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Nutrient-Gene Interaction in Colon Cancer, from the Membrane to Cellular Physiology

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Abstract

The International Agency for Research on Cancer recently released an assessment classifying red and processed meat as “carcinogenic to humans” on the basis of the positive association between increased consumption and risk for colorectal cancer. Diet, however, can also decrease the risk for colorectal cancer and be used as a chemopreventive strategy. Bioactive dietary molecules, such as n-3 polyunsaturated fatty acids, curcumin, and fermentable fiber, have been proposed to exert chemoprotective effects, and their molecular mechanisms have been the focus of research in the dietary/chemoprevention field. Using these bioactives as examples, this review surveys the proposed mechanisms by which they exert their effects, from the nucleus to the cellular membrane. In addition, we discuss emerging technologies involving the culturing of colonic organoids to study the physiological effects of dietary bioactives. Finally, we address future challenges to the field regarding the identification of additional molecular mechanisms and other bioactive dietary molecules that can be utilized in our fight to reduce the incidence of colorectal cancer.

Keywords

n-3 polyunsaturated fatty acids; microRNAs; colonic organoids; membrane organization

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INTRODUCTION

According to the National Cancer Institute, colorectal cancer accounted for 8% of all new cancer cases in 2015, making it the fourth most common cancer diagnosed in the United States (82). In addition, colorectal cancer accounted for 8.4% of all cancer deaths in 2015, the second leading cause of cancer-related death in the United States. Although the rate of new colorectal cancer cases has been dropping on average by 3.1% each year over the last 10 years, the death rates have not changed significantly over the same period (82). These statistics clearly highlight the importance of not only understanding the complex etiology of colorectal cancer, but also of developing effective prevention and treatment strategies for the disease.

The majority (95%) of colorectal cancers begin as noncancerous polyps of the intestinal epithelium on the inner lining of the colon or rectum that have accumulated oncogenic mutations over time (78, 203). Noncancerous polyps may become malignant and transform into adenomatous polyps if left undetected. Approximately 20% of colorectal cancer cases are attributed to patients with two or more first- or second-degree relatives with colorectal cancer (128, 184), suggesting that genetic factors play a minor role in the development of colon cancer. Various environmental factors contribute to the development of cancer. For example, of all cancer-related deaths, 25–30% can be attributed to tobacco, 30–35% are linked to diet, and 15–20% are due to infections (6). In particular for colon cancer, intestinal inflammation (e.g., Crohn's disease and ulcerative colitis) (53, 57) and obesity (19, 139) are additional risk factors that are associated with increased incidence of this form of cancer.

As mentioned above, epidemiological studies have established that diet can play a role in increasing the risk of developing colorectal cancer. In one study comparing US-born with foreign-born Japanese populations, the rate of colorectal cancer for US-born Japanese men was twice that of foreign-born Japanese men. Similarly, US-born Japanese women had a 40% higher incidence compared to foreign-born Japanese women (59). This trend of increased colon cancer risk in association with migration to the United States from another country has been confirmed in other migrating populations (80, 118, 161). Furthermore, studies have documented a correlation between increased colon cancer incidence and per capita consumption of meat, animal protein, and total fat (8, 173). Overwhelming evidence indicates that the consumption of red and processed meat is associated with an increased risk of colorectal cancer (14, 16, 27, 40, 116, 147, 175, 178). Various mechanisms have been proposed to link red and processed meat to colorectal cancer (see Figure 1). The high level of the iron-porphyrin pigment, heme, in red meat has been associated with colorectal cancer in epidemiological studies (11, 117). Heme is poorly absorbed by the small intestine; therefore, dietary heme can accumulate in the colon (225), where it induces colonic injury resulting in hyperproliferation and hyperplasia (86, 87, 188), which may lead to the development of colorectal cancer. With the recent focus on the gut microbiota, it is not surprising to find that heme can alter microbial composition and facilitate the malignant transformation of colonic epithelial cells (84, 85). The second potential compound found in red meat that may increase colorectal cancer risk is heterocyclic amines (40, 79, 148). These compounds are generated when red meat is cooked at high temperatures (41, 91, 201) and are thought to be genotoxic once they enter the cell and are metabolized to compounds that

can interact with DNA to produce DNA adducts, inducing mutations in key oncogenic genes such as adenomatous polyposis coli (*Apc*), β-catenin, and K-Ras (201). A third class of compounds, *N*-nitroso compounds (NOCs), is also found in processed meat (121, 206). Although NOC can be endogenously synthesized from amines and amides with nitrosating agents derived from nitrites, the majority of exogenous exposure comes from the diet (83, 127). These compounds can interact with DNA to promote oncogenic mutations in driver genes (95, 119). Similar to the links of heme and heterocyclic amine with colorectal cancer, epidemiological studies have found a link between NOC and colorectal cancer (47, 125, 229).

The role of diet in promoting and preventing colorectal cancer can be context dependent, beneficial in one situation but detrimental in another context (101). One example of the dual modulatory role of diet in colorectal cancer is folate. This critical B vitamin is involved in the one-carbon transfer, where it is used as a substrate for the synthesis of nucleic acid purine bases and DNA methylation (61, 187). Thus folate plays an important role in regulating cell division, and folate deficiency is linked to many human health diseases, including congenital defects, adverse pregnancy outcomes, and cardiovascular diseases (198, 212). On the basis of these findings, an effort has been made to increase the dietary intake of folate/folic acid through supplementation in the food system (158), resulting in a significant reduction in congenital defects, such as neural tube defect, by as much as 50% postfortification (219). In cancer, however, one therapeutic strategy may be to interrupt DNA synthesis in rapidly replicating cells to suppress tumor growth. Indeed, folate deficiency has been shown to suppress DNA synthesis in neoplastic cells (102, 104). Epidemiological data on folate, however, have suggested that a high level of folate is associated with a reduction in risk of colorectal cancer (73, 97–99, 179). Some clinical studies have also demonstrated efficacy (93, 154, 221), although other clinical studies have shown no or even detrimental effects of folate supplementation on colorectal cancer (37, 58, 124, 214). These results highlight the duality of folate in cancer prevention and promotion. It has been suggested that folate deficiency may increase the risk of neoplastic transformation in normal colonic tissue, but as the disease progresses, folate deficiency may be beneficial in stopping the progression of the malignant transformation (reviewed in 103). In contrast, folate supplementation may be necessary for inhibiting normal colonic tissue from malignant transformation but may be detrimental once adenocarcinoma foci have developed in the colon (101). Folate may play a role in colorectal cancer in a context-dependent manner.

Another compound that has gained considerable attention in preventing colorectal cancer is aspirin, with the US Preventive Services Task Force releasing its systematic evidence review concluding that aspirin appears to reduce the risk of colorectal cancer incidence (35). It is thought that aspirin and other nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase-2 (COX-2), which is often overexpressed in colorectal cancer tissue (26). Even though it is one of the most promising chemopreventive agents, potential serious side effects including gastrointestinal bleeding (25, 199) and cardiovascular events (142, 170) make it less than an ideal agent. Thus the search for innocuous, bioactive compounds for chemoprevention remains important.

