasms. In addition, although further validation is required, folate supplementation may be of particular interest in patients with long-standing inflammatory bowel disease. We are not aware of any reports of Phase III studies with folate as the main intervention and adenoma recurrence or cancer incidence as the main endpoints.

V.B.4. New nutritionally-derived chemopreventive agents

A number of dietary components have demonstrated interesting chemopreventive activity on a variety of preclinical models of colorectal carcinogenesis. Organic selenium compounds appear to be active colorectal chemopreventive agents. *P*-methoxybenzyl selenocyanate and 1,4-phenylenebis (methylene)selenocyate are two synthetic organoselenium compounds which demonstrated substantial protective activity on a rat chemical colorectal carcinogenesis model [196]. Two citrus extracts, perillyl alcohol and auroptene have also been found to reduce tumor incidence and multiplicity in chemical carcinogenesis rodent models [197,198]. The monoterpenoid perillyl alcohol is a metabolic derivative of d-limonene which is present in citrus fruits and other dietary components. Its proposed mode action involves the interference with isoprenylation of small cellular proteins such as $p21^{ras}$. The mechanism of action of auroptene is not fully understood but may involve the induction of phase II metabolic enzymes (blocking activity) as well as the interference with polyamine production through the inhibition of ODC. Other interesting leads under development include the xanthine oxidase inhibitor 1′-acetoxychavicol acetate which is present in the seeds or rhizome of *Langunas galanga*, and the naturally occurring flavonoids diosmin and hesperidin [199,200].

Curcumin, a component of turmeric, deserves special attention. This compound has displayed substantial chemopreventive activity in animal models of colorectal carcinogenesis [201]. It may exert its anticarcinogenic effects through a number of potential mechanisms which include the modulation of COX, modulation of LOX, antiangiogenic activity and antioxidant properties [201,202]. Curcumin's actions on prostanoid metabolism likely involve the modulation of the nuclear factor κB pathway (NFκB). Curcumin has strong antiproliferative effects on established human colon cancer cell models [203]. Preclinical and early clinical pharmacology suggest that curcumin is poorly absorbed through the gastrointestinal tract, making it an attractive compound for use as a chemopreventive of gastrointestinal malignancy [204]. A phase Ia study of a well-defined curcumin preparation is in progress at the University of Michigan and a phase Ib study is in the planning phase.

V.C. Hormones

Epidemiology. Epidemiological data shows a decrease in colorectal cancer mortality in both genders but more substantially in women [205,206]. Grodstein conducted a metaanalytical assessment of the epidemiologic literature. Data from 18 epidemiological studies of postmenopausal hormone therapy and colorectal cancer were pooled and analyzed. A 20% reduction in risk of colon cancer was observed [RR 0.81 (95% CI 0.74–0.86)]. Much of the reduction in risk was limited to current hormone users [RR 0.66 (95% CI 0.59–0.74)] [207]. Hormonal differences have been proposed as an explanation for these differences. Hormone-replacement therapy (HRT) in postmenopausal women and its effects in colorectal cancer endpoints have been evaluated in a handful of studies. Post-menopausal use of hormones was associated with a significant decrease in colorectal cancer mortality in the Cancer Prevention Study II with an overall RR of 0.71 [208]. The degree of protection was higher with current use and with continued use for very long periods of time (over 11 years). In the Nurses' Health Study, a protective effect (decrease in colorectal cancer incidence) was documented with current HRT use. The overall reduction in risk was estimated at 35%. Meta-analytical studies support a protective effect with similar (moderate) levels of protection. The Nurses' Health Study also evaluated the effects of HRT on colon adenoma formation [209]. Hormone replacement therapy was protective against the development of large size adenomas, suggesting that estrogen protective effects may occur during the later phases of carcinogenesis (late adenoma stage).

Postulated mechanisms. Estrogen may exert anticarcinogenic effects in the colon through a number

of possible mechanisms which include a reduction in the production of secondary bile acids, inhibition of insulin-like growth factor levels or through direct effects on the colorectal epithelium [210,211]. A recent molecular epidemiological study suggested that modulation of MSI may in part explain estrogen's anticarcinogenic effects [212].

Preclinical data. A large body of preclinical information has documented the presence of estrogen receptors (ER) in established human cell lines, with a predominance in estrogen receptor beta isoforms [213,214]. Tumors from patients with primary colon cancers have also shown to express ER [215]. Estrogen analogues as well as selective estrogen receptor modulators (SERMs) have produced substantial inhibition in cell growth of established human colon cancer cell lines *in vitro* [216–218]. A number of mechanistic *in vitro* studies are currently undergoing in order to better elucidate the nature of this effects. These studies have supported the involvement of the ER in the regulation of colon cancer cell growth, and have suggeted that signaling through ER-β may be important [219,220]. Preclinical *in vivo* studies have started to look into the effects of estrogenic compounds or SERMs on colon carcinogenesis in chemically-induced rodent models. Ziv et al. studied the effects of tamoxifen on 1,2-dimethylhydrazine (DMH)–HCl-induced colon carcinogenesis in rats. In this study the incidence of DMH-induced colon cancer in rats was reduced by tamoxifen. Whether these effects reflect modulation of estrogen-dependent pathways or were induced through estrogen-independent mechanisms is not clear [221]. Smirnoff et al. reported on the protective effect of estrogen against DMH-induced murine colon carcinogenesis. Tumor multiplicity was significantly decreased by estrogen. In addition, these protective effects were associated with a marked increase in vitamin D receptor (VDR) mRNA and protein expression in normally appearing colonic mucosa. These effects were associated with significant decreases in methylation density of CpG islands in the VDR gene, suggesting that some of the protective effects may relate to reversion of VDR gene silencing. Serum vitamin D and parathyroid hormone levels remained unaffected [222].

Human studies. To our knowledge there are no reports of prospective human chemoprevention studies of estrogenic modulation in high-risk populations for colorectal cancer.

V.D. Polyamine inhibitors

Mechanisms. The polyamines (spermine, spermidine and putrescin), are essential for normal cell growth. The rate-limiting enzyme associated with the synthesis of polyamines is ODC [223]. A number of factors associated with cellular transformation are known inducers of ODC. In addition, suppression of polyamine production through the modulation of ODC results in cell growth inhibition. ODC and the polyamine pathway seem to have an important role in carcinogenesis and their modulation may be associated with a chemopreventive effect [224]. DFMO is the main representative of a class of compounds capable of inhibiting ODC activity.

Preclinical data. DFMO has demonstrated anticarcinogenic activity in *in vitro* cell transformation assays as well as in *in vivo* chemical carcinogenesis models. DFMO is also capable of suppressing cell proliferation in human colon cancer cell models and has displayed inhibitory effects on ACF systems [225,226]. Protective effects in most of these systems have been associated with profound inhibition of ODC activity and substantial depletion of polyamines. Other agents, that seem to act through multiple mechanisms, may also concede anticarcinogenic protection, partly, through the modulation of polyamine levels in intestinal mucosa. Soy protein-rich diets, for instance, were found to reduce mucosal polyamines in male Wistar rats.

Human studies. A phase I study was completed by Love et al. [227] which reported dose limiting ototoxicity for DFMO with doses greater than 1 g/m^2. Subsequently, the same group of investigators conducted a placebo-controlled trial of DFMO in individuals at risk for colorectal cancer (personal history of resected adenomatous polyps or strong family history of colon cancer). The main goals were to assess the effects of this compound on polyamine and ODC levels in different segments of the colorectal mucosa and to evaluate the toxicity associated with 1-year treatment. DFMO was administered at 0.5 g/m^2/day as a single oral dose. Significantly, decreased levels of putrescine and spermidine were found in the rectosigmoid mucosa of individuals treated with DFMO. Similar trends were seen in other anatomical segments (rectum and cecum) but did not reach statistical significance. Modulation of polyamines were observed as early as 3 months after

the initiation of DFMO and persisted until the end of the study. The most worrisome side-effect was hearing loss (12.5%). This was both subjectively reported and audiologically confirmed. Given the hearing side-effects associated with this regimen, the investigators suggested that alternative schedules would need to be evaluated prior to proceeding to phase III studies [228]. A short-term phase IIa trial reported by Meyskens et al. [229] demonstrated that DFMO given at doses as low as 0.10 g/m^2 per day was able to inhibit polyamine production in rectal mucosa after only 1 month. Based on these results a follow-up study was designed in order to establish whether polyamine content in rectal mucosa could be suppressed (without a rebound) for 1 year with doses lower than 0.5 g/m^2. The investigators concluded that a dose of 0.50 g/m^2 was safe and effective, and they recommended this dose level of administration for use in combination phase IIb or single-agent phase III chemoprevention trials [230].

