

ENVIRONMENTAL CARCINOGENESIS

Understanding the Interaction Between Environmental Exposures and Molecular Events in Colorectal Carcinogenesis

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INTRODUCTION

Colorectal carcinoma is one of the second most common malignancies in the western world (1). Although screening methods to detect this disease at an early stage continue to improve (2-4), advances in treatment have failed to raise survival rates of patients with advanced disease at diagnosis (5). Consequently, preventive measures may provide the principal means to profoundly reduce the number of deaths attributed to this disease.

Rapid advances in molecular biology techniques have enhanced our understanding of the genetic basis of the disease and the identification of the specific mutations involved in its natural history. A comprehensive understanding of the role that molecular events play in tumor

development and their interaction with environmental and dietary exposures may help to establish successful preventive strategies. Each molecular event that generates genetic alterations is a possible target of prevention of neoplastic progression. The ability to identify individuals susceptible to specific molecular events and genetic pathways involved in colorectal carcinogenesis because of specific exposures and/or genotypes will enhance our ability to institute appropriate chemopreventive measures (6).

The purpose of this paper is to examine the information, both environmental and genetic, that has been gathered from epidemiologic and experimental studies and explore how they may interact along the adenoma-carcinoma sequence. In addition, we will discuss how acquir-

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ing information on both genetics and environmental exposures could possibly lead to improvements in prevention/intervention of this disease.

EPIDEMIOLOGICAL FACTORS

There is compelling evidence of an environmental component to the development of cancer of the colon-rectum. Not only do incidence rates vary dramatically throughout the world, where 20-fold differences have been reported (1,7), but migrant studies as well as secular trends observed with greater Westernization (8) suggest that these international differences may be partially explained by differences in dietary and other environmental factors (9).

Epidemiological studies, ranging from cross-sectional to analytical designs, consistently show the importance of nutritional factors in colorectal cancer risk. High fat and meat intakes have been associated with greater incidence and mortality, whereas those with diets containing fiber and cereal exhibit an inverse association with risk (10–13). More specifically, red meat intake is consistently associated with an approximate two-fold increase in risk, independent of fat content (14–16). Similarly, vegetable and fruit intakes have been associated with lower risk that does not appear to be due to fiber alone (17). Some studies suggest that certain micronutrients, such as folate (18–20), may play an important role in this reduction.

Other environmental exposures, such as aspirin use

and cigarette smoking, have also been reported to be associated with colorectal cancer development. It is believed that aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) decrease colorectal cancer risk by approximately 50% (21–24). In contrast, cigarette smoking is consistently associated with an increase in colorectal polyp formation (25–30) and possibly with colorectal cancer risk (31–38).

THE ADENOMA-CARCINOMA GENETIC SEQUENCE

Cancer is a disease resulting from the clonal expansion of a genotoxic event in a single somatic cell that induces mutations in important genes. For a normal cell to become malignant, the current body of evidence suggests that it must acquire several characteristics, including activation of oncogenes and inactivation of tumor suppressor genes through mutation (39–42). Studies of successive histopathological stages that differentiate carcinogenic progression suggest that four or five mutations in proto-oncogenes and tumor-suppressor genes may be necessary to overcome growth control and establish a fully neoplastic lesion (43).

The development of colorectal cancer is no exception. Since Vogelstein and Fearon first suggested the adenoma-carcinoma sequence in colorectal carcinogenesis, evidence from a variety of biological fields suggests that it is a multistep process involving mutations of oncogenes and tumor suppressor genes (Fig. 1) (39). Early in the pro-

