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REVIEWS: CURRENT TOPICS

Mouse models for unraveling the importance of diet in colon cancer prevention

Alexandra E. Tammariello[a], John A. Milner[b,*]

[a]Department of Human Nutrition, Foods and Exercise, Virginia Tech, Blacksburg, VA, USA
[b]Nutritional Science Research Group, Division of Cancer Prevention, National, Cancer Institute, National Institutes of Health, Health and Human Services, Rockville, MD 20892, USA

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Abstract

Diet and genetics are both considered important risk determinants for colorectal cancer, a leading cause of death worldwide. Several genetically engineered mouse models have been created, including the Apc^{Min} mouse, to aid in the identification of key cancer related processes and to assist with the characterization of environmental factors, including the diet, which influence risk. Current research using these models provides evidence that several bioactive food components can inhibit genetically predisposed colorectal cancer, while others increase risk. Specifically, calorie restriction or increased exposure to n-3 fatty acids, sulforaphane, chafuroside, curcumin and dibenzoylmethane were reported protective. Total fat, calories and all-trans retinoic acid are associated with an increased risk. Unraveling the importance of specific dietary components in these models is complicated by the basal diet used, the quantity of test components provided and interactions among food components. Newer models are increasingly available to evaluate fundamental cellular processes, including DNA mismatch repair, immune function and inflammation as markers for colon cancer risk. Unfortunately, these models have been used infrequently to examine the influence of specific dietary components. The enhanced use of these models can shed mechanistic insights about the involvement of specific bioactive food and components and energy as determinants of colon cancer risk. However, the use of available mouse models to exactly represent processes important to human gastrointestinal cancers will remain a continued scientific challenge.
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1. Introduction

The worldwide risk of colorectal cancer points to the societal need for effective prevention strategies. Today, colon cancer ranks fourth as the most commonly diagnosed cancer and is the second leading cause of cancer deaths in the United States [1]. According to rates of colon cancer from 2003 to 2005, the lifetime risk of developing colon cancer for men and women is 5.29% [2]. Moreover, colon cancer does not appear to be a consequence of aging, but is intrinsically linked with eating behavior. In fact, up to 90% of these cancer cases appear to relate to lifestyle, with the highest incidence occurring in economically developed countries [3,4].

While substantial evidence exists that diet and exercise are important determinants of colorectal cancer risk, considerable variability is acknowledged [4]. Fiber [5], garlic [6], milk [7] and calcium [8] are some of the dietary components with some of the most compelling information linking eating habits with a probable decrease in the risk of colon cancer. Red meat, including beef, pork, lamb and goat [9], processed meat (smoked, cured, salted, or preserved) [10] and alcohol [11] have been linked with an increase in risk of colon cancer, although the relative importance in determining risk remains largely unresolved [4].

2. Genetics and colon cancer

Although diet is assumed a contributor to colon cancer risk, genetics is also a key determinant. Hereditary predispositions

* Corresponding author. Tel.: +1 301 496 0118; fax: +1 301 480 3925.
E-mail address: milnerj@mail.nih.gov (J.A. Milner).

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can arise from mutations in genes involved in the DNA mismatch repair pathway or in the adenomatous polyposis coli (APC) gene located on chromosome 5, leading to hereditary nonpolyposis colorectal cancer or familial adenomatous polyposis (FAP), respectively [12]. These mutated genes are found in two key pathways; the APC/Wnt pathway and the Tgfβ pathway. The APC gene is a tumor-suppressor gene, and mutations are thought to occur at an early stage in carcinogenesis through the Apc/Wnt pathway. Mismatch repair is known to originate from mutations of MSH2, MLH1, PMS1 or PMS2 genes [13].

It is likely that a single mutation is insufficient to account for most cancer, including colorectal. Several years ago, a cascade of events was proposed to account for the evolution of a neoplasm [14] (Fig. 1). The loss of a single copy of the *APCTS* gene on 5q21 transforms normal colonic epithelial tissue and leads to multiple polyps. A loss of a second copy of the APC allele appears to increase the risk so that each in10^6 epithelial cells develops into a polyp. The APC protein is involved with β-catenin degradation. β-Catenin, in conjunction with the DNA-binding protein TCF-4, is known to function as a transcriptional activator of an array of genes including MMP-7, c-*myc*, cyclin D, CD44, gastrin, COX-2 and PPARδ. Evidence suggests DNA hypomethylation is fundamental to the onset and development of an early adenoma. The K-*ras* oncogene is thought to be involved in the progression from early to intermediate adenomas, since mutations occur in 50% of intermediate and late adenomas, but only about 10% in early adenomas. The K-*ras* oncogene encodes a 21-kDa membrane-bound protein involved in signal transduction, which is part of the GTP family of proteins. Approximately 50% of late adenomas and carcinomas have a mutation of the Thymidylate synthase (TS) gene on 18q, suggesting this gene is important for the evolution of an intermediate adenoma to late adenoma. The TS gene promoter is polymorphic with either two (TSER∗2) or three (TSER∗3) tandem repeats of 28 base pairs which can influence the expression of mRNA for TS. Late adenomas and colorectal cancers also have high mutation rates at the TP53 gene, providing insights into the transformation from late adenoma to carcinoma [14]. This review highlights briefly the evidence that exists about the role of food components as modifiers of one or more of these steps in the cancer process.