V.E. Other agents

Other agents have displayed chemopreventive effects against colorectal carcinogenesis in a variety of experimental systems. These include the dithione oltipraz, the polyphenol ellagic acid, anethole trithione, diallyl disulfide, butylated hydroxyanisole, purpurin, ritin, butyrate, isothiocyanates, phenyl-3-methyl caffeate and other agents. These agents are being evaluated in animal models and some are undergoing phase I testing at this point.

VI. Future directions

Targeted therapeutics. Substantial progress has been made in our understanding of colorectal premalignancy and colon cancer genesis. The molecular genetic models of colorectal carcinogenesis have grown progressively more complex, offering a more detailed albeit incomplete picture of the critical mechanisms that underline the transformation of colonic epithelial cells.

A number of targeted therapies have been developed and are currently being tested in established human malignancies. The demonstration of substantial therapeutic activity associated with the administration of the targeted agent STI-571 in chronic myelogenous leukemia (CML) and in the highly chemotherapy-resistant gastrointestinal stromal tumors (GIST), has provided impetus to this new paradigm [231]. The case of CML may be of relevance to chemoprevention, as STI-571 is substantially more active during the early stages of the disease (chronic phases) and its efficacy is compromised as additional molecular abnormalities are acquired by the malignant clone. As Vogelstein et al. had suggested, fewer molecular abnormalities seem to be required for the development of colon premalignancy (adenomas) [8].

The procurement of new active leads for testing as colorectal cancer chemopreventives will likely involve the redirection of some of the novel targeted agents currently under clinical evaluation in cancer therapeutics for testing as modulators of premalignancy, when mechanistically appropriate. Good examples include the modulators of growth factor receptors, farnesyl transferase inhibitors, modulators of lipooxigenase and inhibitors of angiogenesis. The immunology of colon premalignancy still remains an untapped field in colorectal cancer chemoprevention that deserves attention [232].

Combination chemoprevention. Given the redundancy of signal transduction regulatory mechanisms in transforming or transformed cells, it is reasonable to believe that a single agent will not be sufficiently active to slow or reverse neoplastic transformation. For this reason, combinations of synergistically active chemopreventive agents are contemplated in the future. An interesting example of a rationale combination of chemopreventive agents might be the combination of a COX-2 inhibitor and an epidermal growth factor receptor inhibitor. Preliminary *in vitro* data suggest strong synergism between blocking the epidermal growth factor receptor and COX-2 enzyme [233–235]. EGFR stabilizes COX-2 expression in this model [233,234]. Indeed, *in vivo* data demonstrate strong anticarcinogenesis synergism between COX inhibition and EGFR inhibition in chemical carcinogenesis models [236,237]. Synergistic combinations of NSAIDs and the polyamine inhibitor DFMO are currently under clinical testing [238,239]. It is reasonable to predict that mechanistically based combinations of chemopreventive agents that target different, yet related signal transduction pathways that control cellular proliferation and apoptosis, offer great promise.

Foodstuffs as chemopreventives. Nature offers a large if not the largest drug procurement potential. Yet, chemoprevention development with nutritional agents has followed two distinct pathways – identifying, isolating, purifying and creating 'drugs' from foodstuffs that appear to be cancer protective in epidemiology

studies or diet modification. Chemoprevention trials of micronutrients identified and tested in this manner have been disappointing. Recent data from the Stoner laboratory suggest that foodstuffs can be grown remarkably uniformly from year to year. These food stuffs (strawberries, black raspberries) contain large quantities of micronutrients known to be cancer protective (calcium, ellagic acid, ferulic acid and β-sitosterol). The concentrations of calcium, ellagic acid, ferulic acid and β-sitosterol in lyophilized black raspberries studied by Dr. Stoner's group [240] were 167, 200, 72 and 21 mg/kg respectively in a 10% black raspberry diet. Yet, in similar chemically induced models where inhibition of colon tumors or ACF were observed, the minimum effective concentrations of these compounds were 500, 8,000, 2,000 and 500 mg/kg, respectively [241–243]. This suggests that low levels of multiple compounds, such as those naturally present in black raspberries and strawberries, may have substantial chemopreventive effects. The use of multiple compounds at low doses, especially with different mechanisms of action, is attractive because it reduces the possibility of toxicity related to large doses of single compounds. As individual food components are tested for chemopreventive activity in model systems, it is logical to look for foods that naturally contain multiple components and to test the effects of a whole food containing them in a single system. Thus, the identification of foodstuffs with chemopreventive properties offers a unique opportunity for simplified multiagent therapy through the administration of well-standardized whole foods (as suppose to purified compounds). Fruits and vegetables when administered whole may help to deliver a number of potentially active chemopreventives in one single intervention.

References

1. Greenlee RT, Murray T, Bolden S, Wingo PA: Cancer statistics. CA Cancer J Clin 50: 7–33, 2000
2. Osborne M, Boyle P, Lipkin M: Cancer prevention. Lancet 349(Suppl II): 27–30, 1997
3. Verno SW: Participation in colorectal cancer screening: A review. J Natl Cancer Inst 89: 1406–1422, 1997
4. Wattenberg LW: An overview of chemoprevention: Current status and future prospects. Proceedings of the Society for Experimental Biology and Medicine 216: 133–141, 1997
5. Lippman S, Lee J, Sabini A: Cancer chemoprevention: Progress and promise. J Natl Cancer Inst 90(20): 1514–1518, 1998
6. Heinonen O, Albanes D: The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 330: 1029–1035, 1994
7. Omen G, Goodman G, Thornquist M, Balmes J, Cullen M, Glass AK, et al.: Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 334(18): 1150–1155, 1996
8. Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 61: 759–767, 1990
9. Rubinfeld B, Souza B, Albert I, Muller O, Chamberlain SH, Maziarz FR, et al.: Association of the APC gene product with β-catenin. Science 262: 1731–1734, 1993
10. Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, et al.: Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 253: 665–669, 1991
11. Groden J, Thliveris A, Samowitz W, Carlson M, Gilbert L, Albertsen H, et al.: Identification and characterization of the familial adenomatous polyposis coli gene. Cell 66: 589–600, 1991
12. Fearnhead NS, Britton MP, Bodmer WF: The ABC of APC. Human Molec Genet 10(7): 721–733, 2001
13. Hiltunen MO, Alhonen L, Koistinaho J, Myohanen S, Paakkonen M, Marin S, Kosma VM, Janne J: Hypermethylation of the APC (Adenomatous Polyposis Coli) gene promoter region in human colorectal carcinoma. Int J Cancer 70: 644–648, 1997
14. Munemitsu S, Albert I, Souza B, Rubinfeld B, Polakis P: Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor suppressor protein. Proc Natl Acad Sci USA 92: 3046–3050, 1995
15. Hulsken J, Birchmeier W, Behrens J: E-cadherin and APC compete for the interaction with beta-catenin and the cytoskeleton. J Cell Biol 127: 2061–2069, 1995
16. Korinek V, Barker N, Morin PJ, van Wichen D, deWeger R, Kinzler KW, et al.: Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-1-colon carcinoma. Science 275: 1784–1787, 1997
17. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kuzler KW: Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 275: 1787–1790, 1997
18. Funk CD, Funk LB, Kennedy ME, Pong AS, Fitzgerald GA: Human platelet/erythroleukemia prostaglandin synthase: cDNA cloning, expression, and gene chromosomal assignment. FASEB J 5: 2304–2312, 1991
19. Sigal E: The molecular biology of mammalian arachidonic acid metabolism. Am J Physiol 260: L13–L28, 1991
20. Sirois J, Richards J: Purification and characterization of a novel, distinct isoform of prostaglandin endoperoxide synthase induced by human chorionic gonadotrophin in granulosa cells of rat preovulatory follicles. J Biol Chem 267: 6382–6388, 1992
21. Herschman H: Prostaglandin synthase 2. Biochim Biophys Acta 1299: 125–140, 1996
22. Sano H, Kawahito Y, Wilder R, Hashiramoto A, Mukai S, Asai K, et al.: Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res 55: 3785–3789, 1995
23. Kargman S, O'Neill S, Vickers P, Evans J, Mancini J, Jothy S: Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. Proc AACR 36: 601, 1995

