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Chemoprotective Epigenetic Mechanisms in a Colorectal Cancer Model: Modulation by n-3 PUFA in Combination With Fermentable Fiber

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Abstract Colorectal cancer is the third major cause of cancer-related mortality in both men and women worldwide. The beneficial role of n-3 polyunsaturated fatty acids (PUFA) in preventing colon cancer is substantiated by experimental, epidemiological, and clinical data. From a mechanistic perspective, n-3 PUFA are pleiotropic and multifaceted with respect to their molecular mechanisms of action. For example, this class of dietary lipid uniquely modulates membrane and nuclear receptors, sensors/ion channels, and membrane structure/cytoskeletal function thereby regulating signaling processes that influence patterns of gene expression and cell phenotype. In addition, n-3 PUFA can synergize with other potential chemoprotective agents known to reprogram the chromatin landscape, such as the fermentable fiber product, butyrate. Nutri-epigenomics is an emerging field of research that is focused on the interaction between nutrition and epigenetics. Epigenetics refers to a group of heterogeneous processes that regulate transcription without changing the DNA coding sequence, ranging from DNA methylation to histone tail modifications and transcription factor activity. One implication of the nutri-epigenome is that it may be possible to reprogram epigenetic marks that are associated with increased disease risk by nutritional or lifestyle interventions. This review will focus on the nutri-epigenomic role of n-3 PUFA, particularly DHA, as well as the combinatorial effects of n-3 PUFA and fermentable fiber in relation to colon cancer.

Keywords Fish oil · Colon cancer · Nutri-epigenomics · Chemoprevention · Fermentable fiber · Docosahexaenoic acid

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Introduction

Over the past 25 years, hundreds of published papers have described the effects of polyunsaturated fatty acids (PUFA) on normal and cancer cell types, including differences between n-6 and n-3 PUFA with respect to their mechanisms of action [1–3, 4•]. From this body of work, there is now mounting evidence that n-3 PUFA, namely, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) found in fish and algal oils exert anti-inflammatory properties in the colon, enhance the efficacy of chemotherapeutic drugs, suppress chronic inflammatory biomarkers associated with obesity/diabetes, and reduce colon cancer risk [5–10]. The actions of n-3 PUFA appear to involve multiple mechanisms that link the cell membrane, cytosol, and the nucleus [4•, 11]. For example, n-3 PUFA modulate membrane and nuclear receptors and sensors/ion channels thereby regulating signaling processes that influence patterns of gene expression. These effects appear to be mediated, in part, via the incorporation of n-3 PUFA into cell membranes [4•, 12]. Moreover, these changes in membrane composition can affect membrane order, the formation of lipid rafts, and intracellular signaling processes [2].

With respect to the cell nucleus, nutri-epigenomics is an emerging field of research that is focused on the interaction between nutrition and the epigenome. Epigenetics refers to a group of heterogeneous processes that regulate transcription

without changing the DNA coding sequence. These changes include not only covalent histone modifications, principally acetylation and methylation of lysine residues, but also phosphorylation and ubiquitination, DNA methylation, transcriptional machinery, and noncoding RNA activities [13–15]. Epigenetic marks can exhibit plasticity throughout the life course, albeit to varying degrees, and can be modified by environmental factors including diet [16]. One implication of the interaction between the diet and the epigenome is that it may be possible to reprogram epigenetic marks that are associated with increased disease risk by nutritional or lifestyle interventions. This review will focus on the nutri-epigenomic role of n-3 PUFA, particularly DHA, in relation to colon cancer.