Clearly, bioactive dietary compounds remain largely unexplored for their potential in providing a varied armamentarium for preventing the development of colorectal cancer (Figure 1). Long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs), such as eicosapentaenoic acid (EPA, $20{:}5^{\Delta 5,8,11,14,17}$) and docosahexaenoic acid (DHA, $22{:}6^{\Delta 4,7,10,13,16,19}$), the most notable bioactive components found in fish oil, have been shown to prevent multiple forms of cancer. The increased consumption of fish, n-3 PUFA, EPA, or DHA has been associated with a significant risk reduction for colorectal cancer (69, 100, 105). Additional human studies have shown inverse relationships between intake of n-3 PUFA and risk of colon cancer (143, 180, 204). A meta-analysis of prospective cohort studies and a 22-year prospective study both showed reduced risk of colorectal cancer with increased consumption of fish and fatty acids from fish (68, 77). Additionally, individuals with high serum levels of n-3 PUFA have a decreased risk of developing colorectal cancer (76, 108, 165). Other dietary bioactives, such as curcumin (diferuloylmethane), a yellow color pigment of turmeric (*Curcuma longa* Linn) extracts, have also shown promise in suppressing colorectal cancer in experimental models and placebo-controlled clinical trials (145, 192). Utilizing n-3 PUFA, curcumin, and fermentable fiber as examples, this review highlights some of the putative mechanisms by which dietary bioactives may be used to prevent colorectal cancer, from the cell membrane to the regulation of transcription (Figure 2).

n-3 PUFA AND TRANSCRIPTIONAL MACHINERY

The bulk of dietary PUFAs is in the form of neutral fat or triglyceride and is well absorbed in the small intestine via emulsification, hydrolysis, and micelle formation (15). Following lipoprotein transport, PUFAs are incorporated into cell membrane glycerophospholipids by a remodeling pathway (Lands' cycle) to generate membrane asymmetry and diversity (194). This involves the concerted actions of phospholipases A2 and lysophospholipid acyltransferases.

Cell stimulation by a number of agonists triggers the formation of products of lipid hydrolysis, including DHA and EPA and their oxidative metabolites (63). These lipid mediators have been shown to interact directly with specific ligand-dependent nuclear receptors, including constitutive androstane receptor, hepatocyte nuclear factor 4 alpha (HNF4A), peroxisome proliferator-activated receptor gamma (PPARγ), pregnane X receptor (PXR), and retinoid X receptor alpha (RXRα) (157, 207). In this fashion, n-3 PUFA can regulate the function of nuclear receptors and impact transcriptional machinery. For example, DHA can bind and activate PPARγ, where it can transactivate genes associated with fatty acid transport and β-oxidation to control energy balance by regulating fatty acid homeostasis (163). Interestingly, impaired expression and function of PPARγ are associated with inflammatory bowel disease and colon cancer (52, 227). Therefore, one therapeutic application of n-3 PUFAs may be the activation of PPARγ and its associated fatty acid homeostasis pathways.

The liver X receptors (LXRs) are transcriptional regulators of cholesterol metabolism that control cholesterol uptake into the cell, its subsequent catabolism, and efflux out of the cell (176). This is noteworthy because cholesterol can control cell proliferation, and disruption in

cholesterol metabolism has been associated with the development of colon cancer (164, 172, 228). LXRs also function by heterodimerizing with RXRα (176). The activation of LXRα by n-3 PUFA has been shown in preclinical studies to block proliferation of human colorectal cancer cells and to slow the growth of xenograft tumors in mice (123).

PXR (NR1I2) has been shown to regulate the expression of genes involved in the oxidation, conjugation, and transport of xenobiotics and to promote the metabolism, elimination, and detoxification of chemotherapeutic agents (167). It is noteworthy that the transcription of PXR increases in the presence of n-3 PUFA (114), and PXR can suppress the proliferation and tumorigenicity of colon cancer cells (151). The constitutive androstane receptor (NR1I3) is likewise transcriptionally increased by n-3 PUFAs in epithelial colorectal adenocarcinoma cells and similarly regulates genes involved in xenobiotic detoxification and energy homeostasis (114).

HNF4A maintains epithelial cell function and normal colon physiology via regulation of the balance between proliferation and differentiation, immune function, ion transport, epithelial barrier function, and oxidative stress (3, 22). P1-HNF4A, but not P2-HNF4A, expression is lost in colorectal carcinomas in humans, and it is predicted that treatments that increase nuclear P1-HNF4α protein levels, such as n-3 PUFA treatment, could help slow colon cancer progression (31, 150).

Since the original description of dietary fat as a regulator of gene expression more than a decade ago, many transcription factors have been identified as prospective indirect targets for n-3 PUFA regulation. For example, DHA can increase the activity of cyclic AMP response element-binding protein (CREB) binding protein, E1A-binding protein p300, and MYC and decrease the activity of nuclear factor-kappa B (NF-κB) (NFKB1) and signal transducer and activator of transcription 3 (STAT3) (157). However, DHA does not directly bind to this class of transcription factor. With respect to colon cancer, DHA exhibits a protective suppressive effect against hyperactivated STAT3 and may reestablish the equilibrium between STAT3 and PPARγ (42). The decrease in STAT3 activity may be associated with the ability of n-3 PUFA to trigger PPARγ-RXR heterodimers to localize at their cognate PPAR response elements and exchange corepressors for coactivators such as CREB binding protein and p300 (52).

The cytotoxic effects of DHA are also associated with signaling pathways involving lipid metabolism and endoplasmic reticulum (ER) stress. DHA can deplete free cholesterol in the ER, which leads to ER stress and growth arrest/apoptosis of metastatic tumor cells (92). It has been suggested that these alterations in the sterol content of the ER by DHA mediate growth partly by decreasing nuclear sterol regulatory element-binding protein, an important regulator of lipid homeostasis and cell growth regulation (185). Induction of ER stress mediators by DHA also promotes expression of protein kinase RNA-like endoplasmic reticulum kinase (PERK), which in turn increases translation of activating transcription factors 3, 4, and 6 (92). Furthermore, an elevation in PERK activity can increase levels of ER protein GADD34 (PPP1R15A) and the proapoptotic transcription factor DDIT3/CHOP along with its downstream target TRIB3 (197). The experimental details associated with differentially expressed target genes are described in a recent review (207).

Colon adenocarcinomas exhibit defective expression of the *APC* gene, which is a critical regulator of the Wnt signaling pathway. This and other developmental pathways play an important role in both genetic (familial) and sporadic epithelial cancers (71). From a chemoprevention perspective, in vivo studies demonstrate that fish-oil-derived n-3 PUFAs suppress the formation of intestinal tumors in mice and humans with a defective APC allele (36, 218). The downstream APC signaling oncogenic transcription factor MYC is an important regulator of cell proliferation, and the lack of MYC expression is associated with a reduced number of intestinal adenomas (156). Interestingly, patients with an amplified *MYC* gene and wild-type p53 have a greater response to anticancer therapies (7). In colon cancer cells, DHA increases the level of MYC, which is believed to induce a chemoprotective, proapoptotic phenotype (20).