24. Hecht J, Rovai L, Herschman H: Cyclooxygenase-2 expression and regulation in colonic mucosa, cancer, and cell lines. Proc AACR 36: 598, 1995
25. Eberhart C, Coffey R, Radhika A, Giardiello F, Ferrenbach S, DuBois R: Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 107: 1183–1188, 1994
26. Subbaramaiah K, Telang N, Ramonetti J, Araki R, DeVito B, Weksler B, et al.: Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. Cancer Res 56: 4424–4429, 1996
27. Boolbol S, Dannenberg A, Chadburn A, Martucci C, Guo X, Ramonetti J, et al.: Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res 56: 2556–2560, 1996
28. Oshima M, Dinchuk J, Kargman S, Oshima H, Hancock B, Kwong E, et al.: Suppression of intestinal polyposis in Apc^{-716} knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87: 803–809, 1996
29. DuBois R, Awad J, Morrow J, Roberts L, Bishop P: Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor–alpha and phorbol ester. J Clin Invest 93: 493–498, 1994
30. Tsujii M, DuBois R: Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 83: 493–501, 1995
31. Thiagalingam S, Lengauer C, Leach FS, Schutte M, Hahn SA, Overhauser J, et al.: Evaluation of candidate tumor suppressor genes on chromosome 18 in colorectal cancers. Nat Genet 13: 343–346, 1996
32. Fazeli A, Dickinson SL, Hermiston ML, Tighe RV, Steen RG, Small CG, et al.: Phenotype of mice lacking functional deleted in colorectal cancer (Dcc) gene. Nature 386: 796–804, 1997
33. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, et al.: The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 75: 1027–1038, 1993
34. Leach FS, Nicolaides NC, Papadopoulos N, Liv B, Jen J, Parsons R, et al.: Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 75: 1215–1225, 1993
35. Brenner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, et al.: Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary nonpolyposis colon cancer. Nature 368: 258–261, 1994
36. Thibodeau SN, French AJ, Cunningham JM, Tester D, Burgart LJ, Roche PC, et al.: Microsatellite instability in colorectal cancer: Different mutator phenotypes and the principal involvement of hMLH1. Cancer Res 58: 1713–1718, 1998
37. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, et al.: Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science 268: 1336–1338, 1995
38. Souza RF, Lei J, Yin J, Appel R, Zon TT, Zhou X, et al.: A transforming growth factor beta 1 receptor type II mutation in ulcerative colitis associated neoplasms. Gastroenterology 112: 40–45, 1997
39. Massague J: The transforming growth factor beta family. Annu Rev Cell Biol 6: 597–641, 1990
40. Massague J, Polyak K: Mammalian antiproliferative signals and their targets. Curr Opin Genet Dev 5(1): 91–96, 1995
41. Alexandrow M, Moses H: Transforming growth factor beta and cell cycle regulation. Cancer Res 55: 1452–1457, 1995
42. Oberhammer F, Pavelka M, Sharma S, Tiefenbacher R, Purchio A, Bursch W, Shulte-Hermann R: Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor beta 1. Proc Natl Acad Sci USA 89: 5408–5412, 1992
43. Rorke E, Jacobberger J: Transforming growth factor-beta 1 (TGF beta 1) enhances apoptosis in human papillomavirus type 16-immortalized human ectocervical epithelial cells. Exp Cell Res 216: 65–72, 1995
44. Lin H, Wang XF, Ng-Eaton E, Weinberg R, Lodish H: Expression cloning of the TGF-beta type II receptor, a functional transmembrane serine/threonine kinase. Cell 68: 775–785, 1992
45. Wrana J, Attisano L, Carcamo J, Zentella A, Doody J, Laiho M, Wang X-F, Massague J: TGF-beta signals through a heteromeric protein kinase receptor complex. Cell 71: 1003–1014, 1992
46. Moustakas A, Lin H, Henis Y, Plamondon J, O'connor-McCourt M, Lodish H: The transforming growth factor beta receptors types I, II and III form hetero-oligomeric complexes in the presence of ligand. J Biol Chem 268: 22215–22218
47. Boyd F, Massague J: Transforming growth factor beta inhibition of epithelial cell proliferation linked to the expression of a 53-kDa membrane receptor. J Biol Chem 264: 2272–2278, 1989
48. Laiho M, Weis F, Boyd F, Ignotz R, Massague J: Responsiveness to transforming growth factor-beta (TGF-beta) restored by genetic complementation between cells defective in TGF-beta receptor I and II. J Biol Chem 266: 9108–9112, 1991
49. Laiho M, Weis M, Massague J: Concomitant loss of transforming growth factor (TGF) – beta receptor types I and II in TGF-beta-resistant cell mutants implicates both receptor types in signal transduction. J Biol Chem 265: 18518–18524, 1990
50. Wrana J, Attisano L, Wieser R, Ventura F, Massague J: Mechanism of activation of the TGF-β receptor. Nature 370: 341–347, 1994
51. Grady W, Myeroff L, Swinker S, Rajput A, Thiagalingam J, Lutterbaugh J, Neumann A, Brattain M, Chang J, Kim S-J, Kinzler K, Vogelstein B, Willson J, Markowitz S: Mutational inactivation transforming growth factor beta receptor type II in microsatellite stable colon cancers. Cancer Res 59: 320–324, 1999
52. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan R, Zborowska E, Kinzler K, Vogelstein B, Brattain M, Willson JKV: Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science 268: 1336–1338, 1995