Direct n-3 PUFA Interaction With Nuclear Receptors

DHA and EPA and their oxidative metabolites have been shown to interact with specific ligand-dependent nuclear receptors including CAR, HNF4A, PPARG, PXR, and RXRA (Fig. 1) [17]. In this fashion, n-3 PUFA regulate the function of nuclear receptors and their impact on transcriptional processes. For example, DHA-bound PPARG can be transported to the nucleus where it controls energy balance by regulating fatty acid homeostasis in part via enhancing the expression of genes associated with membrane-bound fatty acid transporting proteins and β-oxidation of fatty acids in peroxisomes and mitochondria [18]. Interestingly, impaired expression and function of PPARG is associated with inflammatory bowel diseases (IBD) and colon cancer [19, 20]. RXRA, which is implicated in cancer chemoprevention, also preferentially binds to n-3 PUFA in colonocytes [21]. Activation of PPARG as well as heterodimers formed with RXR play an important role in the antitumor effects of n-3 PUFAs [19].

LXRs are transcriptional regulators of cholesterol metabolism that control cholesterol uptake into cells, catabolism, and efflux [22]. This is noteworthy because cholesterol can control cell proliferation, and disruptions in cholesterol metabolism have been associated with the development of colon cancer [23–25]. LXRs also function by heterodimerizing with RXRA