3. Mouse models for colorectal cancers

Diverse murine models have been developed to take into account alternatively expressed genes and the pathways to gastrointestinal carcinogenesis, to provide an understanding of the human condition (Fig. 2). Historically, murine models have incorporated carcinogen treatment to initiate colorectal cancer. Azoxymethane (AOM) is a classic carcinogen that

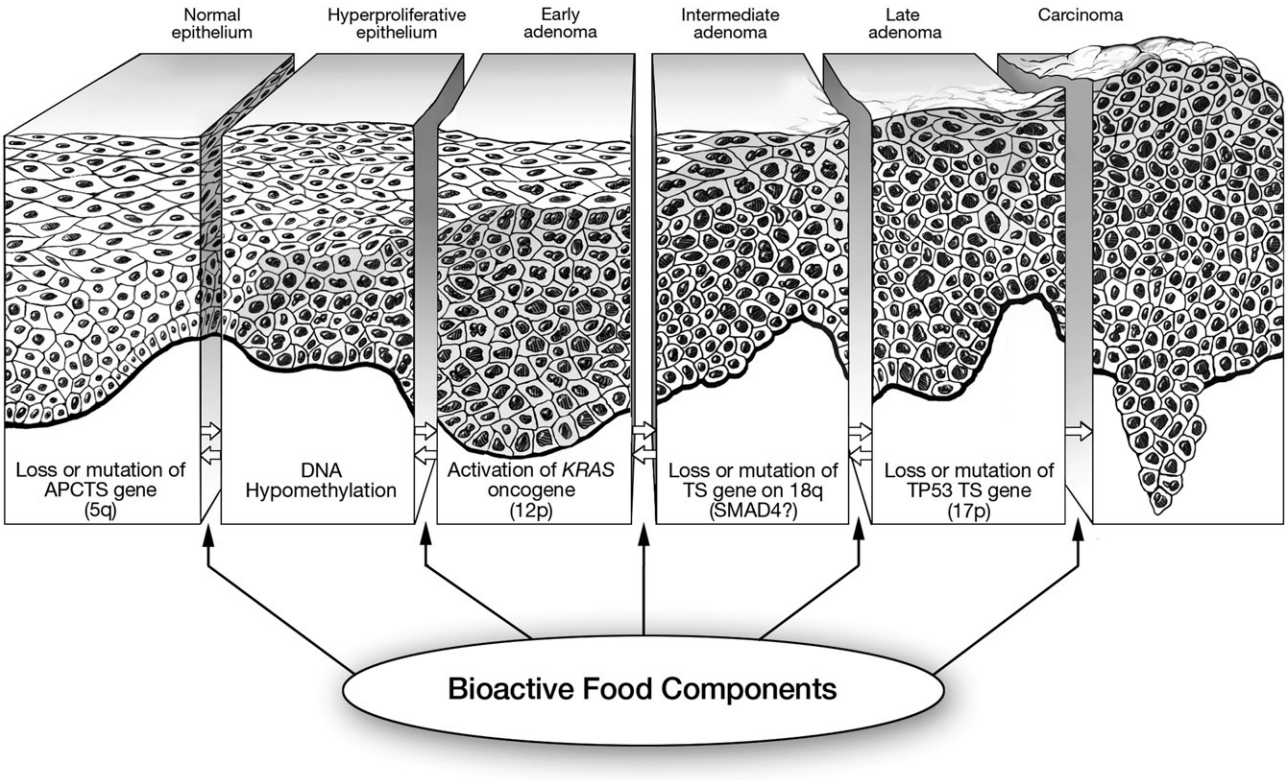

Fig. 1. Developed in 1990, the Vogelstein model pointed to genetic alterations that take place during colon carcinogenesis. This model paved the way to development of murine models for colorectal cancer. Bioactive food components may influence multiple steps in this evolution of the neoplasm.

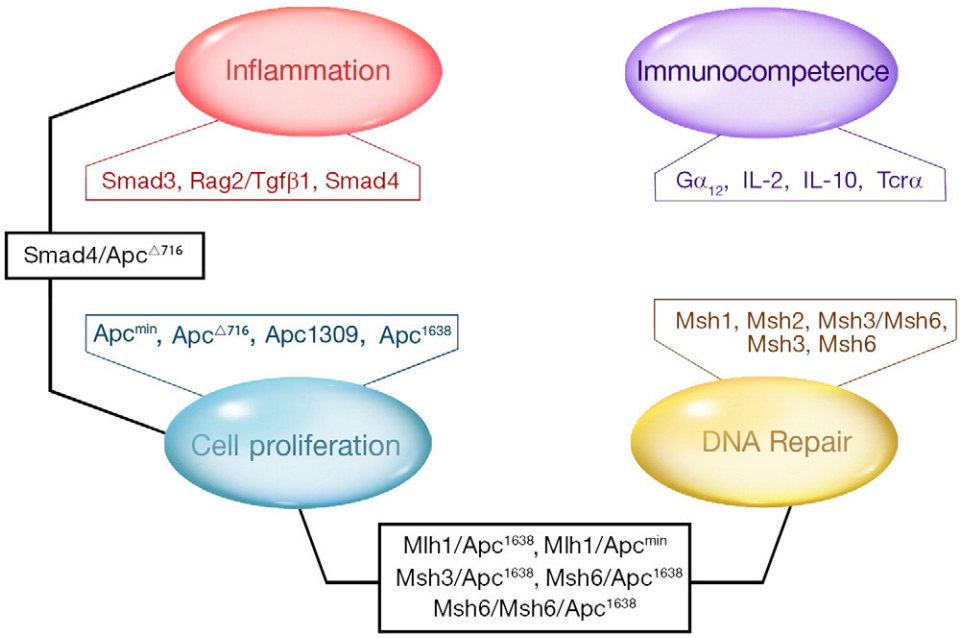

Fig. 2. Mouse models for colorectal cancer can be subdivided into various categories. The individual mouse models represent a specific category, or a combination of categories based on genetic modifications.