53. Parsons R, Myeroff L, Liu B, Willson JKV, Markowitz S, Kinzler K, Vogelstein B: Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. Cancer Res 55: 5548–5550, 1995
54. Thibodeau S, Bren G, Schaid D: Microsatellite instability in cancer of the proximal colon. Science 260: 816–819, 1993
55. Ionov Y, Peinado M, Malkhosyan S, Shibata D, Perucho M: Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 363: 558–561, 1993
56. Markowitz S: TGF-β receptors and DNA repair genes, coupled targets in a pathway of human colon carcinogenesis. Biochim et Biophys Acta 1470: M13–M20, 2000
57. Bird R: Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: Preliminary findings. Cancer Lett 37: 147–151, 1987
58. Siu I, Pretlow T, Amini S, Pretlow T: Identification of dysplasia in human colonic aberrant crypt foci. Am J Pathol 150: 1805–1813, 1997
59. Polyak K, Hamilton S, Vogelstein B, Kinzler K: Early alteration of cell-cycle-regulated gene expression in colorectal neoplasia. Am J Pathol 149: 381–387, 1996
60. Takayama T, Katsuki S, Takahashi Y, Ohi M, Nojiri S, Sakamaki S, et al.: Aberrant crypt foci of the colon as precursors of adenoma and cancer. N Engl J Med 339: 1277–1284, 1998
61. Jen J, Powell S, Papadopoulos N, Smith K, Hamilton S, Vogelstein B, et al.: Molecular determinants of dysplasia in colorectal lesions. Cancer Res 54: 5523–5526, 1994
62. Pretlow T, Brasitus T, Fulton N, Cheyer C, Kaplan E: K-ras mutations in putative preneoplastic lesions in human colon. J Natl Cancer Inst 85: 2004–2007, 1993
63. Smith A, Stern H, Penner M, Hay K, Mitri A, Bapat B, et al.: Somatic APC and K-ras codon 12 mutations in aberrant crypt foci from human colons. Cancer Res 54: 5527–5530, 1994
64. Hamilton S: Pathogenesis of polyps (adenomas). Dis Colon Rectum 26: 413–414, 1983
65. Wargovich MJ, Chen CD, Harris C, Yang E, Velasco M: Inhibition of aberrant crypt growth by non-steroidal anti-inflammatory agents and differentiation agents in the rat colon. Int J Cancer 60(4): 515–519, 1995
66. Reddy B, Rao C, Seibert K: Evaluation of a cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. Cancer Res 56: 4566–4569, 1996
67. Kawamori T, Rao C, Seibert K, Reddy B: Chemopreventive effect of celecoxib, a specific cyclooxygenase-2 inhibitor on colon carcinogenesis. Cancer Res 58: 409–412, 1998
68. Konstantakos A, Siu I, Pretlow T, Stellato T, Pretlow T: Human aberrant crypt foci with carcinoma *in situ* from a patient with sporadic colon cancer. Gastroenterology 111: 772–777, 1996
69. Thurnherr N, Deschner EE, Stonehill EH, Lipkin M: Induction of adenocarcinomas of the colon in mice by weekly injections of 1,2-dimethylhydrazine. Cancer Res 33: 940–945, 1973
70. Narisawa T, Sato T, Hayakawa M, Sakuma A, Nakano H: Carcinoma of the colon and rectum of rats by rectal infusion of *N*-methyl-*N*-nitro-*N*-nitroguanidine. Gann 62: 231–234, 1971
71. Rogers KJ, Pegg AE: Formation of o6-methylguanine by alkylation of rat liver, colon and kidney DNA following administration of 1,2-dimethylhydrazine. Cancer Res 37: 4082–4087, 1977
72. Moon RC, Fricks CM, Schiff LJ: Effects of age and sex on colon carcinogenesis. Proc Am Assoc Cancer Res 17: 23–28, 1976
73. Winneker RC, Tompkins M, Westenberger P, Harris J: Morphological studies of chemically induced colon tumours in hamsters. Exp Mol Pathol 27: 19–34, 1977
74. Pozharisski KM, Kapustin YM, Likhachev AJ, Shaposhnicov JD: The mechanism of carcinogenic action of 1,2-dimethylhydrazine in rats. Int J Cancer 15: 673–683, 1975
75. Ward JM, Yamamoto RS, Brown CA: Pathology of intestinal neoplasm and other lesions in rats exposed to azoxymethane. J Natl Cancer Inst 51: 1029–1039, 1973
76. De Filippo C, Caderni G, Bazzicalupo M, Briani C, Giannini A, Fazi M, Dolara P: Mutations of the Apc gene in experimental colorectal carcinogenesis induced by azoxymethane in F344 rats. Br J Cancer 77: 2148–2151, 1998
77. Maltzman T, Whittington J, Driggers L, Stephens J, Ahnen D: AOM-induced mouse colon tumors do not express full-length APC protein. Carcinogenesis 18(12): 2435–2439, 1997
78. Bissonnette M, Khare S, von Lintig FC, Wali RK, Nguyen L, Zhang Y, Hart J, Skarosi S, Varki N, Boss GR, Brasitus TA: Mutational and nonmutational activation of p21ras in rat colonic azoxymethane-induced tumors: Effects on mitogen-activated protein kinase, cyclooxygenase-2 and cyclin D1. Cancer Res 60(16): 4602–4609, 2000
79. Singh J, Kulkarni N, Kelloff G, Reddy BS: Modulation of azoxymethane-induced mutational activation of ras protooncogenes by chemopreventive agents in colon carcinogenesis. Carcinogenesis 15(7): 1317–1323, 1994
80. Shivapurkar N, Tang Z, Ferreira A, Nasim S, Garett C, Alabaster O: Sequential analysis of K-ras mutations in aberrant crypt foci and colonic tumors induced by azoxymethane in Fischer-344 rats on high-risk diet. Carcinogenesis 15(4): 775–778, 1994
81. Vivona AA, Shpitz B, Medline A, Bruce WR, Hay K, Ward MA, Stern HS, Gallinger S: K-ras mutations in aberrant crypt foci, adenomas and adenocarcinomas during azoxymethane-induced colon carcinogenesis. Carcinogenesis 14(9): 1777–1781, 1993
82. Shivapurkar N, Belinsky SA, Wolf DC, Tang Z, Alabaster O: Absence of p53 gene mutations in rat colon carcinomas induced by azoxymethane. Cancer Lett 96(1): 63–70, 1995
83. Erdman SH, Wu HD, Hixson LJ, Ahnen DJ, Gerner EW: Assessment of mutations in Ki-ras and p53 in colon cancers from azoxymethane- and dimethylhydrazine-treated rats. Mol Carcinog 19(2): 137–144, 1997