DHA can inhibit NF-κB activity (217). This is relevant because NF-κB mediates signaling pathways that control the transcriptional activation of genes important for the regulation of many cellular processes and is aberrantly activated in many types of cancer (130, 138). n-3 PUFA treatment inhibits the expression and activity of NF-κB in many cell types; however, the exact mechanism is not fully understood (197). This has implications in chronic disease management because the DHA-mediated decrease in NF-κB activity has been shown to sensitize tumor cells to gamma-irradiation and promote the induction of apoptosis (227).

Curcumin also has been shown to inhibit cell proliferation, invasion, migration, angiogenesis, and inflammation and to induce cell cycle arrest and apoptosis in various cancers, such as breast, cervical, oral, gastric, melanoma, pancreatic, colon, and prostate (2, 74, 96). It exhibits its anticancer effects in part by regulating genes involved in cellular signaling pathways, including NF-κB, protein kinase B (Akt), mitogen-activated protein kinase, p53, and other pathways (152). Curcumin inhibits gene expression of epidermal growth factor receptor (EGFR) in human colon cancer cells (32) and also binds to the active sites of 5-lipoxygenase and COX-2 and inhibits their activity (169). It is noteworthy that curcumin modulates COX-2 and inducible nitric oxide synthase (163), similar to the effect induced by synthetic nonsteroidal anti-inflammatory drugs but without the side effects associated with these pharmaceutical agents (60).

EFFECTS OF n-3 PUFA, FIBER, AND CURCUMIN ON NONCODING MICRORNAs

High-throughput noncoding microRNA (miRNA) profiling studies have linked aberrant expression of miRNAs to the development of colon cancer (159, 196). For example, miR-21, a well-described “oncogenic” miRNA, is positively correlated with colorectal cancer metastasis (195). Elevated expression of miR-21 has been reported in breast (223), glioblastoma (67), pancreatic (140), and colon (9, 45, 216) cancers. The antiapoptotic properties of this miRNA target several tumor suppressors, including PTEN, PDCD4, BCL2, TIMP3, TGFβR2, SPRY3, and RECK (9, 120, 195, 216). Additionally, dysregulation of miRNA editing has been linked to aberrant EGFR signaling, which interacts with argonaute 2, thereby perturbing miRNA processing from precursor to mature miRNAs (48, 193). The fact that DHA antagonizes EGFR (209), which can modulate miRNA maturation through

phosphorylation of argonaute 2 (48, 193), implicates a potential regulatory molecular mechanism involving fish oil and miRNAs. This epigenetic mechanism of action highlights a potential regulation of miRNAs by bioactive dietary components, and further study is needed to validate it.

The effects of n-3 PUFA on carcinogen-induced miRNA expression profiles during the early stages of colon tumorigenesis in rat colonic mucosa have been previously examined (44–46). The data indicate that translational alterations are far more extensive relative to transcriptional alterations in mediating malignant transformation. In contrast, changes in transcription are more extensive relative to translation in mediating the effects of diet. Therefore, during the early stage of colonic neoplasia, diet and carcinogen appear to predominantly regulate gene expression at multiple levels via unique mechanisms. Table 1 summarizes specific miRNAs modulated by a fish oil diet with respect to their validated mRNA target genes. miR-18a, miR-19b, miR-27b, miR-93, and miR-497 exhibited a coherent response at the total and polysomal mRNA levels by fish oil feeding. miR-132, miR-146b, miR-192, miR-206, and miR-218 were increased by fish oil feeding, with inverse relationships with their target genes. Specifically, azoxymethane treatment did not affect let-7d, miR-15b, miR-107, miR-191, and miR-324-5p expression in the fish-oil-fed animals. DHA in human colorectal adenocarcinoma cells modulated miR-141-3p, miR-221-3p, miR-192, miR-30c, miR-1283, let-7f, miR-181a, and miR-1 (72). Furthermore, DHA treatment of gastric cancer cells increased miR-15b and miR-16, resulting in a downregulation of Bcl-2 and the induction of apoptosis (202).

A diverse population of microbiota colonizes the human gastrointestinal tract. The colon contains approximately 10^{11} cells per gram of contents, representing the densest population of microbes in the healthy adult (51). These predominantly anaerobic microbial populations are metabolically active, producing many metabolites that can exert both protective and pathogenic effects on the host. Some protective metabolites produced by the microbiota include short-chain fatty acids, such as acetate, butyrate, and propionate. A range of gut microbial structural components and metabolites directly interacts with host intestinal cells and stroma to influence nutrient uptake and epithelial resilience (75). For example, we and others have proposed that n-3 PUFA and butyrate, a four-carbon short-chain fatty acid produced during anaerobic fermentation of dietary fiber by endogenous bacteria in the colon, interact to profoundly suppress colon cancer (30, 110, 111). Dietary fish oil and the fermentable fiber pectin can act synergistically to protect against colon carcinogenesis primarily by enhancing apoptosis (110) (Figure 3). Table 2 summarizes animal and cell-line studies examining the synergism between n-3 PUFA and pectin or its fermentation product, butyrate.

The effects of dietary fish oil and pectin on miRNA expression during the early stages of colon tumorigenesis in preclinical models have been examined (46, 190). Table 1 summarizes specific miRNAs modulated by a combination fish oil and pectin diet with respect to their validated mRNA target genes. Surprisingly, miR-21 was decreased by the combination diet compared to the control diet (189, 190). This is noteworthy because, as previously noted, miR-21 is a well-known oncogenic miRNA, and its validated targets, PDCD4 and PTEN, are known tumor suppressor genes (9, 135, 229). Compared to the fish

oil diet, miR-26b, miR-30b, miR-98, miR-130b, miR-182, miR-200c, and miR-203 were uniquely increased by fish oil and pectin combination feeding. Their validated targets in Table 1 are known to promote tumorigenesis.

In colon cancer, curcumin has been shown to modulate tumor suppressor genes and transcription factors. In RKO and HCT116 colon cancer cells, the expression of miR-21, which correlated with the inhibition of activator protein-1 binding to its promoter, was reduced following treatment with curcumin. Consequently, cell proliferation, tumor growth, invasion, and in vivo metastasis were suppressed while the expression of the tumor suppressor PDCD4, a target of miR-21, was upregulated (141). In another study, curcumin induced cell cycle arrest and apoptosis in TE-7 human esophageal cancer cells through downregulation of Notch-1-specific miR-21 and miR-34a and upregulation of tumor suppressor let-7a (200). Curcumin also inhibited the growth of RKO and SW480 colon cancer cells through induction of reactive oxygen species and repression of specificity protein (Sp) transcription factors through downregulation of miR-27a, miR-20a, and miR-17. These miRNAs regulate the Sp repressors zinc finger and BTB domain-containing proteins 4 and 10 (ZBTB4 and ZBTB10) (65). This regulation has important implications because Sp proteins are transcription factors that regulate genes involved in cell death and angiogenesis and are often overexpressed in tumors (1, 24). Moreover, curcumin is known to modulate DNA methylation in colorectal cancer cells (122), and recent advances in microarray and sequencing technologies have reported miRNA genes that are silenced by methylation in cancer (126). It has also been suggested that DHA enhances cell permissiveness to curcumin uptake (4, 137). Therefore, a diet containing both n-3 PUFA and curcumin may suppress colon cancer by acting on different molecular targets. An interesting future frontier will be the pursuit of epigenetic molecular complexes targeted by a combination of pleiotropic chemoprotective bioactive dietary compounds. This approach will likely avoid problems commonly associated with drug rejection.