has been used to promote tumor development that has characteristics similar to human colonic tumors. Other carcinogens used include *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine [15], *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine [16], 1,2-dimethylhydrazine [17], 2-amino-3,4-dimethyllimidazo[4,5-f]quinoline [18], *N*-methyl-*N*-nitrosourea [19], capsaicin, captafol, captan, hydrogen peroxide and *N*-(trichloromethylthio)phthalimide [20]. The chemical carcinogen model is considered less expensive than the genetically engineered mice, but the quantity of carcinogens needed and their relevance to human risk remains an area of active discussion. Human tumors are often mutated at the APC or p53 gene and have a tendency to metastasize, whereas a carcinogen-induced model does not mutate at the same genes and has a low rate of metastasis [20]. These differences between chemically induced models and the human phenotype may depend on the specificity of the carcinogen used to induce the genetic and epigenetic changes. Nevertheless, these carcinogen models have provided important clues about genetic alterations and have assisted with the development of transgenic and knockout models. Collectively, tumors arising from animal models share many histological and genetic features with humans. The major differences between these models and humans are the location of masses in the small of Min mice (compared to the colon in human beings), and the mutation of β-catenin gene in AOM-injected rats (compared to Apc mutations in humans Apc) [21]. Overall, it remains advisable to examine these tumors for their utility in predicting the prevention of the risk and/or behavior of colorectal cancers in humans.

Knockout models take already present genes and replace them with an inactive form that is achieved by disabling the target gene in stem cells [22]. The Apc/Wnt pathway and mismatch repair are two of the more commonly used models. The Tgfβ pathway is recognized to be responsible for autoimmune functions where mutations can lead to the progression of intestinal cancers [23]. Smad-deficient mice are a consequence of mutations in this pathway and are recognized to develop multiple intestinal lesions. Smad3$^{-/-}$ models reliably develop colonic tumors by 6 months of age when produced on a pure 129/Sv background. Genetic background is recognized to influence risk since tumors only develop in 30% of the hybridized Smad3$^{-/-}$ mice [24]. Multiple gene interactions may influence the overall propensity for develop a gastrointestinal tumor. Several new Apc models have been developed recently to focus attention on various components of cell proliferation, epigenetic processes and telomerase activity [25]. These mouse models provide important insights to the genetic/epigenetic controls and potential molecular targets behind human cancers. Unfortunately, a dearth of publications deals with diet as a variable in most of these models.

4. Apc^{Min} mouse model studies

The Apc^{Min} mouse is the pioneer model for colorectal cancer, although admittedly it primarily serves as a model for small intestinal cancers. This genetically engineered model is widely used, and patterns the human condition familial adenomatous polyposis [26]. The Apc^{Min} mouse truncation occurs at position 850 on the Apc gene [20]. Development of the Apc^{Min} mouse has precipitated other models such as the Apc 716, 1309 or 1638, which represent truncations at

Table 1
Dietary components with inhibitory and stimulatory effects on small intestinal tumors in the Apc^{Min} mouse

I. Inhibitory			
Food component	Basal diet[a]	Mean difference	Reference
Bilberry (10%)	AIN-93G	40%	Misikangas et al. [28]
Bowman-Birk Inhibitor	AIN-76A		Kennedy et al. [29]
(.1%)		44%	
(.5%)		39%	
Caffeic-acid phenethyl ester	AIN-76A	63%[b]	Mahmoud et al. [30]
Calorie restriction (60% calories of control)	AIN-76A	60%	Mai et al. [31]
Cellulose	AIN-93G with 20% soybean oil+no fiber		Yu et al. [32]
(5%)		77%	
(10%)		42%	
Chafuroside (10 ppm)	AIN-76A	44%	Niho et al. [33]
Cloudberry (10%)	AIN-93G	34%	Misikangas et al. [28]
Copper (6 ppm)	AIN-93G with 1 ppm copper	48%[c]	Davis et al. [34]
Curcumin			
(.01%)	AIN-76A	64%[b]	Mahmoud et al. [30]
(.2%)	RM3	42%[b]	Perkins et al. [35]
Cyanidin-3-glucoside (.3%)	AIN-93G	51%[d]	Cooke et al. [36]
Dibenzoylmethane (1%)	AIN-76A	49%[c]	Shen et al. [37]
EGCG (.16%)+fish oil (12%)	AIN-76A	53%	Bose et al. [38]
Eicosapentaenoic acid (31 g/kg)	US-17	48%	Petrik et al. [39]
Flaxseed (15%)	AIN-93G	31%	Oikarinen et al. [40]
Guar gum fiber	AIN-93G with 20% soybean oil+no fiber		Yu et al. [32]
(5%)		57%	
(10%)		30%	
Hydroxsymatairesinol (.02%)	Modified AIN-936+2.5% inulin	32%	Oikarinen et al. [41]
Ligonberry (10%)	AIN-93G	42%	Misikangas et al. [28]
Mirtoselect (.3%)	AIN-93G	37%[d]	Cooke et al. [36]
Selenium-enriched broccoli (2.2 g/kg)	AIN-93G+2.2 g/kg low selenium broccoli	29%[c]	Davis et al.[42]
Stearidonic acid (31 g/kg)	US-17	45%	Petrik et al. [39]
Sulforaphane			
(300 ppm)	AIN-76A	25.3%	Hu et al. [43]
(600 ppm)	AIN-76A	47%	Shen et al. [37]
(600 ppm)		47%[c]	
Tricin (.2%)	AIN-93G	36%[c]	Cai et al. [44]
Wheat Bran Fiber	AIN-93G with 20% soybean oil+no fiber		Yu et al. [32]
(5%)		53%	
(10%)		43%	
Wheat Bran oil (2%)	AIN-93G	35%	Sang et al. [45]
White Currant (10%)	AIN-93G	37%	Rajakangas et al. [46]

