Published in final edited form as:
Curr Colorectal Cancer Rep. 2014 December 1; 10(4): 442–452. doi:10.1007/s11888-014-0241-6.

Mechanisms by Which Pleiotropic Amphiphilic *n*–3 PUFA Reduce Colon Cancer Risk

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Abstract

Colorectal cancer is one of the major causes of cancer-related mortality in both men and women worldwide. Genetic susceptibility and diet are primary determinants of cancer risk and tumor behavior. Experimental, epidemiological, and clinical data substantiate the beneficial role of *n*–3 polyunsaturated fatty acids (PUFA) in preventing chronic inflammation and colon cancer. From a mechanistic perspective, *n*–3 PUFA are pleiotropic and multifaceted with respect to their

Compliance with Ethics Guidelines

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.

Conflict of Interest Robert S. Chapkin, Vanessa DeClercq, Eunjoo Kim, Natividad Roberto Fuentes, and Yang-Yi Fan declare that they have no conflict of interest.

molecular mechanisms of action. For example, this class of dietary lipid uniquely alters membrane structure/ cytoskeletal function, impacting membrane receptor function and downstream signaling cascades, including gene expression profiles and cell phenotype. In addition, *n*–3 PUFA can synergize with other potential anti-tumor agents, such as fermentable fiber and curcumin. With the rising prevalence of diet-induced obesity, there is also an urgent need to elucidate the link between chronic inflammation in adipose tissue and colon cancer risk in obesity. In this review, we will summarize recent developments linking *n*–3 PUFA intake, membrane alterations, epigenetic modulation, and effects on obesity-associated colon cancer risk.

Keywords

(*n*–3) PUFA; Colon cancer; Membrane rafts; Cytoskeleton; Epigenetics; Obesity

Introduction

Colon cancer is a major public health concern due to the high prevalence of the disease both globally and in the USA [1]. Colon cancer is third in cancer incidence in both men and women and the second leading overall cause of cancer mortality [2]. Epidemiological and migrant population studies have indicated that environmental factors can influence cancer risk [3]. With respect to the focus of this review, diet is considered a key environmental factor impacting colon cancer risk [4, 5]. This is highly relevant because clinical practitioners are currently searching for toxicologically innocuous cancer chemoprevention approaches that are free of safety problems intrinsic to drugs administered over long periods of time. Since a large body of evidence supports the safety and efficacy of dietary or supplemental *n–3 polyunsaturated fatty acids* (PUFA), e.g., docosahexaenoic acid (*DHA*, $22{:}6^{\Delta 4,7,10,13,16,19}$) and eicosapentaenoic acid (*EPA*, $20{:}5^{\Delta 5,8,11,14,17}$), we propose that *n*–3 PUFA are ideally suited to reduce colon cancer risk. Dietary lipids can alter the cell membrane and tissue fatty acid levels, regulating multiple signaling events, thereby modulating the development of colorectal cancer. It has been shown that tissue fatty acid distribution is related to the incidence of colorectal cancer prognosis [6], with risk increasing by 1–3-fold with respect to *n*–6 PUFA content. In contrast, risk is reduced by 37–87 % with increasing *n*–3 PUFA content in colorectal cancerous tissue.

DHA and EPA are enriched in marine fish oil. DHA is a primary structural component of the human brain, cerebral cortex, skin, sperm, testicles, and retina [7]. The parent substrate, α-linolenic acid (18:3*n*–3; ALA), is found in plant oils, but conversion to EPA and DHA in humans from ALA is low and humans have no other means of synthesizing *n*–3 PUFAs. Therefore, EPA and DHA can be classified as “essential” nutrients.

Dietary administration of *n*–3 PUFA in rodent models of colon carcinogenesis has been demonstrated to reduce colon tumor size and multiplicity, compatible with its chemopreventative activity [8–10]. *n*–3 PUFAs have multiple targets implicated in various stages of cancer development, including cell proliferation, cell survival, angiogenesis, inflammation, metastasis, and epigenetic abnormalities that are crucial to the onset and progression of cancer [11]. In this review, we will focus on three unique aspects of cell

signaling modulation by *n*–3 PUFA: membrane alterations, epigenetic modulation, and impact on obesity-related colon cancer.