Adult stem cells of the colon are of particular interest because they sustain self-renewal and are targets for cancer-initiating mutations (133, 220). Perturbations in adult stem cell dynamics are generally believed to represent an early step in colon tumorigenesis. Recently, several studies have demonstrated the role of miRNAs in the maintenance of colon cancer stem cells. For example, repression of the translation of select miRNAs in stem cells and differentiated daughter cells has been shown to be a means of regulating stem cell renewal and differentiation (56, 66, 94). Although evidence supports the beneficial effects of certain dietary components on suppression of colon cancer cells (46, 190), a comprehensive, comparative analysis of the effects of these dietary agents on colonic stem cells has not yet been conducted (189).

n-3 PUFA, DNA METHYLATION, AND HISTONE MODIFICATIONS

In addition to regulating transcriptional machinery and the expression of miRNAs, dietary bioactives can also exert chemoprotective effects through DNA modification. Genes are silenced by covalently adding a methyl group to cytosine, typically occurring in CpG dinucleotide islands and island shores (89). Typically, in the context of cancer, hypermethylation occurs at tumor suppressors, whereas hypomethylation occurs at tumor

promoters. Another possibility of change in methylation is the presence of alternative RNA transcripts, as shown for the PIP5K1A locus in colon cancer (89). Several studies have shown that fish oil modulates the DNA methylation status of gene promoters. For example, in a high-fat-diet study of mice, fish oil supplementation abolished the decrease in *Pparg2* promoter methylation in skeletal muscle, thereby suppressing the increase in *Pparg2* expression (5). Another study has shown that EPA can decrease methylation at the CCAAT/enhancer-binding protein promoter for C/EBPβ and C/EBPδ to increase their expression in U937 leukemia cells (23). Interestingly, we have demonstrated that n-3 PUFA/DHA and pectin/butyrate reduce cancer risk in part via changes in the promoter methylation state of apoptosis-related genes, leading to induction of proapoptotic gene expression and increased apoptosis in colonocytes (33, 34). Similar to results in mouse models, in clinical studies n-3 PUFA has been shown to influence DNA methylation. In one study of the Yup'ik Alaska Natives, DNA methylation of 27 CpG sites was differentially regulated by n-3 PUFA (10). These findings highlight the effects of n-3 PUFA on regulating gene expression through DNA methylation in both preclinical and clinical studies, although more research is required to pinpoint the exact mechanisms by which n-3 PUFA alters the DNA methylation landscape.

Another epigenetic modification that can regulate gene expression is histone modification, which is composed of an array of posttranslational alterations of the histone tail that is associated with active and repressed gene expression. For example, histone H3 lysine 4 trimethylation is associated with active gene transcription, whereas H3 lysine 9 trimethylation is associated with repressed gene expression (18). We have demonstrated using rat colonic epithelial cells that there are differences in histone modification and proto-oncogene expression between proximal and distal colon (208). Studies are in progress to determine the effect of dietary fat and fiber composition on global histone posttranslational epigenetic programming in preclinical models of colon cancer.

The interaction of dietary fat and fiber-derived compounds in the colonic lumen can substantially impact the metabolism and kinetics of the colon epithelial cell population and suppress inflammation and neoplasia (29, 109, 210). For example, butyrate, formed via fiber fermentation, has pleiotropic effects in the colon (49, 81), acting as a principal energy source and a survival factor for normal colon cells while exerting antiproliferative, differentiation-inducing, and apoptosis-inducing effects in cancer cells (21). In addition to the regulation of basic cytokinetic processes, butyrate has also been shown to affect cell adhesion, morphology, invasiveness, metastasis, oxidative metabolism, and angiogenesis, as well as the activity of different enzymes and transcription factors. These effects are attributed in part to the function of butyrate as a histone deacetylase inhibitor, which mechanistically links it to gene expression (183).

ADDITIONAL MECHANISMS BY WHICH NUTRITIONAL BIOACTIVES EXERT CHEMOPROTECTIVE EFFECTS

The review thus far has focused on the binding of bioactive dietary compounds to transcription factors, or regulating transcription indirectly by regulating miRNA or DNA

methylation. However, n-3 PUFA and its metabolites, as well as other amphiphilic dietary compounds such as curcumin, can modulate the function of membrane-associated receptors and exert additional effects on oncogenic cellular signaling.

The cellular plasma membrane is a heterogeneous composition of lipids and proteins. These lipids have many different head groups (glycerophosphocholine versus sphingomyelin), tail lengths (16:0 versus 18:0), and saturation indices (16:0 versus 16:1). Although membrane proteins have many different characteristics, such as transmembrane, lipid anchoring, and outer versus inner leaflets, recent studies have highlighted that these lipids and proteins are not just randomly distributed but rather are exquisitely organized. In fact, receptor activation can even be controlled by local spatial heterogeneity in ligand concentrations (136). Ultimately, most signals are propagated by the formation of signaling membrane domains composed of lipid-lipid, lipid-protein, and protein-protein interactions of differing size scales, such as micrometer (immunological synapse) and nanometer (Ras-Raf) platforms.

One view is that these membrane proteins exist in a balance of clustered and nonclustered states. The composition and organization of the membrane as well as the influence of cytoskeletal protein-protein interactions can stabilize these domains, favoring a shift of equilibrium to a clustered state. Interestingly, clustered proteins are not always efficient signaling domains because these clusters must be arranged in a particular conformation and be composed of the appropriate binding partners to propagate the signal (12, 132, 144). These membrane domains can be stabilized by transbilayer interactions between outer and inner leaflet lipids as well as the cytoskeleton (168).

Multidrug-resistance cancer cells have unique plasma membrane lipid composition and organization, specifically increased sphingomyelin and rigidity (213, 224). Increased rigidity is associated with increased lipid rafts, which typically facilitate efficient cellular signaling. It appears that these multidrug-resistance cells have remodeled their membrane to be in a state that is more receptive to activation. The plasma membrane, which is considered the outermost part of the cell, must receive and process extracellular signals. Because n-3 PUFAs such as DHA and EPA are physically incorporated into membrane phospholipids (Figure 4*a*), their main effect may originate at the membrane. Other compounds, such as curcuminoids, however, are not a physical part of the membrane. Instead, these compounds can intercalate into the membrane, where they can influence lipid-lipid and lipid-protein interactions (Figure 4*b*) (88). Effects of small-molecule agonists and antagonists can typically be predicted by structure-activity relationships, highlighting binding regions between proteins and said molecule. Unfortunately, it is very difficult to predict the effects of membrane-targeted dietary bioactives because the interaction involves modeling lipid and protein biophysics.