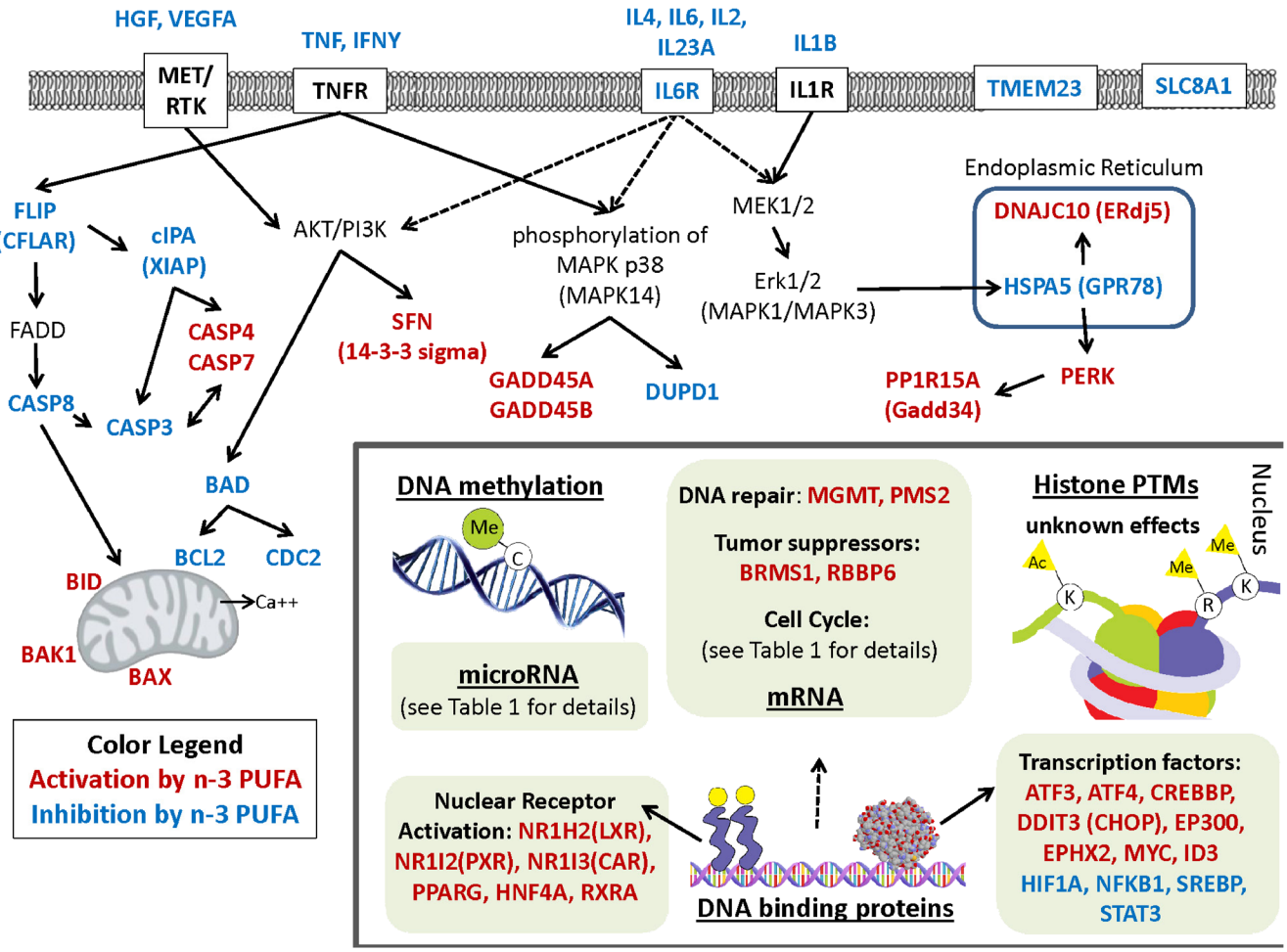

Fig. 1 Epigenetic effects of n-3 PUFA in the colon, intestinal genes that are up- or downregulated by n-3 PUFA at the mRNA and protein levels. *Red font* represents gene upregulation, and *blue font* indicates gene downregulation. Epigenetic levels of regulation in the nucleus are *underlined* Nuclear genes are grouped by classification

and binding to direct repeats with four nucleotide spacers (DR4 elements), termed LXR response elements (LXREs), in the promoter regions of target genes [22]. Interestingly, n-3 PUFA activated LXRα blocks proliferation of human colorectal cancer cells and slows the growth of xenograft tumors in mice [26].

PXR (NR1I2) has been shown to regulate the expression of genes involved in the oxidation, conjugation, and the transport of xenobiotics and promotes the metabolism, elimination, and detoxification of chemotherapeutic agents [27]. The transcription of PXR increases in the presence of n-3 PUFA [28]. This is noteworthy because PXR can suppress the proliferation and tumorigenicity of colon cancer cells [29]. CAR (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 [28].

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 [30, 31]. P1-, 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, could help slow colon cancer progression [32, 33].

Indirect DHA Regulation of Transcription Factors

Since the original description of dietary fat as a regulator of gene expression over 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 CREBBP, EP300, and MYC and decrease the activity of NF-kB (NFKB1) and STAT3 [17]. However, DHA does not directly bind to this class of transcription factors. With respect to colon cancer, DHA exhibits a protective suppressive effect against hyperactivated STAT3 and may reestablish the equilibrium between STAT3 and PPARG [34]. The ability to decrease STAT3 activity may be associated with the ability of n-3 PUFA ligands to trigger PPARG-RXR heterodimers to localize at their cognate PPAR response elements (PPREs) and exchange corepressors for coactivators such as cyclic AMP response element binding protein (CREB) and p300 [20].

The cytotoxic effects of DHA are also associated with signaling pathways involving lipid metabolism and endoplasmic reticulum (ER) stress. DHA induced depletion of free cholesterol in the ER can lead to ER stress, resulting in the growth arrest/apoptosis of metastatic tumor cells [35]. It has been suggested that these alterations in the sterol content of the ER by DHA mediate growth reduction partly by downregulating nuclear SREBP, an important manager of lipid homeostasis and cell growth regulation [36]. Induction of ER stress mediators by DHA also promotes expression of the kinase PERK, which in turn promotes translation of transcription factors ATF3, 4, and 6 [35]. 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 [37]. The experimental details associated with differentially expressed target genes are described in Table 1.

Colon adenocarcinomas exhibit defective expression of the adenomatous polyposis coli (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 [38]. From a chemoprevention perspective, in vivo studies demonstrate that fish oil-derived n-3 PUFA suppress the formation of intestinal tumors in mice and humans with a defective APC allele [39, 40]. The downstream APC signaling oncogene, MYC, is an important regulator of cell proliferation, and the lack of MYC expression is associated with a reduced number of intestinal adenomas [41]. Interestingly, patients with an amplified MYC gene and wild type p53 have a greater response to anticancer therapies [42]. In colon cancer cells, DHA increases the level of MYC, which is believed to induce a chemoprotective, proapoptotic phenotype [43].