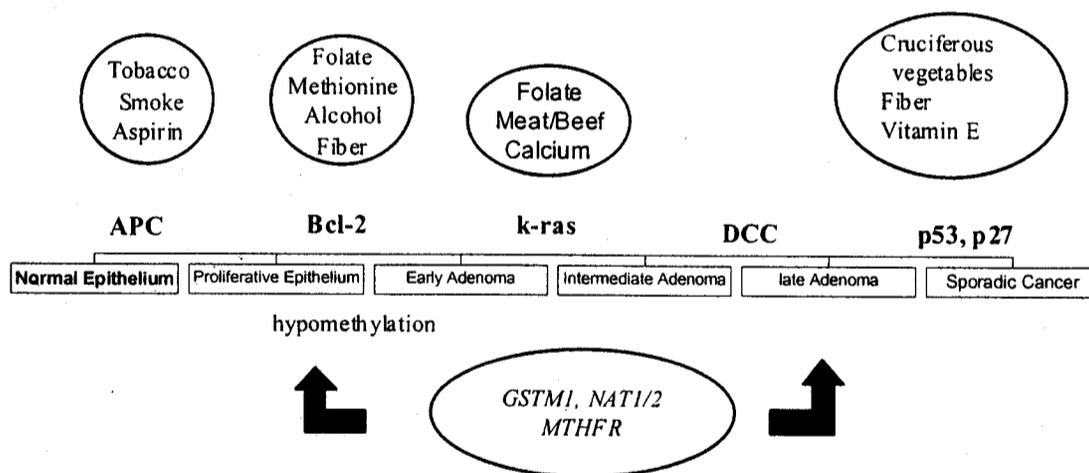


Figure 1. Model of possible links between environmental exposures, inherited susceptibility factors, and somatic alterations in colorectal carcinogenesis.

cess, mutation of the adenomatous polyposis coli gene (*apc* gene; located on chromosome 5q) (44) and alterations in methylation pattern (45) lead to abnormal epithelial proliferation and early adenoma formation. Next, mutations of the *K-ras* gene (located on the short arm of chromosome 12) induce an early adenoma to progress to an intermediate adenoma (46). Genetic instability may also play a role at this stage (47). For a progression of an intermediate adenoma to a late adenoma to occur, a deletion or mutation of the *dcc* (deleted in colorectal carcinoma) gene on chromosome 18q appears to be important (48). Deletion or mutation of the *p53* gene occurs at the transition of an adenoma to a carcinoma (40). These mutations occur in a somewhat preferential order, although there is evidence that this is a heterogeneous process (i.e., other cellular changes may be sufficient for this neoplasm to form) and may be dependent on the cell's intracellular and environmental factors (39,47). Therefore, a tumor's mutations and its genetic pathway (the order in which mutations occur) may be greatly influenced by its endogenous as well as exogenous exposures.

GENE-ENVIRONMENT INTERACTIONS

Early Events

Apoptosis: *apc* Gene and Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Many experimental, clinical, and epidemiological studies suggest that aspirin and other NSAIDs may inhibit the occurrence or growth of colorectal adenomas and carcinomas (21–24,49), although conflicting results have been reported (50). Although several mechanisms of aspirin action have been proposed at various stages of carcinogenesis, it appears that it may act as an *early* disrupter of the adenoma–carcinoma sequence (although sustained use may suggest that NSAIDs act throughout the sequence). A number of recent epidemiological studies have reported 50% reduction in colorectal adenoma development risk among regular users of aspirin (21–24,51), supporting reports in rodents that these drugs inhibit adenomas and early carcinomas of the colon (52).

Various mechanisms have been proposed for this observation, including inhibition of prostaglandin synthesis; perhaps one of the most intriguing is that aspirin and NSAIDs suppress cyclooxygenase-2 (COX-2), producing effects on epithelial proliferation and apoptosis (53,54). The NSAID sulindac has been shown to reduce the number and size of colorectal polyps in patients with

familial adenomatous polyps (FAP) in case series studies (55–57) and two clinical trials (58,59). Because adenomatous polyps in patients with FAP and upwards of 80% of all sporadic colon cancers (31,34,60) share alterations in the *apc* gene, aspirin and specifically COX-2 inhibitors may play a role in promotion of apoptosis through interaction with this gene (61).

Because of the compelling evidence of a chemopreventive effect of NSAIDs, future studies to further elucidate its mechanism are clearly warranted. Experimental studies should further define the pathway(s) of tumor inhibition in animals, and by extrapolation, to humans. More importantly, the appropriate dose, duration, and specific type of aspirin treatment for optimal chemoprotective effects need to be defined. Drugs are currently under development at several pharmaceutical companies with the goal of creating the appropriate chemoprevention strategy.

Bcl-2 and Fiber Intake

Another protein that may be involved in apoptosis early in the disease process is bcl-2 (62,63). Overexpression of the protein is associated with an inhibition of cell death and this overexpression has been observed to occur in most adenomas and in less than one-third of carcinomas, suggesting its importance as an early event in the adenoma–carcinoma sequence (64,65). Sodium butyrate, a fermentation product of dietary fiber in the large intestine, has been shown to induce apoptosis in human colorectal carcinoma (66,67). Mandal et al. (68) showed that overexpression of bcl-2 in colorectal carcinoma DiFi cells resulted in suppression of butyrate-induced apoptosis and enhanced survival in response to butyrate. Therefore, genes associated with apoptosis, such as *bcl-2*, and the factors that affect its regulation may play a critical role in colorectal carcinogenesis.