II. Stimulatory			
Food component	Basal diet	Mean difference	Reference
Apple pomace (20%)	RM1	32%	Mandir et al. [47]
Fat	R20		Wasan et al. [48]
(10%)		28%	
(15%)		47%	
Retinoic-acid (all trans) (10 g/kg)	AIN-76A	133%	Mollersen et al. [49]

[a] More information about the composition of the AIN-93G, AIN-76A, RM3, US17, RM1 and R20 diets is available [35,39, 41,48,50–52].
[b] Total tumors only, predominantly in the small intestine.
[c] Extrapolated from manuscript figure.
[d] Extrapolated from manuscript figure, total tumors only.

other sites in the Apc gene. The Apc^{716} model produces the same effect as the Apc^{Min}, but the onset and number of tumors is more pronounced. Apc^{1638} mice tend to develop the least amount of tumors all together, and $APC^{1309/+}$ develop a slightly higher number of polyps than the Apc^{Min} mouse [27]. While there are no perfect models, varieties of the APC model incorporate conditions that reflect phenotypic responses in humans.

4.1. Dietary factors which decrease risk

These genetic models have been used to assess the effects of dietary components on cancer, most notably in the Apc^{Min} murine model (Tables 1–3). Some essential nutrients appear protective against colon cancer. For example, feeding a 60% calorie-restricted diet resulted in a 60% reduction in small intestinal polyp numbers. Mai et al. [31] found the average number of polyps greater than 2 mm was 57% lower when a caloric restricted diet was provided compared to ad libitum. While not statistically different, this reduction in calories lead to an almost 40% increase in mean colonic polyps [31]. This study generally supports the findings that calorie restriction reduces risk. Mechanistically, calorie restriction is recognized to reduce cell proliferation, enhance rates of apoptosis and reduce inflammation [65]. Changes in one or more of these may account for the change in cancer risk in the ^{Min} model.

In addition to calorie restriction, consumption of some fatty acids appears to reduce colon cancer risk. Various fatty

Table 2
Dietary components with inhibitory and stimulatory effects on colonic tumors in the Apc^{Min} mouse

I. Inhibitory

Food component	Basal diet[a]	Mean difference	Reference
Steridonic acid (31 g/kg)	US17	85%	Petrik et al. [39]
Sulforaphane (600 ppm)	AIN-76A	80%	Shen et al. [37]

II. Simtulatory

Food component	Basal diet	Mean difference	Reference
Fat	R20		Wasan et al. [48]
(10%)		207%	
(15%)		225%	
Retinoic acid (all-trans) (10 g/kg)	AIN-76A	500%	Mollersen et al. [49]
White currant (10%)	AIN-93G	268%	Rajakangas et al. [46]

[a] More information about the composition of the US-17, AIN-76A, R20 and AIN-93G diets is available at [39,48,50,51].

acids including conjugated linoleic acid isomers (CLA), gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), steraridonic acid (SDA), eicosapentaencic acid (EPA) and docosahexaenoic acid (DHA), were examined in a study by Petrik et al. [39] when added to a US17 diet. Adding CLA, GLA or ALA to the diet at 31 g/kg did not change tumor incidence or size. However, SDA and EPA (31 g/kg) resulted in a reduced tumor number and size. DHA addition caused a 38% decrease in tumor number in the small intestine. While an 8% increase in tumor number in the colon occurred, in these animals it was not significant. EPA supplementation significantly reduced small intestinal tumors by 48% and colonic tumors by 30%. SDA addition resulted in a significant 45% fewer small intestinal tumors and was as effective as the drug Sulindac (320 mg/kg) in reducing colonic tumors by 85% [39]. The ratio of n-6 to n-3 fatty acids may be key to determining a response to fatty acids. Bartram et al. [66] suggests when the ratio is low that pro-inflammatory products arising from n-6 fatty acids decreases, as reflected by depressed cell proliferation and an accompanying reduction in visible tumors.

Sulforaphane, a component of cruciferous vegetables, appears to be an effective anti-cancer agent in cell culture, carcinogen-induced and genetic cancer models [67]. When added at 300 or 600 μg/g to an AIN-76A diet for Apc^{Min} mice, it reduced the average number of polyps significantly (25.3% and 47% respectively in the small intestine). In this study, no polyps were observed in the large intestine. The antiproliferative effects were evident since about 5.8% of the polyps in the control group were less than 1 mm, whereas 53.2% and 72.5% were less in the 300- and 600-μg/g treatments, respectively [43]. Sulforaphane has been demonstrated to cause a dose-depended decrease in the proliferation of HT-29 colon cancer and multiple other cells in culture. In addition, sulforaphane is known to induce apoptosis in a variety of mammalian cells, yet appears to be less toxic to normal cells [67,68].

Chafuroside, a flavone derivative in oolong tea, has been reported to cause a significant inhibition of intestinal tumors in the ^{Min} mouse. Supplementation of a powdered AIN-76A diet with 0, 2.5, 5 or 10 mg/g chafuroside resulted in a dose dependent response in intestinal tumors with a 17%, 27% and 44% reduction, respectively. Only the 10 mg/g exposure caused a statistically significant change in tumor number. Chafuroside did not result in a statistically significant change in colon tumor number [33]. Similar to other phenolics in tea, such as epigallocatechin gallate, chafuroside can serve as a free-radical scavenger, reduce inflammation and increase apoptosis [69]. A change in one or more of these cancer related processes might account for its ability to retard tumors in the ^{Min} mouse.