NF-kB activity can be inhibited by DHA [44]. This is relevant because NF-kB 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 [45, 46]. n-3 PUFA treatment inhibits the expression and activity of NF-kB in many cell types; however, the exact mechanism is not fully understood [37]. This has implications in chronic disease management because the DHA-mediated decrease in NF-kB activity has been shown to sensitize tumor cells to gamma-irradiation and promote the induction of apoptosis [19].

DHA-mediated Modulation of Apoptosis Regulatory Pathways

It has been demonstrated that DHA contributes to the downregulation of BCL2, a well-known antiapoptotic molecule [47], which can block lipid peroxidation and thus apoptosis induction. Additionally, DHA induces caspase-dependent apoptosis in colon adenocarcinoma cells and adenoma cells [48]. There is also evidence of upregulation of CASP4 and CASP7 [35] along with increased activation of the intrinsic apoptotic pathway as demonstrated by CASP9 and Bid cleavage [48]. CASP4 activation may also be linked to augmented expression of ER resident factor ERdj5 and downregulation of antiapoptotic GRP78 [49]. The major involvement of the intrinsic apoptotic pathway following DHA treatment is through increased expression and activation of BAX and BAK [37], depolarization of the mitochondrial membrane, and the subsequent release of cytochrome c and Smac/Diablo into the

Table 1 Epigenetic studies examining the effects of n-3 PUFA and fermentable fiber on colonic gene expression

Studies documenting the effects of n-3 PUFA								
Upregulated								
Gene symbols	Reference	Assay type	Carcinogenesis method	Treatment method	Dose	Organism	Level	Cancer stage
FOXO3A,FAF1,BCL10,DFFA,TNFRSF1A, GADD45A, CASP7,MOAP1,DAP3,TNFRSF10B, GADD45B,CASP4, CIP1/P21/CDKN1A,CCNG2, SFN/14–3-3, PPP1R15A/GADD34,TRIB3	Slagsvold et al., 2010 [37]	Genome arrays	Colon adenocarcinoma cell line, SW620	In vitro	DHA 70 μmol/L	Human	RNA	Adenocarcinoma
ATF6,GCLC,OSBP,CAPN7,NPC2,VCP,SOD1,VLDLR, NRF2,XBP1,HSP47,CAPN2,CAMLG,ATF4,PERK, CASP7,PSMD1/RPN2,IP3R1,LDLR,TXNRD1, CASP4,GCLM, GADD34,ATF3,DNAJB1,NPC1, BAG3,HSPA1B,TRIB3, SQSTM1, HSPA1A/B, HMOX1	Jakobsen et al., 2008 [35]	Genome arrays	Colon adenocarcinoma cell line, SW620	In vitro	DHA 70 μmol/L	Human	RNA	Adenocarcinoma
ERDJ5,PERK	Fasano et al., 2012 [49]	Western blot	Colon adenocarcinoma cell line, SW480	In vitro	DHA 30 μmol/L	Human	Protein	Adenocarcinoma
BID,BAK,BAX	Giros et al., 2009 [50]	Western blot	Colon adenocarcinoma cell line, HT-29 and Caco-2	In vitro	DHA 60 μmol/L	Human	Protein	Adenocarcinoma
MYC	Calviello et al., 2005 [43]	Western blot	Colon adenocarcinoma cell line, HT-29 and LS-174	In vitro	DHA 10 μmol/L	Human	Protein	Adenocarcinoma
miR-18a, miR-27b, miR-93, miR-200c, miR-497	Shah et al., 2011 [11]	Low-density array	Azoxymethane (AOM) injection (15 mg/kg bw)	Diet	11.5 % fish oil (ad libitum)	Rat distal colon	RNA	Cancer progression
miR-30c, miR-141	Gil-Zamorano et al., 2014 [91]	q-PCR	Colon adenocarcinoma cell line, Caco-2	In vitro	DHA 200 μmol/L	Human	RNA	Adenocarcinoma
Downregulated								
CCND1,CCND3,CDK2,CDC42,CDC25C,CDC45L, CDC20, CDK4,E2F1,CENPE,AKT1/PKB,BAD, CCNA2,CCNF, CDC25B,TNFRSF1B,CDK2AP, AURKA,BUB1,CDC7, PCNA,BIK,BIRC5,BIRC5, UNG,CCNA2,CCNB2,STMN1, CDC2/CDK1, AURKB,PLK1,NFKB,CHOP	Slagsvold et al., 2010 [37]	Genome arrays	Colon adenocarcinoma cell line, SW620	In vitro	70 μmol/L	Human	RNA	Adenocarcinoma
FDPS,CAT,CAV1,DHCR7,DHCR24,PMVK,TM7SF2, CCND1,HMGCR,SREBP2	Jakobsen et al., 2008 [35]	Genome arrays	Colon adenocarcinoma cell line, SW621	In vitro	DHA 70 μmol/L	Human	RNA	Adenocarcinoma
GRP78	Fasano et al., 2012 [49]	Western blot	Colon adenocarcinoma cell line, SW480	In vitro	DHA 30 μmol/L	Human	Protein	Adenocarcinoma
IL2,IL4,IFNG,TNF,IL6,IL1B	Purasiri et al., 1994 [62]	ELISA	CRC patient serum	Supplement	50 % n-3 PUFA supplement	Human	Protein	Adenocarcinoma
XIAP,FLIP,BAD,BCL2,COX2	Giros et al., 2009 [50]	Western blot	Colon adenocarcinoma cell line, HT-29 and Caco-2	In vitro	DHA 60 μmol/L	Human	Protein	Adenocarcinoma
BIRC5,CTNNB1, MMP7,PPARD,VEGF	Calviello et al., 2007 [67]	Western blot	Colon adenocarcinoma cell line, HCT116 and SW480	In vitro	DHA 10 μmol/L	Human	Protein	Adenocarcinoma
miR-21	Shah et al., 2011 [11]	Low-density array	Azoxymethane (AOM) injection (15 mg/kg bw)	Diet	11.5 % fish oil (ad libitum)	Rat distal colon	RNA	Cancer progression