n–3 PUFA Effects on Membrane Structure/Signaling

Membrane Lipid Rafts

Cellular membranes are composed of a heterogeneous mixture of lipids and proteins, whose distinct order maintains efficient signal transduction. Membrane lipids can undergo phase separations and interact selectively with membrane proteins and with sub-membrane cytoskeletal elements [12]. Lipid rafts are dynamic and small (10–200 nm) membrane microdomains enriched in sphingolipids and cholesterol, which function as sorting platforms for many membrane-associated proteins [13]. Stabilization of these domains is hypothesized to be maintained by lipid and cytoskeletal influences [14•]. Recent evidence suggests that lipid rafts may modulate the malignant transformation process. For example, the levels of lipid rafts are increased in many types of cancer [15–17]. Additionally, lipid rafts mediate cell signaling events that are often constitutively or hyper-activated in cancer [18–20]. There is also evidence suggesting that disruption of lipid rafts in cancer can lead to increased responsiveness to anti-cancer therapies [21]. Additionally, some anti-cancer drugs have beneficial effects through alteration of the protein content of lipid rafts [22]. In colon cancer, lipid rafts have been shown to function in cell death-mediated signaling [23, 24], cell entry/bioavailability of bioactive compounds [25], and localization of key proteins involved in immune response [26].

Membrane Properties Are Altered by Diet

It has been shown that *n*–3 PUFA can alter membrane cholesterol and/or sphingomylin content. For example, DHA and EPA reduce cholesterol synthesis in HT29 colon cancer cells [27•]. Fish oil (FO)-fed mice exhibited a ~46 % reduced cholesterol content in colonic caveolae, specialized rafts enriched in the structure protein caveolin-1, which regulate the clustering of signaling proteins such as Ras and eNOS [28]. In addition, a ~40 % reduction in $CD4^+$ T cell lipid raft sphingomyelin levels was observed in mice fed a FO-supplemented diet [29]. Dietary *n*–3 PUFA are also capable of displacing acylated proteins from lipid raft microdomains in vivo [56] and can alter the size and distribution of cell surface microdomains [30, 41]. *n*–3 PUFA incorporate into membrane phospholipids, primarily glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE), in the *sn*-2 position, creating a highly disordered molecule that is cholesterol-phobic. However, the affinity of DHA-incorporated phospholipids for lipid rafts varies among the different phospholipid subclasses. For example, it has been reported that cholesterol is less soluble in GPE-DHA than in GPC-DHA relative to GPE-oleic acid (OA) and GPC-OA [31–33]. DHA-GPE prefers a non-raft environment, while DHA-GPC prefers a raft environment [34••].

Membrane Lipid Order Modification by *n*–3 PUFA

Lipid raft stability can be assessed by a variety of polarity-sensitive probes such as Laurdan or di-4-ANEPPDHQ. Quantitative imaging of these probes yields general polarization (GP) values, where higher values reflect a higher membrane order [35, 36]. With respect to diet, membrane order is increased in T cell plasma membranes from FO-fed mice or transgenic

mice that produce *n*–3 PUFA [37, 38••]. Similarly, B cells isolated from mice fed FO show an increase in membrane order in cross-linked cells relative to non-cross-linked cells [39]. This is in contrast to the decrease in membrane order reported in Jurkat cells treated with EPA and DHA [40, 41]. A possible explanation for the differences reported in these studies is that malignant transformed Jurkat cell lines may be inherently different from primary T cells with respect to specific plasma membrane properties. These findings are noteworthy because chronic inflammation increases cancer risk [42, 43]. Specifically, T cell-mediated inflammation in the colon has been linked to the onset of inflammatory bowel diseases (IBD) and to colitis-associated cancer (CAC) [44].

To corroborate the effects of *n*–3 PUFA on cell surface microdomain organization, immunogold electron microscopy of plasma membrane sheets coupled with spatial point analysis of validated microdomain markers has been used [45]. Clustering of probes within cholesterol-dependent (H-Ras) or cholesterol-independent (K-Ras) microdomains exhibits differential sensitivities to PUFA treatment. DHA increases clustering of the lipid raft marker, GFP-tH, and has no effect on the non-raft marker, GFP-tK [30]. This indicates that the effect of DHA is mediated through interactions with lipid raft domains.

n–3 PUFA Displace Signaling Molecules from Raft/Membrane Domains

Many proteins involved in colon cancer cell signaling, including receptors and G proteins, localize to lipid rafts [13]. The epidermal growth factor receptor (EGFR) is a tyrosine kinase that plays a critical role in cell proliferation, survival, and resistance to cancer therapy [46, 47]. EGFR requires lipid raft localization for efficient signaling [48, 49]. *n*–3 PUFA, in part through a reduction in membrane cholesterol, displaces EGFR from rafts, leading to an altered phosphorylated state [50, 51, 52••]. This in turn suppresses colonocyte downstream signaling events involving EGFR, such as phosphorylation of ERK1/2, STAT3, and Akt and activation of Ras [52••]. A possible explanation for this reduction in signaling may involve an increase in EGFR ubiquitination and internalization.