Intermediate Events

K-ras Oncogene

Among the oncogenes involved in colorectal tumorigenesis, the *K-ras* gene seems to have a very important role, with a reported prevalence of gene mutations in 35 to 65% of colorectal tumors (69–72). The mechanisms by which *K-ras* and its product affects cell growth and differentiation are not well understood, but this gene codes for a group of guanosine triphosphate (GTP) binding proteins associated with the inner surface of the cell plasma membrane, and may be involved in the signal

transduction pathway (73). Mutations in *K-ras* and the resulting mutant proteins result in altered transmission of these signals from the plasma membrane that ultimately affects the growth and differentiation of the cell.

K-ras seems to be a major target of the mutagenic action of environmental carcinogens (69,73). Several animal models have consistently shown that carcinogenic agents reproducibly induce the same type of tumor carrying the same type of DNA lesion (74,75). Alkylating agents induce the same base substitution, a G to A transition and G to C mutations at the 12th codon in *K-ras* in different animal tumors (69,73,76). The same type of point mutation is predominately observed among *K-ras* mutations detected in human colorectal tumors in the United States (69,76).

A recent study suggests that the endogenous production of alkylating agents, such as N-nitroso compounds (NOC), in fecal specimens increases with increasing red meat consumption (77). G to A transitions of *K-ras*, which are common in colorectal cancer, are characteristic of the effects of such agents (78,79). Whether NOC from meat protein is the primary contributor, or whether it is some other meat constituent needs to be delineated.

Alternative dietary sources of carcinogenic agents that may induce mutations in *K-ras* include fat and calcium intake. Wienstein et al. (80,81) proposed that fat's effect on colorectal carcinogenesis may be due to diacylglycerol (DAG), because intracellular DAG is involved in the cascade that affects *K-ras* activation and ultimately, cell turnover. They hypothesize that high fat consumption in the colonic microflora produce excess intraluminal DAG that will mimic and amplify cell replication signals.

In contrast to dietary fat, one recent study reports that calcium supplementation in humans markedly reduces the production of fecal DAG and accelerates bacterial metabolism of DAG and its precursors (82), possibly supporting reports from several epidemiological studies suggesting that calcium may provide some protection for colorectal cancer risk (83). Moreover, in an experimental animal model using dimethylhydrazine (DMH)-induced colonic tumors, calcium supplementation decreased the number of G to A transitions in the *K-ras* gene in tumors, leading to the speculation that alterations in *K-ras* mutations may be a possible mechanism by which calcium influences colon carcinogenesis (84).

Bautista et al. (85) studied the *K-ras* gene in tumors from 108 colorectal cancer patients using archival tissue and epidemiological data from a previous case-control study conducted on the island of Majorca. The authors report that high consumption of monounsaturated fats,

mostly derived from olive oil, was associated with a statistically significant decrease in the risk of cancer with wild-type *K-ras* genotype, but not of *K-ras* mutated cancers. Consistent with some experimental data, they also found that high calcium intake was associated with a decreased risk of *K-ras* mutated tumors. No associations were observed between *K-ras* mutations and total or saturated fat in their study. Another epidemiologic investigation (86) examined the association between dietary factors and *K-ras* mutations in colorectal adenomas from 678 participants enrolled in a clinical trial evaluating the effects of a wheat fiber supplement on adenoma recurrence. Although no associations between *K-ras* mutations and dietary calcium, total fat, or saturated fat were found, a higher risk of *K-ras* mutations was significantly associated with a lower intake of total folate. These findings suggest that the protective effect of folate in colorectal cancer may be mediated through its effect on *K-ras* mutations. Future studies will need to have the ability to examine the specific *K-ras* mutational spectrum with appropriately developed questionnaires asking about specific hormonal and food exposures.

Late Events

DCC Tumor Suppressor Gene

The *DCC* gene, located on chromosome 18q, is believed to be a tumor suppressor gene (42,87), although a recent report in mouse models seems to suggest otherwise (88). It is a candidate region for this deletion event on the basis of (i) its location within the minimally lost region, (ii) some evidence of mutations within the remaining *DCC* allele, and (iii) frequent loss of *DCC* expression that has been observed.