84. Koesters R, Hans MA, Benner A, Prosst R, Boehm J, Gahlen J, Doeberitz MK: Predominant mutation of codon 41 of the beta-catenin proto-oncogene in rat colon tumors induced by 1,2-dimethylhydrazine using a complete carcinogenic protocol. Carcinogenesis 22(11): 1885–1890, 2001
85. Wang QS, Papanikolau A, Sabourin CL, Rosenberg DW: Altered expression of cyclin D1 and cyclin-dependent kinase 4 in azoxymethane-induced mouse colon tumorigenesis. Carcinogenesis 19(11): 2001–2006, 1998
86. Luceri C, De Filippo C, Caderni G, Gambacciani L, Salvatori M, Giannini A, Dolara P: Detection of somatic DNA alterations in azoxymethane-induced F344 rat colon tumors by random amplified polymorphic DNA analysis. Carcinogenesis 21(9): 1753–1756, 2000
87. Luongo C, Gould KA, Su LK, et al.: Mapping of multiple intestinal neoplasia (Min) to proximal chromosome 18 of the mouse. Genomics 15: 3–8, 1993
88. Moser AR, Pitot HC, Dove WF: A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 247: 322–324, 1990
89. Dietrich WF, Lander ES, Smith JS, et al.: Genetic identification of Mom-1, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse. Cell 75: 631–639, 1993
90. Oshima M, Dinchuk JE, Kargman SL, et al.: Suppression of intestinal polyposis in Apc delta 716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87: 803–809, 1996
91. Fodde R, Edelmann W, Yang K, et al.: A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors. Proc Natl Acad Sci USA 91: 8969–8973, 1994
92. Smits R, Kartheuser A, Jagmohan-Changur S, et al.: Loss of Apc and the entire chromosome 18 but absence of mutations at the Ras and Tp53 genes in intestinal tumors from Apc 1638N, a mouse model for Apc-driven carcinogenesis. Carcinogenesis 18: 321–327, 1997
93. Kelloff GJ, Boone CW, Steele VE, Crowell JA, Lubet R, Sigman CC: Progress in cancer chemoprevention: Perspectives on agent selection and short-term clinical intervention trials. Cancer Res 54(Suppl): 2015s–2024s, 1994
94. Lippman S, Lec J, Lotan R, et al.: Biomarkers as intermediate endpoints in chemoprevention trials. J Natl Cancer Inst 82: 555, 1990
95. Einspahr JG, Alberts DS, Gapstur SM, et al.: Surrogate end-point biomarkers as measures of colon cancer risk and their use in cancer chemoprevention trials. Cancer Epidemiol Biomarkers Prev 6: 37–48, 1997
96. O'Brien MJ, Winauer SJ, Zauber AG, et al.: The national polyp study: Patient and polyp characteristics associated with high-grade dysplasia in adenomas. Gastroenterology 98: 371–379, 1990
97. Hixson LJ, Fennerty MB, Sampliner RE, et al.: Two-year incidence of colonic adenomas developing after tandem colonoscopy. Am J Gastroenterol 89: 687–691, 1994
98. Kelloff GJ, Sigman CC, Johnson KM, Boone CW, Greenwald P, Crowell JA, Hawk ET, Doody LA: Perspectives on surrogate end points in the development of drugs that reduce the risk of cancer. Cancer Epidemiol Biomarkers Prev 9(2): 127–137, 2000
99. Fujii T, Rembacken BJ, Dixon MF, Yoshida S, Axon AT: Flat adenomas in the United Kingdom: Are treatable cancers being missed? Endoscopy 30(5): 437–443, 1998
100. Kudo S, Kashida H, Tamura T, Kogure E, Imai Y, Yamano H, et al.: Colonoscopic diagnosis and management of nonpolypoid early colorectal cancer. World J Surg 24(9): 1081–1090, 2000
101. Muto T, Kamiya J, Sawada T, Konishi F, Sugihara K, Kubota Y, et al.: Small 'flat adenoma' of the large bowel with special reference to its clinicopathologic features. Dis Colon Rectum 28(11): 847–851, 1985
102. Saitoh Y, Waxman I, West AB, Popnikolov NK, Gatalica Z, Watari J, et al.: Prevalence and distinctive biologic features of flat colorectal adenomas in a North American population. Gastroenterology 120(7): 1657–1665, 2001
103. Yashiro M, Carethers JM, Laghi L, Saito K, Slezak P, Jaramillo E, et al.: Genetic pathways in the evolution of morphologically distinct colorectal neoplasms. Cancer Res 61(6): 2676–2683, 2001
104. Shiff SJ, Basil R: The role of cyclooxygenase inhbition in the antineoplastic effects of nonsteroidal antiinflammatory drugs (NSAIDs). J Exp Med 190(4): 445–450, 1999
105. Shureiqui I, Lippman SM: Lipooxygenase modulation to reverse carcinogenesis. Cancer Res 61: 6307–6312, 2001
106. Pegg AE: Polyamine metabolism and its importance in neoplastic growth and as target for chemotherapy. Cancer Res 48: 759–774, 1988
107. Hixson LJ, Garewal HS, McGee DL, Sloan D, Fennerty MB, Sampliner RE, Gener EW: Ornithine decarboxylase and polyamines in colorectal neoplasia and mucosa. Cancer Epidemiol Biomarkers Prev 2: 369–374, 1993
108. Goodman G: The clinical evaluation of chemoprevention agents: Defining and contrasting phase I, II, III objectives. Cancer Res 52: 2752–2758, 1992
109. Einspahr J, Alberts D, Xie T, et al.: Comparison of proliferating cell nuclear antigen *versus* the more standard measures of rectal mucosal proliferation rates in subjects with a history of colorectal cancer and normal age-matched controls. Cancer Epidemiol Biomarkers Prev 4: 359–366, 1995
110. Boland CR, Montgomery CK, Kim YS: Alterations in human colonic mucin occurring with cellular differentiation and malignant transformation. Proc Natl Acad Sci USA 79: 2051–2055, 1988
111. Sam JS, Lynch HT, Burt RW, et al.: Abnormalities of lectin histochemistry in familial polyposis coli and hereditary nonpolyposis colorectal cancer. Cancer 66: 502–508, 1990
112. Hall PA, Coates PJ, Ansari B, Hopwood D: Regulation of cell number in the mammalian gastrointestinal tract: The importance of apoptosis. J Cell Sci 107: 3569–3577, 1994
113. Bedi A, Pasricha PJ, Akhtar AJ, et al.: Inhibition of apoptosis during development of colorectal cancer. Cancer Res 55: 1811–1816, 1995
114. Piazza G, Rahm A, Krutzsch M, et al.: Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis. Cancer Res 55: 3110–3116, 1995

115. Barnes C, Cameron I, Hardman W, Lee M: Non-steroidal anti-inflammatory drug effect on crypt cell proliferation and apoptosis during initiation of rat colon carcinogenesis. Br J Cancer 77: 573–580, 1998
116. Stoner GD, Budd GT, Ganapathu R, DeYoung B, Kresty LA, Nitert M, Fryer B, Church JM, Provencher K, Pamucku R, Piazza G, Hawk E, Kelloff G, Elson P, van Stolk RU: Sulindac sulfone induced regression of rectal polyps in patients with familial adenomatous polyposis. Adv Exp Med Biol 470: 45–53, 1999
117. DuBois RN, Shao J, Tsujii M, et al.: G1 delay in cells overexpressing prostaglandin endoperoxide synthase-2. Cancer Res 56: 733–737, 1996
118. Shureiqui I, Lippman SM: Lipoxygenase modulation to reverse carcinogenesis. Cancer Res 61: 6307–6312, 2001
119. Narumiya S, Fitzgerald GA: Genetic and pharmacological analysis of prostanoid receptor function. J Clin Invest 108(1): 25–30, 2001
120. Watanabe K, et al.: Role of prostaglandin E receptor subtype EP1 in colon carcinogenesis. Cancer Res 59: 5093–5096, 1999
121. Crist K, Wang Y, Lubet R, et al.: P 53 accumulation and K-ras mutations as surrogate endpoint biomarkers (SEBs) for chemoprevention in a rat colon tumor model. Proc Am Assoc Cancer Res 36: A591, 1995
122. Singh J, Kelloff G, Reddy BS: Intermediate biomarkers of colon cancer: Modulation of expression of ras oncogene by chemopreventive agents during azoxymethane induced colon carcinogenesis. Carcinogenesis 14: 699–704, 1993
123. Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP: Alterations in DNA methylation: A fundamental aspect of neoplasia. Adv Cancer Res 72: 141–196, 1998
124. Bird AP: CpG-rich islands and the function of DNA methylation. Nature 321: 206–213, 1986
125. Bird A: The essentials of NA methylation. Cell 70: 5–8, 1992
126. Sakai T, Toguchida J, Ohtani N, Yandell DW, Rapaport JM, Dryja TP: Allele-specific hypermethylation of the retinoblastoma tumor-suppressor gene. Am J Hum Genet 48: 880–888, 1991
127. Bird AP, Wolffe AP: Methylation-induced repression: Belts, braces and chromatin. Cell 99: 451–454, 1999
128. Jones PL, Wolffe AP: Relationships between chromatin organization an DNA methylation in determining gene expression. Semin Cancer Biol 9: 339–347, 1999
129. Herman JG, Merlo A, Mao L, et al.: Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. Cancer Res 55: 4525–4530, 1995
130. Herman JG, Umar A, Polyak K, et al.: Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci USA 95: 6870–6875, 1998
131. Esteller M, Sparks A, Toyota M, et al.: Analysis of adenomatous polyposis coli promoter hypermethylation in human cancer. Cancer Res 60: 4366–4371, 2000
132. Jubb AM, Bell SM, Quirke P: Methylation and colorectal cancer. J Pathol 195: 111–134, 2001
133. Issa JP: CpG-island methylation in aging and cancer. Curr Top Microbiol Immunol 249: 101–118, 2000
134. Logan RFA, Little J, Hawtin PG, Hardcastle JD: Effect of aspirin and non-steroidal anti-inflammatory drugs on colorectal adenomas: Case-control study of subjects particpating in the Nottingham faecal occult blood screening programme. BMJ 307: 285–289, 1993
135. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC: Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. Ann Intern Med 121: 241–246, 1994
136. Kune G, Kune S, Watson L: Colorectal cancer risk, chronic illnesses, operations and medications: Case control results from the Melbourne Colorectal Cancer Study. Cancer Res 48: 4399–4404, 1988
137. Thun MJ, Namboodiri MM, Heath CW Jr.: Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 328: 1593–1596, 1991
138. Paganini-Hill A, Hsu G, Ross RK, Henderson BE: Aspirin use and incidence of large bowel cancer in a California retirement community. J Natl Cancer Inst 83: 1182–1183, 1991
139. Moorghen M, Ince P, Finney KJ, Suhnter JP, Watson AJ, Appleton DR: The effect of sulindac on colonic tumour formation in dimethylhydrazine-treated mice. Acta Histochemica 34(Suppl): 195–199, 1990
140. Pollard M, Luckert PH: Treatment of chemically-induced intestinal cancer with indomethacin. Proc Soc Exp Biol Med 167: 161–164, 1981
141. Pollard M, Luckert PH: Effect of indomethacin on intestinal tumor induced in rats by the acetate derivative of dimethylnitrosamine. Science 214: 558–559, 1981
142. Pollard M, Luckert PH, Schmidt MA: The suppressive effect of piroxicam on autochronous intestinal tumors in the rat. Cancer Lett 21: 57–61, 1983
143. Reddy BS, Maruyama H, Kelloff G: Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteriodal anti-inflammatory drug, during different stages of rat colon tumor development. Cancer Res 47: 5340–5346, 1987
144. Baron J, Tosteson T, Wargovich M, Sandler R, Mandel J, Bond J, et al.: Calcium supplementation and rectal mucosal proliferation: A randomized controlled trial. J Natl Cancer Inst 87: 1303–1307, 1995
145. Reddy BS, Rao CV, Rivenson A, Kelloff G: Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. Carcinogenesis 14: 1493–1497, 1993
146. Kawamori T, Rao C, Seibert K, Reddy B: Chemopreventive effect of celecoxib, a specific cyclooxygenase-2 inhibitor on colon carcinogenesis. Cancer Res 58: 409–412, 1998
147. Oshima M, Dinchuk J, Kargman S, Oshima H, Hancock B, Kwong E, et al.: Suppression of intestinal polyposis in Apc delta 716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87: 803–809, 1996
148. Laboyle D, Fischer D, Vielh P, et al.: Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology 101: 635–639, 1991
149. Nugent KP, Farmer KCR, Spigelman AD, Williams CB, Phillips RKS: Randomized controlled trial of the effect of