Alteration of raft lipid composition displaces the acylated proteins Lck and LAT from lipid rafts in Jurkat cells treated with EPA [53]. This is associated with the inhibition of T cell signaling by reducing phosphorylation of LAT and phospholipase Cγ1 [54]. A brief summary describing how DHA alters membrane order and EGFR signaling is shown in Fig. 1.

Ras proteins are GTPases, which are targeted to the membrane by farnesylation coupled to either palmitoylation, N, H, and K(A), or a polybasic motif, K(B) [55]. Targeting of palmitoylated N- and H-Ras to the plasma membrane is reduced by DHA, with no effect seen on K(B)-Ras [56]. However, activation of all three isoforms of Ras is attenuated by treatment with DHA [52••]. Collectively, the membrane-altering properties of *n*–3 PUFA are significant because EGFR and Ras are major drivers of colon cancer [57]. Currently, attempts to directly target Ras have repeatedly failed [58]; therefore, alternate strategies must be pursued. DHA, through modulation of membrane order, may provide a novel therapeutic strategy that may complement current therapies.

Epigenetic Effects of *n*–3 PUFA

Colon cancer develops through a multistep process that results from the progressive accumulation of mutations and epigenetic alterations in tumor suppressor genes and oncogenes. Epigenetics involves heritable changes in gene expression via posttranslational and posttranscriptional modifications. These modifications typically occur by changes in (i) DNA methylation, (ii) histone modifications, and/or (iii) microRNA (miRNA) expression [59]. These three mechanisms are interconnected to selectively modulate gene expression [60, 61].

i. In cancer cells, two patterns of DNA methylation can be observed. On the one hand, proto-oncogenes or genes implicated in tumor progression are activated due to global hypomethylation or low levels of methylation. On the other hand, genes such as tumor suppressor genes, implicated in tumor eradication, are silenced due to hypermethylation of their promoter regions [62–64].

ii. Histone modification by posttranslational processing of their tails directly affects chromatin structure and function and subsequently influences chromatin-based processes, including gene transcription, DNA repair, and DNA replication [65, 66]. The level of histone acetylation is based on the activity of two types of enzymes, namely histone acetyl transferases (HATs) and histone deacetylases/ lysine deacetylases (HDACs/KDACs), that regulate the conformation of the chromatin structure to facilitate or hinder the association of DNA repair proteins or transcription factors to chromatin. Hyperacetylation of histones thus can lead to transcriptionally active chromatin. In contrast, deacetylation of histones by HDACs typically leads to a closed (heterochromatin-like) chromatin conformation, thus diminishing accessibility for transcription factors [66, 67]. In this way, HATs serve as activators of gene expression whereas HDACs are typically associated with gene inactivation [68].

iii. Another modulator of epigenetic modification involves noncoding miRNAs. miRNAs are key regulators of posttranscriptional control of gene expression. miRNAs are aberrantly expressed or mutated in cancer, suggesting that they may function as a novel class of oncogenes or tumor suppressor genes [69–71].

Diet, miRNAs, and Colon Cancer

The effects of colon carcinogen and dietary *n*–3 PUFA on rodent microRNA expression during the early stages of colon tumorigenesis have been examined [8, 72, 73]. The data indicate that translational alterations are far more extensive relative to transcriptional alterations in mediating malignant transformation. In contrast, transcriptional alterations were found to be more extensive relative to translational alterations in mediating the effects of diet. High-throughput miRNA profiling studies have linked aberrant expression of miRNAs to the development of colon cancer [74, 75]. Specifically, miR-21 is a well-described “oncogenic” miRNA. For example, miR-21 has been positively correlated with colorectal cancer metastasis [76]. Elevated expression of miR-21 has also been reported in colon cancer [8, 77, 78]. miR-21 has anti-apoptotic properties by directly and indirectly targeting several tumor suppressors, PTEN, PDCD4, BCL2, TIMP3, TGFβR2, SPRY3, and

RECK [76–86]. The effects of *n*–3 PUFA on miRNA expression in the gastrointestinal cancers are summarized in Table 1.