The recent development of antibodies to detect the *DCC* protein product as well as the determination of mRNA expression via the use of reverse transcriptase polymerase chain reaction (PCR) in archival paraffin-embedded tumor specimens has allowed investigators to more easily determine the role this gene plays in colorectal carcinogenesis. A number of studies have found that a loss of expression appears to be a hallmark found in colorectal tumors, but not found in normal colonic tissue (89,90). Furthermore, a study by Shibata et al. (91) also found that loss of *DCC* expression was related to poorer survival prognosis. Although its actual involvement in tumorigenesis remains unknown, the evidence to date suggests that it may be important in tumor growth, invasion, and metastasis and studies should aim at identi-

fying any potential environmental influences on genetic alterations and changes in protein expression.

p53 Tumor Suppressor Gene

Recent molecular investigations have indicated that tumor suppressor genes such as the *p53* gene, in particular, are ideal candidates for studies of gene-environment interaction (92). Molecular biologists report that the specific position and nucleotide substitution of *p53* missense mutations divulge meaningful differences among tumors in diverse tissues (93). Jones et al. (94) suggested that there are two general patterns of *p53* mutations: mutations induced by exogenous carcinogens, and those occurring spontaneously due to endogenous mutagens. Carcinogen-induced mutations are caused by direct interaction of carcinogens with DNA, leading to specific point mutations, predominately transversions. For example, a study of *p53* mutations in small-cell lung carcinomas found that most mutations were transversions at G and C residues (95). This suggests that lung cancer results from the direct interaction of carcinogens in cigarette smoke with DNA.

Spontaneous mutations, on the other hand, arise at CpG dinucleotides, which are thought to result from the frequent methylation of the cytosine residue in CpG sites with a high frequency of transitions. These spontaneous cellular endogenous mutations are assumed to result from an endogenous process of the immediate colonic flora, because no exogenous factors have been identified, although one study suggests that specific carcinogens targeting methylated CpG sites may also be involved (96). In colorectal cancer, most *p53* mutations are spontaneous with transitions occurring at CpG sites. The variability exhibited by the *p53* mutational spectra among different carcinogen-related tumor types implies a causal contribution of exogenous and endogenous elements in neoplastic transformation. Therefore, the analysis of the *p53* mutations and their causes, due to various carcinogens, may provide insights into the specific factors contributing to different human malignancies.

In a molecular epidemiological investigation involving colorectal cancer, Ishioka et al. (97) showed that different rates of transversions (G to T) and transitions (C to T at CpG) in the *p53* gene exist between U.S. and Japanese colorectal patients. In Japan, the frequency of transitions and transversions were virtually the same, whereas U.S. patient tumors were predominately transitions. These differences among *p53* mutational patterns suggest a difference between etiological factors underlying colorectal tumor development in Japan and the United States.

Two recent experimental studies investigated *p53* mutation interactions with dietary factors. Makino et al. (98) exposed rats to heterocyclic amines (HCAs), inducing colorectal tumors. Results indicated that *p53* gene mutations were not involved in colorectal neoplasms induced by HCAs. Hague et al. (66) investigated the effect of the dietary factor sodium butyrate, a fermentation product of dietary fiber, in colorectal tumorigenesis. Sodium butyrate induced apoptosis (programmed cell death) in two colorectal adenoma cell lines and a carcinoma cell line in a *p53*-independent pathway. This observation suggested that dietary fiber acts in a protective manner against colorectal tumor development by inducing cell death in cells with DNA damage that have the capability to proliferate to a colorectal neoplasm. Another study investigating *p53* alterations in oral carcinogenesis using a hamster buccal pouch model observed that vitamin E inhibited *p53* mutant protein overexpression (99). In addition, the occurrence of *p53* gene deletion and overexpression is more commonly seen in left-sided carcinomas of the large bowel, the site that appears to be most influenced by environmental factors (100).