Caffeic-acid phenethyl ester (CAPE) and curcumin, common plant phenolics, have also been examined in APC^{Min} mice. Using an AIN-76A diet as a control, the addition of CAPE at .03% and 0.15%, or curcumin 0.1% was found to influence tumor risk. CAPE at 0.15% or curcumin at 0.1% significantly lowered the incidence of intestinal adenomas by approximately 63% [30]. While the minimum quantity of curcumin needed to elicit a response has also been explored using a using a RM3 diet, the findings are difficult to interpret. Feeding a 0.1% curcumin containing diet was ineffective in reducing tumors. Adding 0.2% curcumin lowered small intestinal tumor load by 42%. Although not statistically significant, 0.2% and 0.5% curcumin resulted in a mean colon adenomas that were 30% and 27% lower, respectively [35]. The results of these studies are consistent with the general beliefs that curcumin can serve as an anti-inflammatory, antioxidant and an antimitogenic agent that can influence multiple signaling pathways [70]. While curcumin has many potential molecular targets, it is relatively poorly absorbed [71].

Combinations of different components can sometimes be more effective than providing ingredients independently. This was shown to be the case with a combination of sulforaphane and dibenzoylmethane (DBM), a minor component derived from licorice and β-diketone analogue of curcumin [37]. The combination proved to be most effective by decreasing intestinal tumors and polyps. The response to combining both at 300 mg/g and 1.0% was as effective as twice the concentration of each provided independently to an AIN-76A diet [37]. While DBM has been reported to inhibit the expression of several cytochrome P450s, other mechanisms may be involved. Studies using DBM in a 7,12 dimethylbenz(a)anthracene (DMBA)-induced mammary tumor model suggest that it can reduce proliferation rates in the mammary gland and the uterus of Sencar mice [72]. Studies by Shen at al. [37] suggest the down-regulation of prostaglandin E_2 or leukotriene B_4 levels may be partially available along with an inhibition of COX-2 expression and thus a change in inflammation may be involved.

Table 3
Food components that have been reported to Influence intestinal tumors

Food component	Basal diet[a]	Small intestine Δ no.	Colon Δ no.	Reference
Alpha Linolenic acid (31 g/kg)	US17	+8%	−38%	Petrik et al. [39]
Anthocyanin (800 mg/l)	Modified AIN-93G	+24%	−15%	Kang et al. [53]
Apple Pomace (20%)	RM1		−10%	Mandir et al. [47]
Arachidonic Acid (15 g/kg)	AIN-93G+ 15 g/kg oleic acid	−29%	−50%	Petrik et al. [39]
Beef	AIN-93G	+50%	+80%	Mutanen et al. [54]
Bilberry (10%)	AIN-93G		+83%	Misikangas et al. [28]
Bovine lactoferrin	AIN-93G			Ushida et al. [55]
(.2%)		−15%	−11%	
(2%)		−20%	−23%	
Bowman-birk inhibitor	AIN-76A			Kennedy et al. [29]
(.1%)			−36%	
(.5%)			−38%	
Calcium	AIN-93G+ 1000 IU/kg Vitamin D			Huerta et al. [56]
(2.5 g/kg)		−8%	+10%	
(10 g/kg)		+22%	+15%	
Calorie restriction (60% calories of control)	AIN-76A		+40%	Mai et al. [31]
Chafuroside	AIN-76A			Niho et al. [33]
(2.5 ppm)		−17%	+10%	
(5 ppm)		−27%	−28%	
(10 ppm)			−4%	
Cherries (200 g/kg)	Modified AIN-93G	−27%	−10%	Kang et al. [53]
Cloudberry (10%)	AIN-93G		+50%	Misikangas et al. [28]
Conjugated linoleic acid (31 g/kg)	US17	+21%	−23%	Petrik et al. [39]
Conjugated linoleic acid isomer	RM1			Mandir et al. [57]
C9t11		+28%[b]	−51%[b]	
T10c12		−1%[b]	−61%[b]	
C9t11+t10c12		+12%[b]	−66%[b]	
Copper (6 ppm)	AIN-93G+ 1 ppm copper		+73%[b]	Davis et al. [34]
Curcumin				
(.1%)	RM3		+25%[b]	Perkins et al. [35]
(.2%)			−13%[b]	
Cyanidin-3-glucoside	AIN-93G			Cooke et al. [36]
(.03%)		−9.5%[c]		
(.1%)		−25%[c]		
Dibenzoylmethane (1%)	AIN-76A		−58%[c]	Shen et al. [37]
Docosahaenoic acid (31 g/kg)	US17	−38%	+8%	Petrik et al. [39]
EGCG				
(.08%)	AIN-93G	−57% (females)	−10% (males)	Ju et al. [58]
(.16%)	AIN-76A	−45% (males)		Bose et al. [38]
(.16%)		−18%		
Eicosapentaenoic acid (31 g/kg)	US17		−30%	Petrik et al. [39]
Fish oil (12%)	AIN-76A	+3%		Bose et al. [38]
Fish oil concentrate K85	AIN-76A			Paulsen et al. [59]
(.4%)		−39%	+5%	
(1.25%)		−26%	−40%	
(2.5%)		−37%	−55%	
Flaxseed (defatted) (.5%)	AIN-93G	−10%	−35%	Oikarinen et al. [60]
Folate	Amino acid defined diet with 2 mg/kg folate			Song et al. [61]
(0 mg/kg)		−68%		
(8 mg/kg)		−12%		
(20 mg/kg)		−67%		
Fruit and vegetable mixture (19.5%)	Muracon-SSP/tox	−18%	+48%	Van Kranen et al. [62]
Gamma-linolenic acid (31 g/kg)	US17	+25%	−15%	Petrik et al. [39]
Hydroxsymatairesinol (.02%)	Modified AIN-936+ 2.5% inulin		+55%	Oikarinen et al. [41]
Indole-3-carbinol	AIN-76A			Kim et al. [51]
(100 ppm)		−8%	−40%	
(300 ppm)		−5%	−6%	
Ligonberry (10%)	AIN-93G		−28%	Misikangas et al. [28]
Mirtoselect	AIN-93G			Cooke et al. [36]
(.03%)		−8%[c]		
(.1%)		−20%[c]		
Oat bran (10%)	AIN-93G	+33%		Mutanen et al. [54]
Resveratrol	AIN-93G			Zeigler et al. [63]
(4 mg/kg)		+6%[b]		
(20 mg/kg)		+13%[b]		
(90 mg/kg)		+18%[b]		
Rye bran				
(10%)	AIN-93G	−25%	−20%	Mutanen et al. [54]
(10%)	Modified AIN-936+ 2.5% inulin	−9%	+7%	Oikarinen et al. [60]
Tricin (.2%)	AIN-93G		+50%[b]	Cai et al. [44]
Wheat bran (10%)	AIN-93G	−1%	+20%	Mutanen et al. [54]
Wheat bran oil (2%)	AIN-93G	−35%	−38%	Sang et al. [45]
White currant (10%)	AIN-93G	−37%		Rajakangas et al. [46]