Combinatorial Properties of *n*–3 PUFA with Other Bioactive Agents

It has been demonstrated that dietary FO and fermentable fiber work synergistically to protect against colon carcinogenesis, primarily by enhancing apoptosis [87, 88, 89]. Curcumin, a well-known epigenetic modifier [90–95], with anti-oxidant [96], anti-inflammatory [97], anti-proliferative [98], and anti-angiogenic [99] properties, has also been shown to have synergetic anti-cancer effects when combined with fish oil. For example, we have reported that the combination of fish oil and curcumin can antagonize NFκB activation in the mouse colon following the induction of chronic inflammation [100]. The synergetic effect of DHA and curcumin has also been shown to block insulin-induced colon carcinoma proliferation [101] and inhibit DMBA-induced mammary tumorigenesis in mice [102].

It has also been shown that *n*–3 PUFA can modulate Wnt signaling, which plays a central role in the physiology and malignant transformation of intestinal stem cells, by suppressing colonocyte nuclear beta-catenin levels [103, 104••, 105, 106, 89]. This is important because perturbations in adult stem cell dynamics are generally believed to represent an early step in colon tumorigenesis [107, 108].

Link Between Obesity and Colon Cancer

Up to 14 and 20 % of all cancer-related deaths may be attributed to obesity in men and women, respectively [109]. Epidemiological data indicate that the risk of colon cancer is strongly associated with increasing body mass index (BMI) [110]. Similar to smoking and a history of colon polyps, a BMI value >25 also significantly increases the risk of colon cancer [111]. In addition to human data, several rodent models have been utilized to demonstrate a link between obesity and increased colon cancer. Studies in mice typically range from 6 to 20 weeks and normally utilize a 32–60 % high-fat lard-based diet resulting in an increased number of colon tumors and aberrant crypt foci as well as increased cell proliferation and reduced apoptosis [112, 113•, 114]. Based on these findings in combination with the rising prevalence of diet-induced obesity, there is an increased need to understand the link between diet, obesity, and colon cancer.

Inflammatory Adipokines

Obesity disrupts the dynamic role of the adipose tissue in energy homeostasis, resulting in the alteration of adipokine signaling and the development of chronic inflammation [115]. Adipose tissue is primarily comprised of mature adipocytes but also contains endothelial cells, adipocyte precursors, fibro-blasts, and immune cells. Excess delivery of nutrients to adipose tissue in obesity results in an increase in adipose tissue mass, followed by an increase in immune cell infiltration and thereby an altered production of proinflammatory adipokines (increased IL-1β and leptin, IL-6 and reduced adiponectin), ultimately contributing to the progression of chronic inflammation [116]. M2 macrophages are typically found in the adipose tissue of lean individuals, whereas obese individuals display an increase in M1 macrophages and a shift to a proinflammatory state [117, 118]. Increased

infiltration of M1 macrophages in obese mice is also associated with systemic changes in T cell subsets, particularly inflammatory Th1 and Th17 cells [119••]. Studies in both humans and mouse models have provided a clear link between inflammation and cancer [120–122]. The chronic low-grade inflammation associated with obesity may play a key role linking excess adipose tissue, altered adipokine status, and the development of colon cancer by providing a favorable niche for tumor development. Interestingly, diet-induced obese mice that have increased numbers of colonic tumors also have elevated circulating levels of several cyto-kines and adipokines [113•]. Changes in adipokine status may impact cancer cell growth, as the adipose tissue itself has the ability to secrete several tumor-promoting molecules such as growth factors, proinflammatory cytokines, and adipokines. The most well-characterized adipokines relevant to colon cancer are adiponectin and leptin. These adipokines have been demonstrated to play a role in cell growth, proliferation, apoptosis, angiogenesis, invasion, and migration.

Adiponectin is one of the most abundant protein secreted by adipocytes [123] and can be found in circulation at 2–20 μg/ml [124]. Circulating levels of adiponectin are inversely associated with BMI and visceral adiposity as well as many chronic diseases such as diabetes, cardiovascular disease, and cancer [124–127]. Epidemiological evidence suggests that low adiponectin levels are correlated with the risk of colon cancer [128–130]. Supporting evidence for the protective role of adiponectin against colon cancer has also been demonstrated in mice and cell culture studies. Reported beneficial actions of adiponectin include decreased cell proliferation [131–133], increased apoptosis, reduced number and size of colonies, and decreased adhesion and invasion [133, 134•]. Most impressively, adiponectin appears to reduce the number of polyps, aberrant crypt foci, and tumor size in diet-induced obese mice [132, 134•]. From a mechanistic perspective, adiponectin appears to exert its beneficial actions by promoting phosphorylation of AMPK and LKB1, resulting in changes in cell cycle and inflammatory (p21, p27 cyclin E, STAT3, VEGF, mTOR) pathways [131, 133, 134•].