A criticism related to the physiological effects of dietary agents is that the bioavailability of most bioactive dietary compounds is very low and therefore membrane enrichment is low. However, from the standpoint of membrane biophysics, even small mole percentage increases of compounds can have drastic effects on biophysical properties of membranes (134). The magnitude and directionality of these effects depend on biophysical properties of the compounds themselves as well as those of the membrane. The colon is unique because

many compounds that are not “bioavailable” and, therefore, not found in circulation are still bathing the colonic epithelium from the luminal side. Therefore, as opposed to the blood pathway shown in Figure 4*a* for PUFA effects on cell membranes, curcumin acts through an intestinal pathway, with the curcuminoid bioactive, for the most part, escaping absorption in the small intestine and being delivered to the colon intact (90), where it can become incorporated into membranes of the colonic epithelium and exert its physiological effects (Figure 4*b*). In comparison, fermentable fiber acts through a microbial pathway (Figure 4*c*), being converted to its bioactive form, butyrate, by the action of intestinal microbiota in the lumen of the colon.

NOVEL TECHNIQUES TO STUDY NUTRIENT-GENE INTERACTION IN COLON CANCER

The intestinal epithelium is one of the most rapidly renewing tissues in vertebrate organisms. Self-renewal of the colonic epithelium is driven by the proliferation of stem cells and their progenitors located in crypts. In 2009, Sato et al. (182) established a long-term primary culture to generate epithelial organoids (enteroids) with crypt- and villus-like epithelial domains representing the complete census of progenitor and differentiated cells, mimicking the situation encountered by cells in the intestinal crypt niche (Figure 5). Later, they adapted the culture conditions to grow similar epithelial organoids from mouse colon and human small intestine and colon. The three-dimensional culture system of the native human colonic epithelium recapitulates the topological hierarchy of stem cell–driven tissue renewal, opening the methodological door for ex vivo studies designed to examine the effects of bioactive dietary compounds on colonic crypt metabolism (153).

From the perspective of membrane-derived bioactives, it has been shown that prostaglandin E_2 supports the growth of chicken embryo intestinal organoids in three-dimensional culture (160). Interestingly, we have provided evidence that exogenous prostaglandin E_3, derived from n-3 PUFA, has diminished ability to support colonic stem cell expansion in mouse colonic organoids relative to prostaglandin E_2, derived from n-6 PUFA, a known promoter of colon tumorigenesis (54, 191). The ability of bioactive compounds to alter colonic stem cell lineage and proliferation in this three-dimensional organoid culture system ex vivo strongly suggests that primary intestinal organoid cultures have widespread application for elucidating the molecular mechanisms of nutrient action on gut biology.

Evidence suggests that targeting cancer cell energy metabolism might be an effective therapeutic approach for selective ablation of malignancies. The Seahorse Extracellular Flux Analyzer is a novel platform designed to perform metabolic profiling of mitochondria, cells, or tissue. Using the Seahorse Analyzer, we have demonstrated that select bioactives known to affect gastrointestinal function and cancer risk can alter colonic mitochondrial function, both in vivo in crypts and in ex vivo organoid culture, by increasing respiration-induced proton leak, thereby inducing apoptosis, a marker of colon cancer risk (55).

The organoid culture system is applicable to basic and translational research. By using either CRISPR-Cas9 or lentivirus, Matano et al. (131) engineered diverse oncogenic mutations in organoids derived from normal colon, facilitated by selective culture conditions that

encouraged the maintenance of the mutations. During tumorigenesis, niche factors often become dispensable, leading to a less stringent culture condition for cancer organoids as compared to wild-type organoids. Established cancer organoids can be xenotransplanted to recapitulate histopathology of the parental tumor from which they are derived. Cancer organoids reflect genetic lesions and gene expression patterns, opening up the possibility of in vitro drug testing for the prediction of clinical treatment response in patients (181).

An elegant model for examining changes in the colonic epithelium due to various intervention therapies is the noninvasive monitoring of gene expression in exfoliated colonocytes. Our group has developed methodology to extract host mRNA from stool samples for the purpose of RNA sequencing to examine gene expression profiles of the host organism (107). Indeed, we have demonstrated that the effects of chemotherapeutic diets on epithelial cell expression can be monitored noninvasively throughout the tumorigenic process (33). Because the sample is easily acquired, repeated sampling over time or after repeated treatments is possible, such as in a crossover intervention study. With this method, conflation of host transcriptome profiles with changes in microbiome can be achieved (50, 186).

FUTURE CHALLENGES

Although this review has focused on the pleiotropic mechanisms by which n-3 PUFA may be chemoprotective against colon cancer, two systematic reviews of n-3 PUFA on cancer risk qualitatively concluded that there is inadequate (129) or limited (70) evidence to suggest an association between long-chain n-3 PUFA intake and colorectal cancer risk. This discrepancy may be due to the source of n-3 PUFA, from fish oil, purified EPA, DHA, or a combination of the two n-3 PUFAs. This can result in variable ratios of EPA and DHA, and the administration of different doses. Consistent use of n-3 PUFA formulation, with particular attention to the ratio of EPA and DHA, as well as the standardization of the dose, may help resolve the discrepancy in the efficacy of n-3 PUFA in preventing colorectal cancer. Furthermore, work from our group suggests that the combined effects of n-3 PUFA in fish oils and highly fermentable fibers may act synergistically to enhance the chemopreventive potential, in part, by increasing apoptosis (Figure 3 and Table 2) (28, 33, 39, 109–111, 149, 177, 211). These results are in agreement with a recent prospective nested case-control study demonstrating significant colorectal cancer risk reduction among Seventh Day Adventist pescovegetarians, whose diets are high in both dietary fiber and n-3 PUFA-rich fish (HR: 0.57; 95% CI: 0.40, 0.82) (149). We postulate that the failure to address an interaction between dietary fat and fiber and their subtypes may explain why the interactive chemoprotective effects of n-3 PUFA and fermentable fiber have been obscured in prospective cohort studies.

Although this discussion has focused on n-3 PUFA and some of the nutrient-nutrient interactions, other diets may be chemoprotective against colorectal cancer as well. Indeed, the so-called Mediterranean diet, consisting of a high intake of vegetables, fruits, nuts, and olive oil, and a low consumption of red meat, may be beneficial in preventing colorectal cancer (174). It has been shown that Mediterranean countries have decreased colorectal cancer rates compared to Western countries (38, 205). Bioactive dietary molecules found in

vegetables and fruits, such as epigallocatechin gallate in green tea (222), proanthocyanidins in apples and cocoa beans (146), genistein in soybeans (166), and sulforaphane in cruciferous vegetables such as broccoli (64), have also emerged as bioactive dietary compounds that may exert chemoprotective effects against colon cancer (Figure 1). More research on these and other emerging compounds is required to fully elucidate their chemoprotective efficacy.