Table 1 (continued)

Studies documenting the effects of n-3 PUFA								
Upregulated								
Gene symbols	Reference	Assay type	Carcinogenesis method	Treatment method	Dose	Organism	Level	Cancer stage
Studies documenting the effects of n-3 PUFA-fermentable fiber combination								
Upregulated								
RBBP6,ID3,BRMS1,MTMR4,MGMT,PMS2	Cho et al., 2011 [80]	Genome arrays	Azoxymethane (AOM) injection (15 mg/kg bw)	Diet	11.5 % fish oil, 6 % pectin (ad libitum)	Rat distal colon	RNA	Tumor
miR-19b, miR-26b, miR-203	Shah et al., 2011 [11]	Low-density array	Azoxymethane (AOM) injection (15 mg/kg bw)	Diet	11.5 % fish oil, 6 % pectin (ad libitum)	Rat distal colon	RNA	Cancer progression
Downregulated								
HIPK2,FEM1B,SLC8A1,PTHR2,DUPD1,IL6R, MFN1, SMOC1,TMEM23,HGF,IL23A,STX1A, ADAM3,PPP1R7, CYP2S1,NRN1,MMP2,SNIP	Cho et al., 2011 [80]	Genome arrays	Azoxymethane (AOM) injection (15 mg/kg bw)	Diet	11.5 % fish oil (ad libitum)	Rat distal colon	RNA	Tumor
PGES2,CTNNB1,PPARD	Vanamala et al., 2008 [57]	Western blot	Azoxymethane (AOM) injection (15 mg/kg bw)	Diet	11.5 % fish oil (ad libitum)	Rat colon	Protein	Tumor

cytosol [50]. Once these factors are released from mitochondria, apoptosis is accelerated [51]. These findings have been confirmed both in vitro [52] as well as in vivo [53].