These findings prompted our laboratory to determine the association of dietary factors with *p53* mutant protein overexpression in an epidemiological case-control study of 163 nonfamilial colorectal cancer patients and 326 controls (101,102). We observed an increased risk for the development of *p53*-negative tumors in patients eating a diet high in meat and beef. Because *p53* mutation and overexpression is a relatively late occurrence in the progression to tumorigenesis, beef/meat may initiate or promote mutations in oncogenes (e.g., *ras*) much earlier in the continuum to colorectal cancer in tumors that do not require *p53* overexpression. Our analysis also suggested a protection of *p53* alterations in patients with a high intake of cruciferous vegetables, fiber, and vitamin E (103). A case-control study of 106 colorectal patients by Obrador et al. (104) also detected a protective effect for *p53* alterations with cruciferous vegetables. Because *p53* mutations in colorectal tumors are endogenous in nature and occur primarily as transitions (G:C to A:T) at CpG nucleotides due to deamination of 5-methylcytosine residues (94), oxygen radicals may promote the rate of deamination of deoxynucleotides (105). Therefore, these data suggest that antioxidant effects of cruciferous vegetables may alter deamination rates and reduce the risk of *p53* mutation/overexpression.

Another case-control study of 185 colon cancer cases and 259 controls evaluated the association between dietary factors and *p53* abnormalities, using *p53* protein overexpression and *p53* mutational analysis (106). Al-

though no differences in risk were observed when cases were compared by p53 alterations for specific meat food groups, an increase in risk was observed for saturated fat (odds ratio [OR] per 16.1 g/day, 1.46; 95% confidence interval [CI], 1.08–1.97) and total fat intake (OR per 37.3 g/day, 1.94; 95% CI 1.32–2.85) for cases without p53 overexpression. However, no differences in risk for either p53 pathway were observed for cruciferous vegetable intake.

p27^{Kip1} Expression

Another protein that is involved in cell-cycle regulation and may have an important role in colorectal cancer development is the cyclin-dependent kinase inhibitor (CDKI) *p27^{Kip1}*. Although mutations in the *p27^{Kip1}* gene are rarely observed (107,108) and its protein expression is mainly regulated at post-transcriptional levels through its translation and degradation (109,110), various studies have amassed evidence for its importance in controlling cell growth. In human cell lines, an inverse correlation between the expression of *p27^{Kip1}* and the degree of tumor malignancy has been observed in human colorectal tumors (111), and a similar inverse relationship has been observed in molecular epidemiologic studies (112,113). Not only has loss of *p27^{Kip1}* expression been demonstrated to play a role in the prognosis of colorectal cancer, Thomas et al. (114) reported that a downregulation of *p27^{Kip1}* may confer metastatic potential to these tumors, suggesting that this loss of expression is a late event. Our recent study assessing the association of *p27^{Kip1}* expression and cigarette smoking supports this finding; we found no association between a loss of *p27^{Kip1}* expression and cigarette smoking exposure, suggesting that this loss may be occurring late in the adenoma–carcinoma sequence (115).

The relationships between environmental exposures and genetic alterations are unknown. These examples illustrate the further need to study oncogenes, tumor suppressor genes, and apoptotic pathways. These studies may aid to elucidate our understanding of the relationship between environmental factors and the genome, and ultimately contribute to improved prevention and early detection of colorectal carcinoma.

Other Susceptibility Genes

Mismatch Repair Genes

One class of modifier genes that plays a role in colorectal carcinogenesis is DNA repair genes. Their primary duty is to maintain the integrity of the genome by remov-

ing lesions, such as O⁶-methylguanine (O⁶-meG), created by chemicals and other environmental agents. Left unrepaired, these lesions can contribute to the development of multiple and sequential genetic alterations that are relevant to the carcinogenic process.

An example of how mutations in DNA mismatch repair (*MMR*) can confer risk is the autosomal dominantly inherited syndrome, hereditary nonpolyposis colorectal cancer (HNPCC) (116). Germline mutations in five *MMR* genes (*hMSH2*, *hMLH1*, *hPMS1*, *hPMS2*, and *hMSH6*) have been found in HNPCC patients (117). Similarly, a case-control study reported an increased risk in sporadic colorectal cancer with reduced expressions in *hPMS1* and *hPMS2* (118).

Additional evidence supports the importance of DNA *MMR* genes in the development of sporadic colorectal tumors. Epigenetic inactivation of *hMLH1* in through DNA hypermethylation is associated with microsatellite instability (MSI) observed in sporadic colorectal cancer (119). Note that a marked reduction in MSI in colorectal cancer cells deficient for *hMLH1*, *hMSH2*, and *hMSH6* was observed with exposure to NSAIDs (120), suggesting that the expression of these genes is modifiable by exogenous agents.