Leptin is another hormone produced and secreted predominantly by adipocytes, and it is involved in regulating body weight by modifying appetite and energy expenditure [135]. A positive association has been made between leptin and colorectal adenoma in men [130]. Men with the highest leptin concentrations (11–70 ng/ml) had a 3.3-fold increase in risk of colorectal adenoma as compared to those with leptin levels between 1 and 5 ng/ml [136]. In colonic epithelial cells, leptin induces proliferation in a VEGF-dependent manner [137]. In vivo, leptin-deficient mice have reduced colonic tumor size as well as reduced cell proliferation and increased apoptosis [138]. The phosphorylation of STAT3 and upregulation of other inflammatory mediators (IL-6, IL-1β, and CXCL1) appear to be involved in the negative effects of leptin on tumor size [138, 139]. It is also important to note that these two adipokines may interact with each other to influence tumor development [140, 141], an interaction that may be further promoted by obesity.

Inflammatory Cytokines

As mentioned above, the obese adipose tissue becomes infiltrated by immune cells which secrete several inflammatory cytokines, further contributing to the chronic inflammatory

environment in obesity. This proinflammatory profile can contribute to the risk of colon cancer. For example, IL-1β has been shown to promote sphere formation and increases in mRNA expression of genes that promote stemness [142, 143]. Cell proliferation and phosphorylation of STAT3 in colon cancer cell lines are induced by IL-6 treatment [131]. Mice deficient in IL-17a display reduced tumor size and number, which is associated with reduced IL-6, IFN-gamma, TNF-alpha, phosphorylated STAT3, and beta-catenin [144].

n–3 PUFA Reduce Obesity-Related Colon Cancer Risk

Since colon cancer development involves adipose-mediated chronic inflammatory processes [145], the adoption of therapeutic strategies to decrease obesity-associated colon tumorigenesis merits consideration. One example of an anti-inflammatory therapeutic is *n*–3 PUFA. Reductions in the local adipose inflammatory environment have been reported in both humans and rodents treated with *n*–3 PUFA [119••, 146••]. In mouse models, FO supplementation increased total serum adiponectin levels in diet-induced obese mice and was further enhanced by combination treatment with thiazolidinedione. These changes were associated with a reduction of macrophage infiltration in epididymal white adipose tissue and inflammatory TNF-alpha and MCP1 [147]. Furthermore, fish oil supplemented to a high-fat diet prevented diet-induced obesity, dyslipidemia, hyperinsulinemia, as well as obesity-induced adipocyte hypertrophy and macrophage accumulation in adipose tissue, resulting in increased circulating adiponectin levels [148]. Our laboratory and others have demonstrated that *n*–3 PUFA are protective against colon tumorigenesis [119••, 149–151] and suppress inflammatory immune cell populations in the colon and adipose tissue [119••, 146••]. Interestingly, in humans, the ratio of *n*–3/*n*–6 PUFA was significantly reduced in the visceral white adipose tissue of obese individuals with colorectal cancer and these individuals also exhibited an upregulation of STAT3 and decreased PPAR-gamma and adiponectin levels as compared to normal-weight cancer-free individuals [152••]. The authors were also able to demonstrate that treatment with DHA resulted in a significant reduction in phosphorylated STAT3 and IL-6 in adipocytes from obese colorectal cancer patients [152••].

Conclusion

There is a growing body of experimental, epidemiological, and preclinical evidence indicating that *n*–3 PUFA, mainly DHA and EPA, are protective against colon tumorigenesis [9, 153–157]. Establishing a causal role of *n*–3 PUFA in colon cancer prevention would have a major translational impact because these dietary nutrients are safe, well tolerated [158], and relatively inexpensive and provide additional health benefits, such as reduction in mortality [159]. In addition, the ingestion of *n*–3 PUFA in combination with other agents, such as fermentable fiber and curcumin, may improve their efficacy in colon cancer prevention/therapy. Herein, we have summarized three major mechanisms where *n*–3 PUFA modulate cancer risk, including membrane lipid order and downstream signaling, epigenetic modulation, and obesity-induced inflammation. Overall, these mechanisms explain some of the actions of an important dietary chemoprotective agent.