Butyrate and propionate, produced in the colon by fermentation of dietary fiber by microbes in the human colon, are histone deacetylase inhibitors, regulating host transcriptome at the epigenetic level (21, 183). Not surprisingly, diet can change the microbial population in the colon (43, 215), and recent studies have begun to profile the changes to the microbiota associated with n-3 PUFA intake (155, 226). The continued study of how dietary bioactive molecules alter the microbiota as a chemoprotective strategy is needed for a full understanding of the dietary-host-microbiome nexus.

The overwhelming majority of colorectal cancers are initiated by activating mutations/deletions in the Wnt pathway (13, 106, 171). From a physiological standpoint, the Wnt signaling pathway is essential for the maintenance of the intestinal stem cell niche (112, 113, 115, 162). Therefore, environmental factors, which are capable of modulating Wnt signaling, will likely have a unique and central role in the physiology and pathology of the intestinal stem cell. A growing body of literature supports the hypothesis that dietary bioactive compounds (e.g., n-3 PUFA, folate, fermentable fiber) can modulate Wnt signaling by suppressing colonocyte nuclear β-catenin levels (17, 62). Nonetheless, our understanding of the mechanisms by which nutrient-gene interaction influences stem cells and colon cancer is still poorly developed. Therefore, certain issues in regard to colonic stem cells need to be addressed. For example, are the stem cell number and location fixed? Do these change during malignant transformation? Can a chemoprotective diet influence these events? These questions and others proposed in this review highlight that the interplay between diet and chemoprevention has not been fully explored and that more questions remain to be addressed in order to fully understand how diet can be used in the prevention of colon cancer.

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Glossary

Apc	adenomatous polyposis coli gene
NOCs	*N*-nitroso compounds
COX-2	cyclooxygenase-2
PUFAs	polyunsaturated fatty acids
EPA	eicosapentaenoic acid

DHA	docosahexaenoic acid
PPARγ	proliferator-activated receptor gamma
PXR	pregnane X receptor
RXRα	retinoid X receptor alpha
LXRs	liver X receptors
NF-κB	nuclear factor-kappa B
STAT3	signal transducer and activator of transcription 3
ER	endoplasmic reticulum
PERK	protein kinase RNA-like endoplasmic reticulum kinase
EGFR	epidermal growth factor receptor
miRNA	microRNA
Sp	specificity protein

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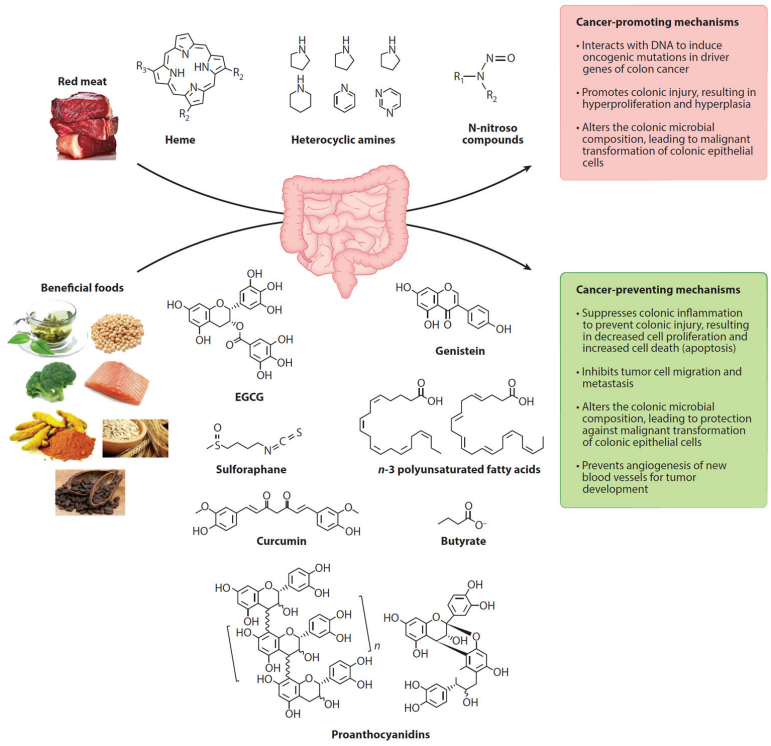

Figure 1.

Effects of diet on promoting and preventing colorectal cancer. Compounds found in the diet have been linked to the development of colorectal cancer through the induction of DNA adducts, leading to mutations in oncogenic genes and promoting hyperproliferation and hyperplasia. Compounds found in many fruits, vegetables, and fish can counteract cancer-promoting compounds in a pleiotropic manner and therefore should be incorporated in a healthy human diet. Abbreviation: EGCG, epigallocatechin gallate.

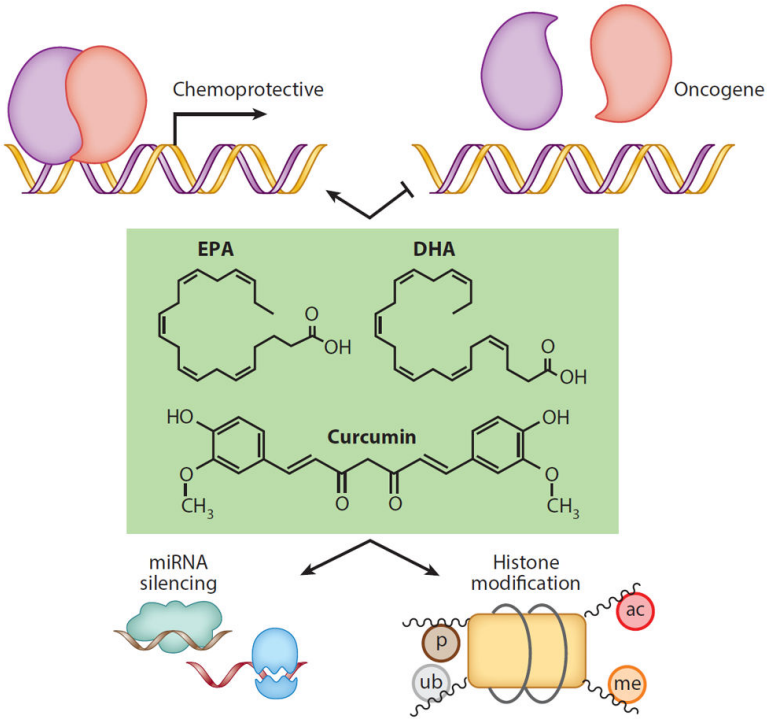

Figure 2.

Potential mechanisms by which bioactive dietary molecules mediate chemoprotective effects in the nucleus. Bioactive dietary molecules such as EPA, DHA, and curcumin can activate transcription factors that regulate chemoprotective genes or inhibit transcription factors that drive oncogenesis. These molecules can also affect miRNA silencing as well as alter posttranslational modification of histones. Abbreviations: ac, histone acetylation; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; me, histone methylation; miRNA, microRNA; p, histone phosphorylation; ub, histone ubiquitination.