Another DNA repair gene of interest is the O⁶-methylguanine DNA-alkyltransferase (*MGMT*) gene. It has long been noted that alkylation of DNA at the O⁶-position of guanine is a critical event that can lead to induction of mutations. To counteract such effects, *MGMT* transfers methyl groups from such methylated moieties of the DNA to its own molecule, repairing DNA lesions in a one-step process. Its activities provide protection against alkylating agents, such as N-nitroso compounds found in meat products and in chemotherapeutics.

DNA repair deficiency leading to genetic instability may be caused by exposure to environmental toxicants and is supported by several studies (121–123). Hospital personnel exposed to antineoplastic drugs were deficient in *MGMT* activities (121). These observations suggest that environmental exposures that damage DNA and affect DNA repair capacity may have enormous implications for chemoprevention as well as in chemotherapeutic strategies for those individuals with a susceptibility to DNA repair defects.

Metabolizing Enzymes

Although susceptibility to cancer due to germline and somatic alterations of oncogenes and tumor suppressor genes is directly involved with tumorigenesis, another group of cancer susceptibility genes may influence the

rate at which these alterations develop. These genes are involved in the regulation of activation and detoxification of both endogenous and exogenous exposures, and may affect the normal processes of oncogenes and tumor-suppressor genes. Because various dietary carcinogens, as well as cigarette smoking, appear to be associated with colorectal cancer risk, studies examining the gene-environment interactions between these exposures and metabolic polymorphisms may be important. Three promising areas of investigation include: the 5,10-methylenetetrahydrofolate (*MTHFR*) gene, the N-acetyltransferase 1 and 2 (*NAT1/2*) genes, and *GSTM1* gene.

Methylation: Folate and MTHFR

Alteration in DNA methylation is one of the earliest events in the pathway to colorectal neoplasia, occurring in small adenomas and throughout progressive stages of neoplastic development (124). These alterations include a reduction in the overall genomic methylation as well as possible regional increases in methylation that lead to variations in gene expression and/or increases in mutation rates via spontaneous deamination. Activation of specific oncogenes via hypomethylation is believed to be one way in which changes in methylation pattern can alter cancer risk. Another is hypermethylation of cytosine-phospho-guanine (CpG)-rich areas of the genome, leading to inactivation of tumor-suppressor gene activity (125).

The apparent protective effect of fruits and vegetables in colorectal cancer prompted several epidemiological investigations to examine whether a methyl-deficient diet may be a risk factor for human colorectal neoplasia, because fruits and vegetables are often high in folate. Giovanucci et al. (18,20,126) observed that individuals with a diet consisting of high alcohol, low methionine, and low folate intake were at increased risk for both adenomas and colorectal cancer. Not only have several other epidemiological studies reported similar associations (20,126-128), but increase in spontaneous genetic damage has also been reported to be associated with exceptionally low values of plasma folate (129,130).

Further evidence that a methyl-deficient diet may be a risk factor in colorectal carcinogenesis comes from animal data. Administration of a methyl-deficient diet in rodents both induces and promotes the development of a variety of tumors including colorectal cancer (131). It is hypothesized that methyl deficiencies restrict the synthesis of S-adenosylmethionine (SAM), a necessary factor for DNA methylation, leading to potentially oncogenic changes (132).

An elegant example of how genes and environmental exposures interact to alter cancer risk has been reported with the *MTHFR* gene. *MTHFR* codes for an enzyme critical in regulating the metabolism of folate to a form that is necessary for methionine synthesis (133). *MTHFR* irreversibly converts 5,10-methylenetetrahydrofolate, the methyl donor in deoxythymidine monophosphate (dTMP) synthesis, from deoxyuridine monophosphate (dUMP) to 5-methyltetrahydrofolate, the primary methyl donor for the remethylation of homocysteine to methionine. Thus, *MTHFR* plays an important role in both methylation and DNA synthesis.

A common polymorphism (677C → T) in the *MTHFR* gene, identified in 20-40% of Caucasians, reduces the activity and thermolability of the enzyme (134). Homozygotes of this polymorphism have elevated plasma homocysteine levels and a reduced level of plasma folate (135). In addition, associations with an increased risk of neural tube defects (136) and a decreased risk of colorectal cancer (19,137) have been observed with this common polymorphism.