[a] More information about the composition of the US17, AIN-93G, RM1, AIN-76A, RM3, amino-acid defined and Muracon-SSP/tox diets is available [35,39,50–52,62,64].

[b] Extrapolated from manuscript figure.

[c] Extrapolated from manuscript figure, total tumors only predominantly in small intestine.

4.2. Stimulation of risk

Although some dietary components are protective against models for colon cancer, others appear stimulatory. For example, total fat calories are often considered to increase risk, which also appears to be the case in the Apc^{Min} mouse. Small intestinal tumor number increased significantly (28%) when a basal diet was increased from 3% to 10% total fat. Increasing the fat content to 15% resulted in a 47% increase compared to the basal diet. Results were even greater in the colon where a 207% increase in tumors occurred when the diet was increased to 10% and 15% compared to the basal respectively [48]. As indicated earlier, the type of fat provided can also influence risk in these genetically predisposed mice. Epidemiological evidence suggests that saturated fat promotes increased colon cancer risk and that fats from vegetable sources tend to influence risk inversely, although considerable literature variability occurs [73].

All-trans retinoic acid, a vitamin A derivative thought to be involved with gene transcription regulation, may also contribute to increased formation of intestinal tumors. In the colon and rectum, dietary retinol may increase risk, but few studies have been completed to date. Foods that contain exaggerated amounts of retinol tend to be high in dietary fat, thus making interpretation difficult [74]. A recent study aimed at examining retinoic acid (5 and 10 mg/kg) as a supplement to an AIN-76A diet, suggested it might promote tumors in ^{Min} mice [49]. A dose-dependent increase in both small intestinal and colonic tumors was observed. The 10 mg/kg fortified diet resulted in a greater than twofold increase in the number of small intestinal tumors, and a fivefold increase in the number of colonic tumors. Mollersen et al. [49] concluded that the increase in tumors might be due to increased transcriptional regulation because there were no signs of local toxicity or an inflammatory reaction. Several genes are known to be regulated by retinoic acid; however, the specific targets are unknown in the mouse intestine and further investigations are warranted [49].

Studies aimed at examining caffeine [58], resveratrol [63], quercetin and rutin [30] have found no ill consequences in terms of intestinal tumorigenesis. Feeding pectin (10%) with a high (74%) and low (37%) methoxylation tended to promote an earlier onset of tumors and an increase in size, but overall tumors were only marginally influenced [75].

4.3. Cell proliferation models

The $Apc^{\Delta 716}$ knockout mouse is similar to the Apc^{Min} mouse, with the difference being in the spot of genetic mutation. This mouse is still representative of FAP, but develops more tumors than the Apc^{Min} [27]. In one study with these mice, DHA addition to the diet did not affect tumor numbers in male mice, but decreased them by 69% in females compared to controls [76]. Another study used this model to examine the effects of dietary fat and fiber. A low-risk diet, containing 5% fat and 20% wheat fiber decreased polyp number in the small intestine by 36%, and by 64% in the colon compared to the high risk diet which contained 20% fat and 0.25% wheat fiber [77]. There were no differences in polyp size in this study. Overall, these results again support the general belief that a diet high in fat calories increases the risk of colorectal cancer, where diets high in dietary fiber and thus lower in fat calories decreases risk. The mechanisms of fiber in cancer prevention are not fully understood but may relate to increase cell apoptosis and differentiation [78].

The Apc^{1638} model (crossed with the p21 knockout) was used to evaluate the impact of a Western-style diet that is high in fat and phosphate and low in calcium and vitamin D on tumor incidence [79]. The survival of those fed the Western-style diet was less, again suggesting a possible protection due to calcium and vitamin D [79]. Calcium and vitamin D are recognized to each induce differentiation, as well as apoptosis. To date, there is only limited evidence suggesting protective effects of vitamin D on colon cancer, and much of the data are inconsistent and/or nonsignificant [80,81]. Although the influence of calcium on risk in human beings remains intriguing, the use of these models may assist in explaining inconsistencies in the literature [82].

The Apc^{1638} mouse has also been crossed with $Scad^{-/-}$ mice, which are deficient in short-chain fatty acid metabolism. The $Scad^{-/-}$ mutation reduces apoptosis by 98% in the proximal colon, and by 50% in the duodenum and distal colon compared to the wild type. This mutation also reduced the proportion of apoptotic cells to proliferating cells in the entire region of the intestinal tract. However, diet can influence this response as evidence by a slight increase in the number of tumors per mouse with the $Scad^{-/-}$ mutation fed a diet fortified with 120 g/kg hard red wheat bran compared to the wild type mice, but not statistically significant [83]. Sixteen cohort and case-control studies have investigated the impact of dietary fiber on colorectal cancer with variable results [4]. The 2007 WCRF report suggest the evidence for the involvement of fiber is limited-suggestive [4].