sulindac on duodenal and rectal polyposis and cell proliferation in patients with familial adenomatous polyposis. Br J Surg 80: 1618–1619, 1993
150. Giardiello FM, Hamilton SR, Krush AJ, et al.: Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med 328: 1313–1316, 1993
151. Steinbach G, Lynch PM, Phillips RKS, et al.: The effect of celecoxib a cyclooxygenase-2 inhibitor in familial adenomatous polyposis. N Engl J Med 342: 1946–1952, 2000
152. Keller JJ, Offerhaus GJ, Polak M, et al.: Rectal epithelial apoptosis in familial adenomatous polyposis patients treated with sulindac. Gut 45: 822–828, 1999
153. Spagnesi M, Tonelli F, Dolara P, et al.: Rectal proliferation and polyp recurrence in patients with familial adenomatous polyposis after sulindac treatment. Gastroenterology 106: 362–366, 1994
154. Block G, Patterson B, Subar A: Fruit, vegetables and cancer prevention: A review of the epidemiological evidence. Nutr Cancer 18(1): 1–29, 1992
155. Leclercq C, Valsta LM, Turrini A: Food composition issues – implications for the development of food-based dietary guidelines. Public Health Nutr 4(2B): 677–682, 2001
156. Bostick RM, Potter JD, Sellers TA, McKenzie DR, Kushi LH, Folsom AR: Relation of calcium, vitamin D, and dairy food intake to incidence of colon cancer among older women. The Iowa Women's Health Study. Am J Epidemiol 137: 1302–1317, 1993
157. Tseng M, Murray SC, Kuper LL, Sandler RS: Micronutrients and the risk of colorectal adenomas. Am J Epidemiol 144(11): 1026–1027, 1996
158. Kampman E, Goldbohm RA, van den Brandt PA, Van't Veer P: Fermented dairy products, calcium and colorectal cancer in the Netherlands Cohort Study. Cancer Res 54(12): 3186–3190, 1994
159. Little J, Logan RF, Hawtin PG, Hardcastle JD, Turner ID: Colorectal adenomas and diet: A case-control study of subjects participating in the Nottingham faecal occult blood screening programme. Br J Cancer 67: 177–184, 1993
160. Neugut AI, Horvath K, Whelan RL, Terry MB, Garbowski GC, Bertram A, Forde KA, Treat MR, Waye J: The effect of calcium and vitamin supplements on the incidence and recurrence of colorectal adenomatous polyps. Cancer 78(4): 723–728, 1996
161. Bergma-Kadijk JA, van't Veer P, Kampman E, Burema J: Calcium does not protect against colorectal neoplasia. Epidemiology 7(6): 590–597, 1996
162. Bostick RM, Potter JD, Fosdick L, et al.: Calcium and colorectal epithelial cell proliferation. A preliminary randomized, double-blind, placebo-controlled clinical trial. J Natl Cancer Inst 85: 132–141, 1993
163. Newmark HL, Wargovich MJ, Bruce WR: Colon cancer and dietary fat, phosphate, and calcium: A hypothesis. J Natl Cancer Inst 72: 1323–1325, 1984
164. Pence BC: Role of calcium in colon cancer prevention: Experimental and clinical studies. Mutat Res 290: 87–95, 1993
165. Lipkin M, Newmark H: Effect of added dietary calcium on colonic epithelial-cell proliferation in subjects at high risk for familial colonic cancer. N Engl J Med 313(22): 1381–1384, 1985
166. Wargovich MJ, Isbell G, Shabot M, Winn R, Lanza F, Hochman L, Larson E, Lynch P, Roubein L, Levin B: Calcium supplementation decreases rectal epithelial cell proliferation in subjects with sporadic adenoma. Gastroenterology 103(6): 1994–1995, 1992
167. Cats A, Kleibeuker JH, van der Meer R, Kuipers F, Sluiter WJ, Hardonk MJ, Oremus ET, Mulder NH, de Vries EG: Randomized, double-blinded, placebo-controlled intervention study with supplemental calcium in families with hereditary nonpolyposis colorectal cancer. J Natl Cancer Inst 87: 598–603, 1995
168. Stern HS, Gregoire RC, Kashtan H, Stadler J, Bruce RW: Long-term effects of dietary calcium on risk markers for colon cancer in patients with familial polyposis. Surgery 108(3): 528–533, 1990
169. Baron JA, Tosteson TD, Wargovich MJ, Sandler R, Mandel J, Bond J, Haile R, Summers R, van Stolk R, Rothstein R: Calcium supplementation and rectal mucosal proliferation: A randomized controlled trial. J Natl Cancer Inst 87(17): 1303–1307, 1995
170. Lupton JR, Steibach G, Chang WC, O'brien BC, Wiese S, Stoltzfus CL, Glober GA, Wargovich MJ, McPherson RS, Winn RJ: Calcium supplementation modifies the relative amounts of bile acids in bile and affects key aspects of human colon physiology. J Nutr 126(5): 1421–1428, 1996
171. Alberts DS, Ritenbaugh C, Story JA, Aickin M, Rees-McGee S, Buller MK, Atwood J, Phelps J, Ramanujam PS, Bellapravalu S, Patel J, Bextinger L, Clark L: Randomized, double-blinded, placebo-controlled study of effect of wheat bran fiber and calcium on fecal bile acids in patients with resected adenomatous colon polyps. J Natl Cancer Inst 88(2): 81–92, 1996
172. Baron JA, Beach M, Mandel JS, van Stolk RU, Haile RW, Sandler RS, Rothstein R, Summers RW, Snover DC, Beck GJ, Frankl H, Pearson L, Bond JH, Greenberg ER: Calcium supplements and colorectal adenomas. Ann NY Acad Sci 889: 138–145, 1999
173. Burkitt DP: Epidemiology of cancer of the colon and rectum. Cancer 28: 3–13, 1971
174. Reddy BS, Hedges AR, Laaksko K, et al.: Metabolic epidemiology of large bowel cancer: Fecal bulk and constituents of high-risk North American and low-risk Finnish population. Cancer 42: 2832–2838, 1978
175. Hill MJ: Dietary fibre and human cancer: Epidemiological data. Adv Exp Med Biol 427: 27–34, 1997
176. Lupton JR: Is fiber protective against colon cancer? Where the research is leading us. Nutrition 16(7–8): 558–561, 2000
177. Eastwood MA, Hamilton D: Studies on the absorption of bile salts to non-adsorbed components of diet. Biochim Biophys Acta 152(1): 165–173, 1968
178. Kritchevsky D: Epidemiology of fibre, resistant starch and colorectal cancer. Eur J Cancer Prev 4: 345–352, 1995
179. D'Argenio G, Cosenza V, Cave MD, et al.: Butyrate production from dietary fibre and protection against large