Two published reports from nested case-control studies conducted in separate prospective cohorts of male health professionals (Physician's Health Study and Health Professional Follow-Up Study) have examined the relationship between the 677C → T *MTHFR* polymorphism and risk of colorectal cancer, particularly in conjunction with dietary intake (19,137). The data suggest that dietary methyl supply is critical for those individuals who are homozygous for the variant *MTHFR* allele (valine/valine) (i.e., they obtain the most protection from high intake of folate, vitamin B₆, and vitamin B₁₂). It appears that when the dietary methyl supply is high, these individuals may be at a reduced risk for colorectal cancer because the higher levels of 5,10-methylenetetrahydrofolate may prevent imbalances of nucleotide pools during DNA synthesis. In contrast, when the methyl supply is low, this protective effect is negated in this group. It has been postulated that these individuals may be less able to compensate due to the depletion of 5-methyltetrahydrofolate (hence, the production of methionine), allowing for alterations in DNA methylation to occur. Although the associations observed were weaker, similar findings between this genotype, diet, and colon cancer have been reported in a large case-control study by Slattery et al. (138).

However, results from a study by Chen et al. (139) found no association between *MTHFR* and adenoma risk, suggesting that *MTHFR* does not play a role in the development of colorectal neoplasia until much later. Further evidence supporting this observation is the significant in-

crease in the number cases with lymph node involvement (i.e., metastasis) among patients who are homozygous variant for this *MTHFR* allele (140).

Because anywhere from 8 to 15% of the Caucasian population are homozygous variant for this *MTHFR* polymorphism, the public health impact may not be trivial. Preliminary data suggest that there may be a survival difference by genotype (141), where the clinical effectiveness of leucovorin therapy may be reduced in individuals who are homozygous for the variant allele. More importantly, because the influence of this polymorphism and its resulting genotypes appear to be modifiable through behavioral changes in dietary habits, it may be one area that could be targeted in preventing this disease.

NAT and Heterocyclic Amines

The relatively consistent finding that higher meat consumption increases colorectal cancer risk has prompted studies to identify agents that may be genotoxic. Heterocyclic amines and polycyclic aromatic hydrocarbons (PAHs) have been identified as pyrolysis products formed during various cooking and barbecuing processes in beef and other food (142,143), and they have been found to induce colorectal tumors in rats and mice (144–147). Similarly, frequent consumption of well-done meats by humans has been positively associated with colon cancer in some studies (148–150), but not in others (151,152).

An enzyme system that is involved in the metabolism of aromatic amines, such as 4-aminobiphenyl and HCAs, is the N-acetyltransferase family. In humans, two independently regulated genes, *NAT1* and *NAT2* (153), encode acetylation activity and both gene loci are known to be phenotypically and genotypically polymorphic. Acetylator phenotype may, depending on the interaction, predispose or protect individuals to certain cancers associated with arylamine exposures.

A number of recent case-control studies have tried to investigate this gene-diet interaction among acetylator status (as defined by *NAT2* genotype), meat intake, and colorectal cancer risk. Initial studies based on phenotypic assays reported an increased risk ranging from 1.8 to 2.5 (154–156) among individuals with the rapid acetylator phenotype. This has been supported also by some animal models (157–159), but not by others (160). The mechanism by which this may occur is through the increase in catalysis of O-acetylation of the N-hydroxy metabolites of aromatic amines, resulting in the formation of N-acetoxyarylamines that can form DNA adducts (158).

More recent studies utilizing PCR-based genotypic

assays, in general, have not supported this association (161–168), although the conclusions have been inconsistent (169,170). In addition, patients with FAP have been reported to show significant reduced activity of *NAT2* (171). One reason for this discrepancy may be that the gene-environment interaction observed phenotypically could be due to *NAT1*, which shares some substrate specificity with *NAT2* (161,172), and is expressed at higher levels in the colonic epithelial cells than *NAT2* (173).

It has been reported that individuals with the variant *NAT1*10* allele have a 2-fold increase in colorectal cancer risk (172). Such an association has not been substantiated in a recent study of colorectal adenomas (167) or in colorectal cancer (161,168). However, an acetylation role in colorectal carcinogenesis cannot be excluded, because a 5- to 6-fold increase in colorectal cancer risk was observed among men who were rapid acetylators for both *NAT1* and *NAT2* in the presence of higher meat consumption in the latter study.