5. DNA repair models

Another set of models was developed to examine another hereditary condition known as nonpolyposis colorectal cancer, namely, DNA-base repair genes. These genes are involved in the maintenance of genomic integrity. In eukaryotes, the initiation of the repair process requires subsets of three different MutS homologs: MSH2, MSH3 and MSH6. These three MutS homologs form heterodimeric complexes consisting of MSH2–MSH6 (termed MutSα) and MSH2–MSH3 and often referred to as MutSβ. $Msh2^{-/-}$ mice are often used to examine inflammation variables [84]. $Msh3^{-/-}$ mice are frequently used as gastrointestinal tumor models, as well as for inflammation induced mucosal hyperplasia [85].

When crossing the Apc mouse with an Msh2 mouse, the new murine model still has the mutation at codon 850 of the *Apc* gene, but results in almost 4 times as many intestinal tumors as the Apc^{Min} mouse in about half of the time. In one study, the effects of folate were studied by using an amino-acid defined diet. Folate was provided at either 0- or 8-mg/kg diet. The addition of folate was accompanied by a 2.7-fold decrease in the number of small intestinal adenomas. In the colon, there was a 67% reduction in the number of tumors, although this value was not statistically different [61]. Although folate has been examined, little compelling evidence demonstrates cancer inhibition or promotion. It is possible that timing of folate addition is critical in the response, such that a deficiency promotes an earlier onset of tumors while administration once the tumor exists may promote its proliferation. In preclinical and clinical studies, folate addition appears to have a biphasic response [86], increasing risk when inadequate and when provided in excess, although unexplainable inconsistencies are evident.

Other models in this category include the Msh1, Msh3, Msh6 and Msh3/Msh6. Unfortunately, a search of the literature did not identify any which have been examined in response to specific dietary components. Combination models representing cell proliferation and DNA repair include $Mlh1/Apc^{1638}$, $Mlh1/Apc^{Min}$, $Msh3/Apc^{1638}$, $Msh6/Apc^{1638}$ and $Msh6/Msh6/Apc^{1638}$, which have also not yet been studied as a function of dietary exposures.

5.1. Immunocompetence models

An additional class of models has been developed to examine the role of immune function. These interleukin-2 (IL-2) and interleukin-10 (IL-10) knockout models reveal spontaneous inflammation leading to adenocarcinomas, similar to the human condition of irritable bowel disease as an antecedent to colon cancer [87–90]. Folate and iron have been examined in the IL-2 knockout model for their impact on cancer risk. Dysplastic colorectal lesions, signified by loss of epithelial cell polarity, hyperchomatic and pleiomorphic nuclei, and an increased number of mitotic figures, were observed in the colons of 1 of 12 (8.3%) IL-2 (−/−) mice fed an AIN76A diet. Dysplasia was not observed in the colons of the 12 mice that received weekly iron injections (intraperitoneal injections as iron-dextran at 12 mg/kg body weight). Dysplasia was detected in 4 of 11 (36.4%) of the mice fed a diet with twofold iron content than the basal diet [91]. Overall and similar in behavior to folate, the benefits/risk associated with iron appear to be influenced by the total exposure and that excessive exposures may counteract the benefits occurring at lower intakes. There are some studies that suggest iron does increase cancer in humans, but the quality of the evidence is limited at best [92,93].

The IL-10 knockout mice have been used to study the impact of fatty acids including corn oil, fish oil and olive oil on colonic neoplasm. A decreased risk was associated enhanced olive oil ingestion, which was further reduced by fish oil [94]. As mentioned earlier, the ratio of n-3 to n-6 fatty acids may be key in determining the magnitude of the overall response. Higher n-3 fatty acid consumption is thought to inhibit cyclooxygenase 2 activity, thereby decreasing proinflammatory agent production [66,95].

Two additional models representing immunocompetence, T cell receptor alpha and $G\alpha_{12}$, are currently available. The Tcr is a molecule found on the surface of T lymphocytes that is, in general, responsible for recognizing antigens bound to major histocompatibility complex molecules. Heterotrimeric G proteins are known to function to relay information from cell surface receptors to intracellular effectors. Recently $G\alpha_{12}$ was found to function as a molecular regulator responding to extracellular stimuli NF-E2-related factor 2, which is involved in a protective responder to oxidative stress. Unfortunately, dietary variables have not been studied using these models.

6. Inflammation models

Inflammation is a process that is modifiable by diet habits. Diet modifications are especially important when involving disorders such as inflammatory bowel disease or Chron's disease where certain foods can modify the severity of this condition, which may possibly lead to cancer [96]. Calorie restriction [65] and supplementation with chafuroside [69] or curcumin [70] have been shown to decrease inflammation in other types of murine models. A series of mouse models including Smad3, Smad4 and Rag2/Tgfβ1 have been developed but have not yet been incorporated into studies involving diet. The same is true for a combination model of $Smad4/Apc\Delta^{716}$ representing inflammation and cell proliferation, but available evidence suggests such investigations are needed.

7. Other considerations

Colon cancer risk is an international issue. Murine models provide an excellent starting point for understanding the linkages between diet, genetics and colorectal cancer, but have not been adequately examined to date. In a general Medline search about 1600 manuscripts were found that deal with mouse models and colon cancer, of which only a little over 100 were examined diet as a variable. Additionally, there are 1075 papers about carcinogens and colon cancer, with only about one third evaluating diet. Undeniably, bioactive food components need to be examined more extensively using the currently available models to explore mechanisms of action and overall outcomes in terms of cancer processes and thus molecular targets.