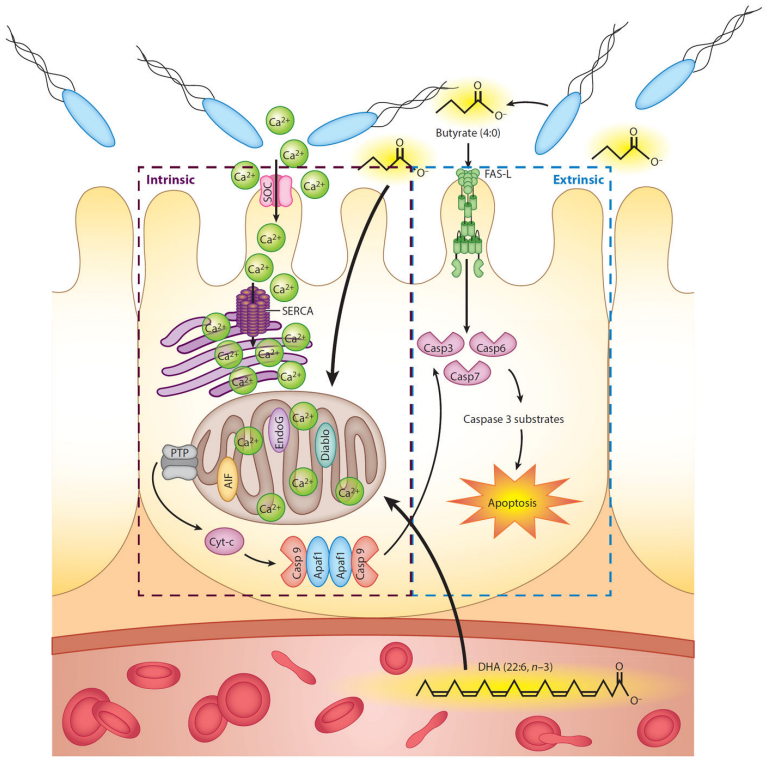

Figure 3.

Proposed mechanisms by which the interaction of dietary long-chain n-3 polyunsaturated fatty acids from fish oil and butyrate from bacterial fermentation of dietary fiber may reduce colon tumorigenesis. Butyrate induces colonocyte apoptosis via a nonmitochondrial, Fas-mediated, extrinsic pathway. Docosahexaenoic acid (DHA) and butyrate, in combination, synergistically perturb intracellular Ca^{2+}, stimulating mitochondrial Ca^{2+} uptake. This directly or indirectly decreases cytosolic Ca^{2+} and promotes store-operated channel (SOC)-mediated entry via plasma membrane channels. Mitochondrial Ca^{2+} accumulation subsequently triggers the opening of the permeability transition pore (PTP) and release of proapoptotic molecules, such as cytochrome c, and other factors, such as apoptosis-inducing factor (AIF) and second mitochondrial activator of caspases (smac/Diablo). Together, these effects culminate in the induction of procaspases and downstream caspases (Casp) that execute cellular apoptosis. Abbreviations: Apaf1, apoptotic protease-activating factor 1; Cyt-c, cytochrome c; EndoG, endonuclease G; SERCA, sarcoendoplasmic reticulum Ca^{2+} ATPase.

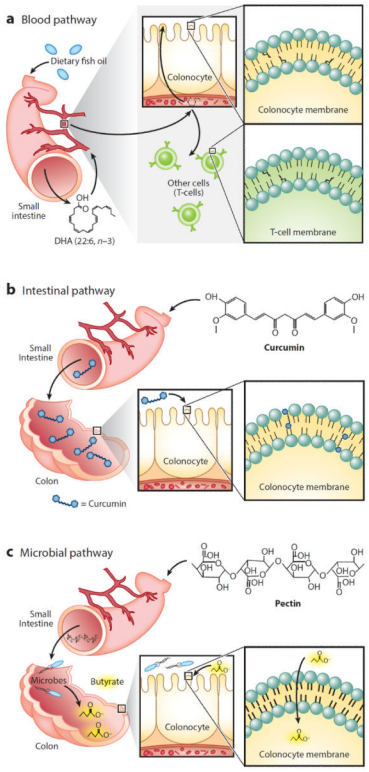

Figure 4.

Multiple pathways for the delivery of dietary bioactives to colonocytes. (*a*) Blood pathway. Polyunsaturated fatty acids are delivered to colonocytes and other cell types, such as T cells, through the bloodstream after digestion and absorption from the small intestine into the portal vein. In the colonocyte, the fatty acids are incorporated into phospholipids in the plasma membrane. Abbreviation: DHA, docosahexaenoic acid. (*b*) Intestinal pathway. Curcumin is poorly bioavailable and hence is transported, intact, to the colon, where it can intercalate between phospholipids in the plasma membrane of colonocytes. (*c*) Microbial pathway. Pectin is not digestible by human enzymes and therefore transits to the colon, where gut microbes ferment it to produce butyrate, which is rapidly taken up by colonocytes.

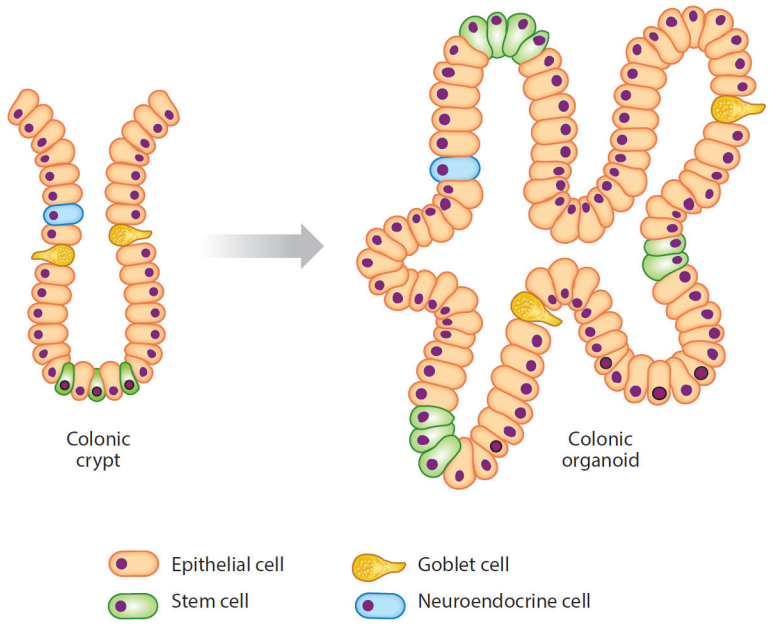

Figure 5.

Culturing of colonic organoids. Colonic crypts, isolated from the colon by ethylenediamine tetra-acetic acid (EDTA) treatment, are cultured in Matrigel 3D matrix in media containing a mixture of recombinant growth factors. All cell lineages in the crypt are recapitulated in the developing organoid.