n-3 PUFA can also act as efficient modulators of both the level and activity of endogenous caspase inhibitors. For example, DHA and EPA decrease XIAP (an X-linked inhibitor of apoptosis protein) at both the protein and mRNA levels, which may in part explain their antineoplastic effects [50]. High XIAP expression correlates with poor clinical outcome and resistance to chemotherapy and radiotherapy in different colon cancer cell lines [50]. DHA also downregulates mRNA and protein levels of two other inhibitors of apoptosis, survivin (BIRC5), and livin (BIRC7) in cancer cells [37]. Furthermore, the immediate and dramatic downregulation of FLIP (CFLAR), a potent inhibitor of caspase-8 (CASP8) activation, appears to be linked to the induction of apoptosis in colon cancer cells following DHA and EPA supplementation [50].

DHA can inhibit the expression of antioxidant enzymes or deplete cells of antioxidants [54]. It has also been suggested that DHA may have anti-inflammatory/proapoptotic effects in colon cancer cell lines by inhibiting the expression and activity of a key rate-limiting cyclooxygenase enzyme, COX-2 [55]. This is noteworthy because COX-2 is often overexpressed in colon tumors and is able to confer a pro-inflammatory niche, which contributes to epithelial cell resistance to apoptosis [56, 57]. Activation of NF-kB and the PPAR-BCL2 feedback loop may control the life-death continuum in colon cells and has been associated with the expression of COX-2 [56]. Chemoprotective suppression of the activation of NF-kB by DHA reduces the production of pro-proliferative eicosanoids produced by COX-2 [58]. Moreover, DHA may suppress tumor cell growth directly by inhibition of the COX-2 derived metabolite, PGE_2, which stimulates cell proliferation and suppresses apoptosis [57]. However, it is possible that DHA may also act via mechanisms independent of COX-2 inhibition [59] because suppression of tumor growth also occurs in cell lines that do not express COX at the protein level. Moreover, the growth of these cells in culture and in nude mice is not affected by overexpression of COX-1 or COX-2 [60]. Additional DHA-dependent proapoptotic mechanisms impacting colon adenocarcinomas include the upregulation of several growth arrest DNA-damage-inducible proteins such as GADD445A and GADD45B, likely through the stimulation of p38 MAPK phosphorylation [37].

Modulation of Cytokines and Growth Factors

Cytokines, including IL1β, IL2, IL4, IFNγ, and TNFα increase in the early stages of carcinogenesis. n-3 PUFA suppression of NF-kB activity is at least partly responsible for the reduction in cytokine levels, including IL2, IL4, IFNγ, TNFα,

IL6, and IL1β [61, 62]. These cytokines (IL1β, TGFβ, TNFα, and IL6) further regulate transcription factor, e.g., HNF4A, function through modulation of proteosomal degradation, DNA binding affinity, transcriptional activity, and co-factor interaction [63•]. Thus, n-3 PUFA cytokine regulatory control can extend to ion transport, epithelial barrier function, and oxidative stress via effects on this transcription factor.

The protective role of n-3 PUFA can also be attributed to an increase in the expression of TGFβ through inhibition of the Akt pathway in intestinal epithelial cells [64] and fat-1 transgenic mice [65]. This is noteworthy because the reduction of TGFβ expression increases chemical-induced colon carcinogenesis [66]. Furthermore, both EPA and DHA decrease the growth of colon tumors by reducing VEGF and TNFα expression through inhibition of ERK1/2 phosphorylation and hypoxia-induced factor HIF1α protein expression [67].