Acknowledgments

This work was supported by the American Institute for Cancer Research (AICR); Cancer Prevention and Research Institute of Texas (CPRIT); National Institutes of Health grants CA129444, CA168312, and P30ES023512; and USDA–NIFA Grant Designing Foods for Health 2010-34402-20875. The authors would also like to thank Tim Hou for formatting assistance.

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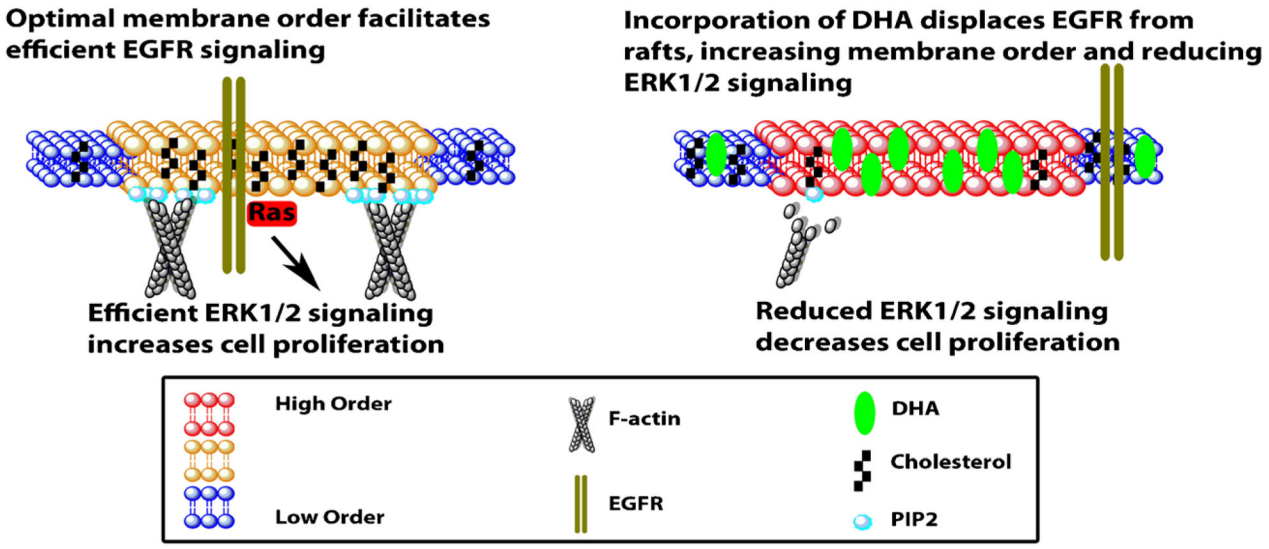

Fig. 1.

DHA alters membrane order and EGFR signaling. DHA-containing phospholipids incorporate into plasma membrane raft and non-raft domains [34••]. Because of DHA's low affinity for cholesterol, DHA incorporated into the raft domain reduces cholesterol levels and displaces cholesterol from plasma membrane rafts [28, 31–33]. This reduction of cholesterol displaces EGFR from the raft into the non-raft domain, causing an increase in phosphorylation, and paradoxically decreasing downstream Ras and ERK1/2 signaling, leading to reduced cell proliferation [28, 52••]. DHA also modulates the levels and localization of a critical signaling lipid, phosphoinositide 4,5-bis-phosphate (PIP_2) [160••]. This in turn reduces filamentous actin remodeling as well as activation of cytoskeletal regulators, Rac1 and Cdc42, which in turn reduces cell migration [157, 160••]. Cholesterol and the cytoskeleton are major contributors to maintaining membrane order. Interestingly, both these factors are reduced by *n*–3 PUFA, yet membrane order is increased in cells isolated from FO-fed animals [37, 38••, 39]. This may be attributed to a FO-dependent reduction of two highly disordered fatty acids, arachidonic acid (AA) and *n*-nervonoylsphingomyelin (C24:1), which could result in a net increase in membrane order [29, 161, 162]

Table 1

miRNAs are differentially regulated by DHA

Cell/tissue	Upregulated miRNA in gastrointestinal cancers	References
Rat colon	miR-132	[163]
	miR-146b	
	miR-192	
	miR-206	
	miR-218	
	let-7d	[8]
	miR-15b	
	miR-107	
	miR-191	
	miR-324-5p	
Human colorectal adenocarcinoma cells	miR-1	[164]
	miR-30c	
	miR-141-3p	
	miR-181a-5p	
	miR-192	
	miR-221-3p	
	miR–1283	
	Let-7f	
Gastric cancer cells	miR-15b	[165]
	miR-16	