bowel cancer in a rat model. Gastroenterology 110: 1727–1734, 1996
180. Hill MJ: Cereals, cereal fibre and colorectal cancer risk: A review of the epidemiological literature. Eur J Cancer Prev 7(Suppl 2): S5–S10, 1998
181. Alberts DS, Martinez ME, Roe DJ, et al.: Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. N Engl J Med 342: 1156–1162, 2000
182. Schatzkin A, Lanza E, Corle D, et al.: Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. N Engl J Med 342: 1149–1155, 2000
183. Glynn SA, Albanes D, Pietinen P, Brown CC, Rautalahti M, Tangrea JA, Gunter EW, Barrett MJ, Virtamo J, Taylor PR: Colorectal cancer and folate status: A nested case-control study among male smokers. Cancer Epidemiol Biomarkers Prev 5: 487–494, 1996
184. White E, Shannon JS, Patterson RE: Relationship between vitamin and calcium supplement use and colon cancer. Cancer Epidemiol Biomarkers Prev 6(10): 769–774, 1997
185. Su LJ, Arab L: Eating patterns and risk of colon cancer. Am J Epidemiol 148(1): 4–16, 1998
186. Freudeheim JL, Graham S, Marshall JR, Haugey BP, Cholewinski S, Wilkinson G: Folate intake and carcinogenesis of the colon and rectum. Int J Epidemiol 20(2): 368–374, 1991
187. Lashner BA, Heidenreich PA, Su GL, Kane SV, Hanauer SB: Gastroenterology 97(2): 255–259, 1989
188. Lashner BA: Red blood cell folate is associated with the development of dysplasia and cancer in ulcerative colitis. J Cancer Res Clin Oncol 119(9): 549–554, 1993
189. Ma J, Stampfer NJ, Giovanucci E, et al.: Methylenetetrahydrofolate reductase polymorphism, detary interactions, and risk of colorectal cancer. Cancer Res 57: 1098–1102, 1997
190. Chen J, Giovanucci E, Hankinson SE, et al.: A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. Carcinogenesis 19: 2129–2132, 1998
191. Giovannucci E, Stampfer MJ, Colditz GA, et al.: Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Natl Cancer Inst 85: 875–883, 1993
192. Cravo M, Fidalgo P, Pereira A, et al.: DNA methylation as an intermediate biomarker in colorectal cancer: Modulation by folic acid supplementation. Eur J Cancer Prev 3: 473–479, 1994
193. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC: Alcohol, low-methionine – low folate diets, and risk of colon cancer in men. J Natl Cancer Inst 86(4): 265–273, 1995
194. Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE, Willett WC: Multivitamin use, folate and colon cancer in women in the Nurses' Health Study. Ann Intern Med 129(7): 517–524, 1998
195. Kim YI, Baik HW, Fawaz K, Knox T, Lee YM, Norton R, Libby E, Mason JB: Effects of folate supplementation on two provisional molecular markers of colon cancer: A prospective randomized trial. Am J Gastroenterol 96(1): 184–195, 2001
196. Reddy B, Rivenson A, El-Bayoumy K, Upadhyaya P, Pittman B, Rao C: Chemoprevention of colon cancer by organoselenium compounds and impact of high- and low-fat diets. J Natl Cancer Inst 89: 506–512, 1997
197. Reddy B, Xan C, Samaha H, Lubet R, Steele V, Kelloff G, Rao S: Chemoprevention of colon carcinogenesis by dietary perillyl alcohol. Cancer Res 57: 420–425, 1997
198. Tanaka T, Kawata K, Kakumoto M, Hara A, Murakami A, Kuki W, et al.: Citrus auraptene exerts dose-dependent chemopreventive activity in rat large bowel tumorigenesis: The inhibition correlates with suppression of cell proliferation and lipid peroxidation and with induction of Phase II drug metabolizing enzymes. Cancer Res 58: 2550–2556, 1998
199. Tanaka T, Kawabata K, Kakumoto M, Makita H, Matsunaga K, Mori H, et al.: Chemoprevention of azoxymethane-induced rat colon carcinogenesis by a xanthine oxidase inhibitor, 1′-acetoxychavicol acetate. Jpn J Cancer Res 88: 821–830, 1997
200. Tanaka T, Makita H, Satoh K, et al.: Chemoprevention of azoxymethane-induced rat colon carcinogenesis by the naturally-occurring flavonoids, diosmin and hesperidin. Carcinogenesis 18: 957–965, 1997
201. Rao CV, Rivenson A, Simi, B, Reddy BS: Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer Res 55: 259–266, 1995
202. Huang MT, Lou YR, Ma W, Newmark HL, Reuhl KR, Conney AH: Inhibitory effects of dietary curcumin on forestomach, duodenal and colon carcinogenesis in mice. Cancer Res 54: 5841–5847, 1994
203. Hanif R, Qiao L, Shiff S, Rigas B: Curcumin, a natural plant phenolic food additive inhibits cell proliferation and induces cell cycle changes in colon adenocarcinoma cell lines by a prostaglandin-independent pathway. J Lab Clin Med 130: 576–584, 1997
204. Cheng AL, Lin JK, Hsu MM, Shen TS, Ko JY, Lin JT, Wu MS, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, Lai MK, Pu YS, Pan MH, Wang UJ, Tsai CC, Hsieh CY: Phase I chemoprevention clinical trial of curcumin. Proc Am Soc Clin Oncol 17: 558a, 1998
205. Grodstein F, Newcomb PA, Stampler MJ: Postmenopausal hormone therapy and the risk of colorectal cancer: A review and meta-analysis. Am J Med 106(5): 574–582, 1999
206. Wysowski DK, Golden L, Burke L: Use of menopausal estrogens and medroxyprogesterone in the United States, 1982–1992. Obstet Gynecol 85: 6–10, 1995
207. Fernandez E, La Vecchia C, Braga C, et al.: Hormone replacement therapy and risk fo colon and rectal cancer. Cancer Epidemiol Biomarkers Prev 7: 329–333, 1998
208. Calle EE, Miracle-McMahill HL, Thun MJ, Heath CW Jr.: Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausal women. J Natl Cancer Inst 87: 517–523, 1995
209. Grodstein F, Platz EA, Giovannucci E, Colditz GA, Kantzky M, Fuchs C, Stampfer MJ: Postmenopausal hormone use and risk of colorectal cancer and adenoma. Ann Intern Med 128: 705–712, 1998
210. McMichael AJ, Potter JD: Reproduction, endogenous and exogenous sex hormones, and colon cancer: A review and hypothesis. J Natl Cancer Inst 65: 1201–1207, 1980