Table 1

miRNAs differentially regulated by DHA, fiber, or curcumin in the colon

Bioactive component	Differentially regulated genes	Validated mRNA targets	Cell line/organism	Target mRNA-involved pathway	Reference
n-3 PUFA	*miR-18a*	Runx1	Rat colon	ERK-MAPK, Wnt/β-catenin, PTEN, apoptosis, EMT	190
	miR-19b	Arid4b, Arpc3, Hipk3			
	miR-27b	Cxcl12, Runx1			
	miR-93	Sqstm1, Stat3, Vegfa			
	miR-497	Bcl2, Bcl2ls, Eln			
	miR-132	Arhgap32, Btg2, Ep300, Foxo3, Kdm5a, Mecp2, Mmp9, Nr4a2, Paip2, Ptbp2			
	miR-146b	Nfkb1, Sirt1			
	miR-192	Zeb2			
	miR-206	Bdnf, Fstl1, Gja1, Hdac4, Hhip, Id1, Id2, Id3, Igsf5, Mmd, Notch3, Pax7, Pola1, Ptplad1, Spry1, Timp3, Utrn			
	miR-218	Onecut2			
	let-7d	Sept3			46
	miR-15b	Bcl2, Ccne1			
	miR-107	Bace1, Serbp1			
	miR-191	Mxi1, Riok3			
	miR-324	Smo			
	miR-1	Calm1, Calm2, Gata4, Hdac4, Igf1, Mef2a	Human colorectal adenocarcinoma cells	EMT, cancer cell migration, invasion, tumor suppressor	72
	miR-30c	Ccne2, Celsr3, Egfr, HSPA4, Mdm2, Mtdh, Runx1, Smad1, Snai1, Timp3, Twf1, Vimentine			
	miR-141	Cdh11, Dlx5, Elavl4, Klf5, Mapk14, PTEN, Slc25a3, Stk3, Zeb1, Zeb2			
	miR-181a	Acvr2a, Bcl2, Cbx7, Cd69, Egr1, Gata6, Hoxa, Msx2, Rgs4, Runx1, Tbr1, Tcra, Tgfbi, Tox			
	miR-192	Zeb2			
	miR-221	Arnt, Bnip3, Cdkn1b, Ddit4, Kit, Psmd9, Timp3			
	miR-1283				
	let-7f				
	miR-15b	Arl2, Bcl2l2, Dedd	Human gastric cancer cells		202
	miR-16	App, Arl2, Bcl2, Bcl2l2, Cadm1, Ccnd1, Ccne1, Ccnt2, Cd40, Fgf2, Jag1, Jun, Mdm4, Slc6a4, Vegfa,Wnt3a			

Bioactive component	Differentially regulated genes	Validated mRNA targets	Cell line/organism	Target mRNA-involved pathway	Reference
n-3 PUFAs plus pectin	*miR-16*	Arl2, App, Bcl2, Ccnd1, Jag1, Jun, Mdm4, Vegfa, Wnt3a	Rat colon	Adenocarcinoma, mTOR, PI3K/AKT, apoptosis, EMT	190
	miR-21	Eif4e3, Fasl, Peli1, Pdcd4, Pten, Reck, Smad7, Spry1, Spry2, Tgfbr3, Tnfaip8l2, YOD1, Yy1			
	miR-26b	Lef1, PDE4B			
	miR-27b	Cxcl12, Mef2c, Pparg, Runx1, Smad3, Smad4, Smad5			
	miR-30b	Aicda, Snai1			
	miR-93	Sqstm1, Stat3, Vegfa, VSIVgp2			
	miR-98	Acvr1b, Il6, Mmp11			
	miR-130b	Meox2, Tgfb1			
	miR-182	Adcy6, Clic5, Fbxw7, Foxo3, Tbx1			
	miR-200c	Bmi1, Flt1, Mapk14, Nog, Reln, Sox2, Vldlr, Zeb1, Zeb2, Zfpm2			
	miR-203	Cav1, Ctnnb1, Rbm44, Snora62, Trp63, TCF4, Vcan, ZNF148			
	miR-206	Adar, Bdnf, Clcn3, Eif4e3, Fn1, Fstl1, Fzd7, Gja1, Hdac4, Hhip, Hmgb3, Id1, Id2, Id3, Igsf5, Meox2, Mmd, Nfat5, Notch3, Pax7, Pdcd10, Pola1, Ptplad1, Rarb, Sh3bgrl, Spry1, Smarcb1, Smarcd2, Timp3, Utrn			
Curcumin	*mir-21*	AP-1, Eif4e3, Fasl, Pdcd4, Peli1, Pten, Reck, Smad7, Spry1, Spry2, Tgfbr3, Tnfaip8l2, YOD1, Yy1	Human colorectal adenocarcinoma cells	Apoptosis, proliferation, inflammation, Wnt, EGFR	141
	mir-34a	Actb, Bcl2, Bcl6, Ccnd1, Dll1, Foxp1, Gas1, Notch-1, Pofut1, Ppp1r10, Sema4b, Sirt1, TGIF2, Trem2, Vcl, Vegfb	Human esophageal cancer cells		200
	mir-17	Specificity protein transcription factors, Bcl2l11, Mapk14, Rbl2, Sqstm1, Stat3, Tgfbr2	Human colon carcinoma cell		65
	mir-20a	App, Bmp4, Ep300, Fgf10, Hbp1, Mapk14, Osr1, Pten, Shox2, Sqstm1, Stat3, Tbx3, Tgfbr2, Trp53inp1, ULK1, Vegfa, Zbtb7a, Zfpm2			
	mir-27a	Alp, Bmp2, Bmpr1a, Creb1, Fbxw7, Il10, Odc1, Ppara, Pparg,			

Bioactive component	Differentially regulated genes	Validated mRNA targets	Cell line/organism	Target mRNA-involved pathway	Reference
		Prdm16, Runx1, Runx2, Smad9, Spp1, Srm, Tcirg1			

Abbreviations: EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ERK, extracellular-signal-regulated kinase; MAPK, mitogen-activated protein kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PUFAs, polyunsaturated fatty acids.

Table 2

n-3 Polyunsaturated fatty acids and butyrate act synergistically in the colon

Cell-line experiments			
Cell line	**Treatment and duration**	**End point**	**Reference**
YAMC	0–200 mM DHA for 72 hours plus 0–10 mM butyrate for the final 6–24 hours	Mitochondrial Ca^{2+} and apoptosis (nucleosomal fragmentation assay)	111
HCT-116	50 mM DHA for 72 hours plus 5 mM butyrate for the final 12 hours	Apoptosis (TUNEL assay)	110
Animal studies			
Animal	**Diet component (% by weight)**	**AOM (mg/kg body weight)**	**End point**
Rat	11.5% fish oil plus 6% pectin	15 mg/kg × 2 injections	miRNA quantification
Rat	11.5% fish oil plus 6% pectin	15 mg/kg × 2 injections	miRNA quantification
Mouse	11.5% fish oil plus 6% pectin	15 mg/kg × 4 injections	miRNA quantification, apoptosis, ACF

Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; DHA, docosahexaenoic acid; miRNA, microRNA.