Effects of DHA on the Cell Cycle

There is some evidence that DHA has a selective dose-dependent growth inhibitory effect on colon cancer but not normal colonic cells [68]. Several key genes involved in the regulation of both the G1 and G2 phases of the cell cycle are affected by DHA treatment in colon cancer. Generally, molecules involved in cell cycle progression, such as Cdc25c, Cdc25b, Cdc20, CDK1, CDK2, and cyclins D, A, and B, are downregulated [37] by DHA incubation as compared to control. In comparison, genes involved in cell cycle arrest such as cyclin-dependent kinase inhibitors (CDKN1A, CDKN1B, CDKN1C, CDKN2A) and stratifin are upregulated by DHA [69]. Some studies additionally show that activated PXR inhibits the proliferation and tumorigenicity of colon cancer cells by targeting the cell cycle at the G(0)/G(1) cell phase via modulation of the p21 (WAF1/CIP1) and E2F/Rb signaling pathways [29]. In addition, in some cell contexts, DHA induces cell cycle arrest and downregulates the nuclear form of sterol regulatory element-binding proteins (SREBP1 and 2) in colon cancer cell lines, indicating a possible relationship between disturbances in lipid homeostasis and cell cycle arrest [35, 36, 43]. While a large number of mechanisms are linked to DHA anti-proliferative effects in cancer, several reports have focused on whether p53 protein plays a role in DHA-induced growth inhibition. DHA inhibits the growth of p53-wildtype colon cell lines as well as of those with inactivating p53 mutations; thus, its action does not seem to be dependent on p53 status [43].

Optimal Chemoprevention: Interaction of DHA With Butyrate

It has been proposed by us and others that n-3 PUFA and butyrate (fiber fermentation product) interact in the colon to profoundly suppress colon cancer [70–72]. Interaction of dietary fiber-derived compounds in the colonic lumen can have a substantial impact on the metabolism and kinetics of the colon epithelial cell population and suppress inflammation and neoplasia [73–75]. For example, butyrate, a four-carbon short-chain fatty acid, is produced during anaerobic fermentation of dietary fiber by endogenous bacteria present in the colon. This agent has pleiotropic effects in the colon [76, 77]. It acts as a principal energy source and a survival factor for normal colon cells, whereas it exerts antiproliferative and differentiation- and apoptosis-inducing effects in cancer cells [78]. In addition to the regulation of basic cytokinetic processes, butyrate has also been shown to affect cell adhesion, morphology, invasiveness, metastasis, oxidative metabolism, angiogenesis, and the activity of different enzymes and transcription factors. These effects are linked in part to butyrate's function as a histone deacetylase inhibitor, which mechanistically links it to gene expression [79].

Studies published by our group describe the protective effects of fish oil containing DHA, compared to corn oil and its interaction with fiber using rat and mouse model colon carcinogenesis models [2, 6]. These data demonstrate that the combination of n-3 PUFA and butyrate (fermentable fiber) treatment maximally enhances cell cycle arrest, by inhibiting expression of cell cycle genes (Table 1), shifting the balance between differentiation and apoptosis depending on the cell transformation status of the model [75, 80, 81]. These findings demonstrate that dietary n-3 PUFA and fermentable fiber can act synergistically to protect against colon carcinogenesis primarily by enhancing the deletion of DNA-damaged cells [57, 71, 72, 82, 83].

Temporal gene expression profiles from exfoliated rat colonocytes have revealed at the cancer initiation stage that fish oil plus fermentable fiber (FO/F) downregulates the expression of genes involved with cell adhesion and enhances apoptosis compared to the non-chemoprotective control of corn oil plus cellulose (CO/C) [80]. In addition, at the cancer progression stage, the expression of genes involved in cell cycle promotion is downregulated while DNA mismatch repair genes, MGMT and PMS2, are upregulated. FO/F also increases apoptosis and the expression of genes that promote apoptosis at the tumor stage [80]. The chemoprotective gene profiles at the tumor stage include the upregulation of the proapoptotic inhibitor of DNA binding ID3 and tumor suppressors BRMS1 and RBBP6, downregulation of antiapoptotic genes HGF and TMMEM23, and downregulation of cytokine signaling, IL23A and receptor IL6RA [80]. Signal transduction-related genes such as MAPK, DUPD1 and PPP1R7, and calcium signaling receptor SLC8A1 were also downregulated [80]. In addition, the chemotherapeutic effect of the FO/F dietary extends to translational activation of the xenobiotic metabolizing phase I enzyme EPHX2 and tumor suppressor retinoblastoma-associated protein RB1. These novel findings demonstrate that the effects of the

chemotherapeutic (FO/F) diet on epithelial cell gene expression can be monitored noninvasively throughout the tumorigenic process by analysis of exfoliated colonocytes.