211. Ma J, Pollak MN, Giovannucci E, et al.: Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 91: 620–625, 1999
212. Slattery ML, Potter JD, Curtin K, Edwards S, Ma KN, Anderson K, Schaffer D, Samowitz WS: Estrogens reduce and withdrawal of estrogens increase risk of microsatellite instability-positive colon cancer. Cancer Res 61(1): 126–130, 2001
213. Arai N, Strom A, Rafter JJ, Gustafsson JA: Estrogen receptor beta mRNA in colon cancer cells: Growth effects of estrogen and genistein. Biochem Biophys Res Com 270(2): 425–431, 2000
214. Fiorelli G, Picariello L, Martineti V, Tonelli F, Brandi ML: Functional estrogen receptor beta in colon cancer cells. Biochem Biophys Res Com 261(2): 521–527, 1999
215. Alford TC, Do HM, Geelhoed GW, Tsangaris NT, Lippman ME: Steroid hormone receptors in human colon cancers. Cancer 43(3): 980–984, 1979
216. Lointier P, Wildrick DM, Boman BM: The effects of steroid hormones on a human colon cancer cell line *in vitro*. Anticancer Res 12(4): 1327–1330, 1992
217. Ziv Y, Gupta MK, Milsom JW, Vladisavljevic A, Kitago K, Fazio VW: The effect of tamoxifen on established human colorectal cancer cell lines *in vitro*. Anticancer Res 16(6B): 3767–3771, 1996
218. Picariello L, Fiorelli G, Brandi ML, Galli G, Malentacchi C, Montali E, Bigozzi U, Ficari F, Tonelli F: *In vitro* bioeffects of the antiestrogen LY117018 on desmoid tumor and colon cancer cells. Anticancer Res 17(3C): 2099–2104, 1997
219. Nakayama Y, Sakamoto H, Satoh K, Yamamoto T: Tamoxifen and gonadal steroids inhibit colon cancer growth in association with inhibition of thymidylate synthase, survivin and telomerase expression through estrogen receptor beta mediated system. Cancer Lett 161(1): 63–71, 2000
220. Ranelletti FO, Ricci R, Larocca LM, Maggiano N, Capelli A, Scambia G, Benedetti-Panici P, Mancuso S, Rumi C, Piantelli M: Growth-inhibitory effect of quercetin and presence of type-II estrogen-binding sites in human colon-cancer cell lines and primary colorectal tumors. Int J Cancer 50(3): 486–492, 1992
221. Ziv Y, Fazio VW, Kitago K, Gupta MK, Sawady J, Nishioka K: Effect of tamoxifen on 1,2-dimethylhydrazine-HCl-induced colon carcinogenesis in rats. Anticancer Res 17(2A): 803–810, 1997
222. Smirnoff P, Liel Y, Gnainsky J, Shany S, Schwartz B: The protective effect of estrogen against chemical induced murine colon carcinogenesis is associated with decreased CpG island methylation and increased mRNA and protein expression of the colonic vitamin D receptor. Oncol Res 11(6): 255–264, 1999
223. Gamet I, Cazenave Y, Trocheris V, et al.: Involvement of ornithine decarboxylase in the control of proliferation of the HT29 human colon cancer cell line. Int J Cancer 47: 633–638, 1991
224. Luk GD, Baylin SB: Ornithine decarboxylase as a biologic marker in familial colonic polyposis. N Engl J Med 311: 80–83, 1984
225. Thompson H, Ronan A: Effect of D,L-2-difluoromethylornithine and endocrine manipulation of the induction of mammary carcinogenesis by 1-methyl-1-nitrosurea. Carcinogenesis 7: 2003–2006, 1986
226. Boyle JO, Meyskens FL Jr., Garewal HS, Gerner EW: Polyamine contents in rectal and buccal mucosa in humans treated with oral difluoromethylornithine. Cancer Epidemiol Biomarkers Prev 1: 131–135, 1992
227. Love R, Carbone P, Verma A, et al.: Randomized phase I chemoprevention dose seeking study of α-difluoromethylornithine. J Natl Cancer Inst 85: 732–737, 1993
228. Love RR, Jacoby R, Newton MA, Tutsch KD, Simon K, Pomplun M, Verma AK: A randomized, placebo-controlled trial of low-dose alpha-difluoromethylornithine in individuals at risk for colorectal cancer. Cancer Epidemiol Bio Prev 7(11): 989–992, 1998
229. Meyskens FL, Emerson SS, Pelot D, Meshkinpour H, Shassetz LR, Einspaar J, et al.: Dose de-escalation chemoprevention trial of α-difluoromethylornithine in patients with colon polyps. J Natl Cancer Inst 85: 732–736, 1994
230. Meyskens FL, Gerner EW, Emerson S, Pelot D, Durbin T, Doyle K, Lagerberg W: Effects of α-difluoromethylornithine on rectal mucosal levels of polyamines in a randomized, double-blinded trial for colon cancer prevention. J Natl Cancer Inst 90(16): 1212–1218
231. O'Dwyer ME, Druker BJ: STI571: An inhibitor of the BCR-ABL tyrosine kinase for the treatment of chronic myelogenous leukaemia. Lancer Oncol 1: 207–211, 2000
232. Meyskens F: Principles of human chemoprevention. Hematol Oncol Clin North Am 12(5): 1–7, 1998
233. Coffey R, Hawkey C, Damstrup L, Graves-Deal R, Daniel V, Dempsey P, et al.: Epidermal growth factor receptor activation induces nuclear targeting of cyclooxygenase-2, basolateral release of prostaglandins, and mitogenetis in polarizing colon cancer cells. Proc Natl Acad Sci USA 94: 657–662, 1997
234. Sheng H, Shao J, Dixon DA, Williams CS, Prescott SM, DuBois RN, et al.: Transforming growth factor-beta1 enhances Ha-ras-induced expression of cyclooxygenase-2 in intestinal epithelial cells via stabilization of mRNA. J Biol Chem 275(9): 6628–6635, 2000
235. Sheng H, Shao J, Hooton EB, Tsujii M, DuBois RN, Beauchamp RD: Cyclooxygenase-2 induction and transforming growth factor beta growth inhibition in rat intestinal epithelial cells. Cell Growth Differ 8(4): 463–470, 1997
236. Torrance CJ, Jackson PE, Montgomery E, Kunzler KW, Vogelstein B, Wissner A, Nunes M, Frost P, Discafani CM: Combinatorial chemoprevention of intestinal neoplasia. Nat Med 6(9): 1024–1028, 2000
237. Gupta RS, DuBois RN: Combinations for cancer prevention. Nat Med 6(9): 974–975, 2000
238. Jacoby RF, Cole CE, Tetsch K, Newton MA, Kelloff G, Hawk ET, Lubet RA: Chemoprevention efficacy of combined piroxicam and difluoromethylornithine treatment of

Apc mutant Min mouse adenomas, and selective toxicity against Apc mutant embryos. Cancer Res 60(7): 1864–1870, 2000
239. Carbone PP, Douglas JA, Larson PO, Verma AK, Blair IA, Pomplun M, Tutsch KD: Phase I chemoprevention study of piroxicam and alpha-difluoromethylornithine. Cancer Epidemiol Biomarkers Prev 7(10): 907–912, 1998
240. Harris G, Gupta A, Nines R, Kresty L, Habib S, Frankel W, et al.: The effects of lyophilized black raspberries on azoxymethane-induced colon cancer and 8-hydroxy-2′-deoxyguanosine levels in the Fischer 344 rat. Nutr Cancer 40(2): 125–133, 2001
241. Li H, Kramer P, Lubet R, Steele V, Kelloff G, Pereira M: Effects of calcium on azoxymethane-induced aberrant crypt foci and cell proliferation in the colon of rats. Cancer Lett 124: 39–46, 1998
242. Deschner EE, Cohen BI, Raicht RF: The kinetics of the protective effect of beta-sitosterol against MNU-induced colonic neoplasia. J Cancer Res Clin Oncol 103(1): 49–54, 1982
243. Rao C, Tokumo K, Rigotty J, Zang E, Kelloff G, Reddy B: Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, a-difluoromethylornithine, 16-a-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. Cancer Res 51: 4528–4534, 1991

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