Numerous studies have examined dietary components ranging from everyday essential nutrients, to specific compounds found in rare plants. Even with the wide range of studies that have been conducted using the varieties of

models, there is little compelling evidence showing the effect of dietary components. The best inhibitory results were seen with caloric restriction, n-3 fatty acids, sulforaphane, chafuroside and curcumin. The data demonstrating all-trans retinoic acid and dietary fat as promoters were rather convincing.

There may well be a maximum effective dose of a dietary component to suppress colorectal cancer. Exposures below or beyond this point may change the magnitude or direction of the response. This is illustrated with cellulose intakes in Apc models. A high-fat modified AIN-93G diet supplemented with 5% cellulose was found to inhibit small intestinal tumors by 77%, but when the dose was increased to 10% cellulose, small intestinal tumor inhibition was reduced to only 42% [32]. Likewise, providing chafuroside at 5 ppm in the AIN-76A diet reduced colonic tumors by 28% and only by 4% when 10 ppm was used [33]. An even more dramatic response occurred with indole-3-carbinol. Providing 100 mg/kg reduced colonic tumors by 40%, but only by 6% when the dose was increased to 300 mg/kg [51]. This data raises the perplexing issue about how to provide guidance about the ideal exposure of bioactive food components to reduce risk.

In addition to the dose of a bioactive food component, nutrient-nutrient interactions may increase inhibitory effectiveness. For example, EGCG at a concentration of 0.16% in the AIN-76A diet inhibited only 18% of small intestinal tumors (Table 1). When EGCG at 0.16% in the AIN-76A diet was mixed with fish oil at a concentration of 12%, tumor inhibition increased to 53% [38].

The quantity of the bioactive food component provided is important, as well as duration of exposure. As indicated in Tables 1 to 3, a dose-response relationship is observed with a variety of dietary components including chafuroside, sulforaphane, dietary fat calories and resveratrol, and in several cases, a maximum benefit was observed. Study design makes it difficult to draw conclusions from these dietary interventions but in most cases, it appears that many food components serve as deterrents, rather than "magic bullets" to prevent tumors within these susceptible mouse models. Undeniably, greater attention is needed to evaluate adequately the impact of duration of exposure on the magnitude of the response in these genetic models.

These preclinical models may shed important information about why some bioactive food components appear to produce a "U"-shaped response in cancer risk including b-carotene, iron and folic acid. The timing of a response to folic acid in Min mice is highly dependent on when it is provided. Early intervention to eliminate a deficiency dramatically reduces the number of neoplasm but when added later in the life of the Apc mouse the number of tumors increases markedly [61]. Recently, findings suggest that a subset of society may be placed at risk as a result of the fortification of the food supply with folic acid [97]. At least part of this inconsistency in response may relate to the existence or absence of a preneoplastic lesion.

8. Conclusions

Several genetically engineered models, which reflect key elements of the human colon cancer condition, are available to investigate the role of diet. The discrepancies between the small and large bowel in the currently available models raise important concerns about the need for additional well-characterized models [20,21,25]. The use of newer germline targeted models with a deletion of Apc exon 14 may avoid some concerns since more severe colonic tumors are present in these mice. Typically these $Apc^{14/+}$ mice develop 5–15 polyps compared to 0.4–4 in other Apc models [98]. Undeniably, the use of these models is necessary to evaluate effective dosages and to explore side-effects or adverse effects [20,25]. Human studies are frequently lengthy and costly and require knowledge about optimal dosages and ill-consequences. The use of surrogate or end-point biomarkers in human studies that arise from well controlled animal studies should help to decrease both cost and risk. Since there is no single surrogate biomarker has been validated as the gold standard for colon cancer the use of multiple models that predict cancer processes seems prudent. Overall, despite the inconsistencies between mice and humans, genetic models are a cost-effective and safe tool to discover the relationship between diet and cancer risk and tumor behavior.

Microbes within the gastrointestinal tract may also contribute to variation between these animal models and observations in humans. Multiple food components may not only modify the composition of microbes within the gastrointestinal tract, but also influence their rates of metabolism and their dynamic relationship with cellular processes occurring with the intestinal mucosal. For example, the bacterial formation of butyrate in the colon for digestion of fructo-oligosaccharides is thought to possibly increase apoptosis. Emerging evidence about interactions among dietary components, the microbiome and mammalian genetics raises intriguing questions that warrant attention [99].

Recent advances hold promise for the development of several types of imaging technologies that will permit the detection of subtle changes in the colon tissue. Coupling this technology with appropriate models holds promise to shed new light on when dietary interventions are most appropriate for reducing the risk and biological behavior of intestinal neoplasia [100]. These new findings will not only unravel the mysteries associated with diet and colon cancer but also will assist in developing appropriate intervention strategies for reducing cancer burden. Unquestionably, this information will have profound implications nationally and internationally.

There are still many unexplained processes in the pathway of diet to cancer, and with additional research, the models can continue to be modified to more closely mimic human beings. Future research needs to investigate which dietary components are most important modifiers of critical targets in the cancer process and under what circumstances.

Maximum effective exposures and nutrient-nutrient and nutrient-gene interactions, along with optimum duration of exposure are fundamental to unraveling the importance of diet in determining colon cancer risk. Models that can examine the impact of genetic and epigenetic factors on eating behaviors, digestibility, absorption, metabolism and/or elimination will provide a clearer picture of those who will likely benefit most from dietary intervention and those who might be placed at risk.

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