Combinatorial Effects of n-3 PUFA and Fermentable Fiber on Non-Coding microRNAs

High throughput microRNA (miRNA) profiling studies have linked aberrant expression of miRNAs to the development of colon cancer [84, 85]. Dysregulation of miRNA editing has been linked to aberrant epidermal growth factor receptor (EGFR) signaling, which interacts with argonaute 2 (AGO2), thereby perturbing miRNA processing from precursor to mature miRNAs [86, 87]. The fact that DHA antagonizes EGFR in cancer cells by increasing receptor internalization and degradation [88] implicates a potential regulatory molecular mechanism involving fish oil and miRNAs. Further study is needed to validate this epigenetic mechanism of action.

Recently, the effects of colon carcinogen and the combination of dietary fish oil and fermentable fiber (pectin) on rodent microRNA expression during the early stages of colon tumorigenesis have been examined [11, 89]. miRNAs modulated by fish oil in colon cancer in both human and rodent models have also been reported [11, 89, 90•]. Specific miRNAs influenced by fish oil treatment or the highly chemoprotective combination of fish oil and pectin diet are summarized with respect to their validated mRNA target genes in Table 1. miR-18a, miR-27b, miR-30c, miR-93, miR-141, miR-200c, and miR-497 were increased by fish oil feeding, while miR-21 was decreased compared to control diet [91]. This is noteworthy because miR-21 is a well-known "oncogenic" miRNA, and its validated targets PDCD4 and PTEN are known tumor suppressor genes [92–94]. In comparison, miR-19b, miR-26b, and miR-203 were increased by fish oil and pectin combination feeding compared to control diet (corn oil plus cellulose diet) [11]. This is noteworthy because their validated targets, HIPK3, ARID4B, ARPC3, LEF1, RUNX1, CXCL12, TRP63, and ZFP281, are known to promote tumorigenesis.

Conclusion

n-3 PUFA are an ideal colon cancer chemotherapeutic because (1) they are toxicologically innocuous and free of safety problems intrinsic to drugs administered over long periods of time, (2) they are relatively inexpensive, and (3) they provide additional health benefits, such as reduction in mortality [40, 95, 96]. In addition, the ingestion of n-3 PUFA with other agents such as fermentable fiber and curcumin may improve their efficacy in colon cancer prevention/therapy [97–99].

From an epigenetic perspective, there is still much to be discovered in terms of the effects of n-3 PUFA in the colon at the chromatin state level. From a chemoprevention perspective, not only can dietary choices modify the epigenome but also intimate knowledge of the mechanisms involved could help tailor nutritional intervention to specific individuals. Along these lines, recent work has begun to focus on n-3 PUFA effects on DNA methylation with respect to colon cancer risk [100•, 101]. Although a substantial body of work exists regarding the effects of n-3 PUFA on cytokines and the resolution of chronic inflammation, studies addressing the specifics of these effects in terms of colon cancer cells are limited [61, 62, 64, 67]. In the future, personalized chemoprevention will be based on individual nutritional requirements and susceptibility to disease, including anatomical considerations such as differences in proximal versus distal colonic tumorigenesis [15, 102].

As discussed in this review, the breadth of n-3 PUFA effects on epigenetic regulation in colon cancer is wide and complex. As described in Table 1 and furthermore illustrated in Fig. 1, a wide array of potential pathways, molecular interactions, and mechanisms are modulated by n-3 PUFA. An interesting future frontier will be the pursuit of epigenetic molecular complexes targeted by chemoprotective n-3 PUFA in combination with fiber.

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Compliance with Ethics Guidelines

Conflict of Interest Karen Triff, Eunjoo Kim, and Robert S. Chapkin declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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