Numerous reasons may explain some of these discrepant findings. One major limitation that has plagued the epidemiological studies is the small samples from which conclusions have been drawn. In addition, inadequate or nonexistent exposure information may have hampered researchers' ability to detect true associations. Accurate exposure information on cooking methods and HCA measures are needed to gain a better understanding of the interaction, if it exists. Another source of error that may have contributed to the inconsistencies observed is the misclassification of assay results. There is the possibility that other unidentified or under-represented alleles that contribute to acetylation rate (i.e., may have functional differences) need to be accounted for before an association can be observed. Clearly, additional studies will need to be conducted to resolve these questions.

GSTM1 and Cigarette Smoking Exposure

GSTM1 belongs to the *mu* subclass of a large family of phase II enzymes involved in detoxification and conjugation of electrophiles in chemical carcinogenesis (174,175). This enzyme is involved in metabolizing PAHs found in tobacco smoke, and individuals lacking *GSTM1* activity are believed to be at increased risk of lung and bladder cancers (176–178). Because of recent prospective studies reporting tobacco smoking as an important risk factor for development of colorectal adenoma and carcinoma (22,23), it has been hypothesized that this gene may be an important modifier in the adenoma-carcinoma pathway.

To date, a number of case-control studies have exam-

ined the potential importance of *GSTM1* in colorectal adenomas and carcinomas development. An initial report by Zhong et al. (179) estimated that there was a 1.7-fold increase in risk in proximal colon cancer among individuals with the null *GSTM1* genotype. Since then, the reported results have been conflicting. A number of studies reported observing no association between the null genotype and colorectal cancer risk (170,180–183); however, a few subsequent studies have observed a weak positive association (168,184), including a statistically significant increase with distal colon tumors among Japanese patients (185).

Even when environmental exposures such as cigarette smoking (168,182,185) and meat consumption (170) were taken into account, these conclusions did not change. One reason for the lack of observed association might be that the interaction between *GSTM1* and smoking is confounded by dietary factors, such as cruciferous vegetable intake (186,187). In addition, the power for subgroup analyses in these studies may be limited by sample size.

With up to 50% of the population lacking *GSTM1* activity (188), on a theoretical level, the identification of susceptible individuals may be possible. Future studies examining the interaction of *GSTM1*, smoking, and intake of cruciferous vegetables may help clarify this gene's potential role in colorectal carcinogenesis.

CONCLUSION

An understanding of the role that molecular events play in tumor development and their interaction with environmental and dietary exposures may help to establish preventive strategies because each molecular event is a possible target for prevention of neoplastic progression. As this review has illustrated, a number of areas have been identified as potentially modifiable factors. The current body of data suggests that folate intake may be a prime candidate for such a colorectal cancer prevention strategy. Future studies should be as large and generalizable as possible (e.g., include minority populations) and conducted over a long period of time to be truly informative. These studies should further evaluate the association between folate intake and colorectal cancer, by site and stage of disease, and should also better delineate the dose–response relationship. Should the evidence support such a prevention strategy, folate intake may need to be increased either through vitamin supplementation or through greater consumption of fortified foods, particularly among those who are homozygous variant for the

MTHFR polymorphism. Because of the relative higher prevalence of this genetic variation, the attributable risk may be substantial. It will be of interest to see whether changes in colorectal cancer incidence are observed in the distant future, particularly with folic acid fortification of baked goods that took effect on January 1, 1998.

Other exposures, such as aspirin, may be more difficult to institute as a public health initiative, due to the potential side effects. However, future studies are still warranted and should be conducted to determine how aspirin use or specific COX-2 inhibitors may be implemented as a preventive strategy. As the appropriate dose and duration of aspirin treatment for optimal chemoprotective effects are defined through future studies, the goal of creating the appropriate chemoprevention strategy may be realized.

It is important to realize that the studies examined here were often the first to examine specific hypotheses, and these associations must be examined using causal criteria (i.e., biological plausibility) rather than based on statistical significance alone. Before a hypothesis is supported, numerous studies must consistently show similar results. Both the strength of the association and a dose–response relationship aid in determining the meaning of the supposition. Unfortunately, with the myriad mechanisms involved in tumorigenesis, any result can be incorporated to explain almost any hypotheses.

Clearly, further research is greatly needed to clarify the complex mechanisms involved in colorectal carcinogenesis. Future studies should be designed to answer some of the following questions: (i) How the multitude of genes and environmental agents interact along the adenoma–carcinoma pathway; (ii) which alterations are unique to particular exposures, if any; and (iii) perhaps, most importantly, how can the difficulties in examining the temporal relationship within this pathway be addressed? All of these questions must be answered with consistent findings before any specific hypothesis can be embraced.

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