HHS Public Access

Author manuscript

Biochim Biophys Acta. Author manuscript; available in PMC 2018 September 01.

Published in final edited form as:

Biochim Biophys Acta. 2017 September ; 1859(9 Pt B): 1668–1678. doi:10.1016/j.bbamem.2017.03.014.

Emerging Role of Chemoprotective Agents in the Dynamic Shaping of Plasma Membrane Organization

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Abstract

In the context of an organism, epithelial cells by nature are designed to be the defining barrier between self and the outside world. This is especially true for the epithelial cells that form the lining of the digestive tract, which absorb nutrients and serve as a barrier against harmful substances. These cells are constantly bathed by a complex mixture of endogenous (bile acids, mucus, microbial metabolites) and exogenous (food, nutrients, drugs) bioactive compounds. From a cell biology perspective, this type of exposure would directly impact the plasma membrane, which consists of a myriad of complex lipids and proteins. The plasma membrane not only functions as a barrier but also as the medium in which cellular signaling complexes form and function. This property is mediated by the organization of the plasma membrane, which is exquisitely temporally (nanoseconds to minutes) and spatially (nanometers to micrometers) regulated. Since numerous bioactive compounds found in the intestinal lumen can directly interact with lipid membranes, we hypothesize that the dynamic reshaping of plasma membrane organization underlies the chemoprotective effect of select membrane targeted dietary bioactives (MTDBs).

Keywords

n-3 PUFA; Dietary bioactives; membrane order; membrane therapy; cancer prevention

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Introduction

Chronic diseases and conditions such as heart disease, stroke, cancer, type 2 diabetes, obesity and arthritis account for the vast majority of health spending in the U.S. While today's situation is grave, the chronic disease crisis looms even larger in the future. This is supported by claims that if the current trends continue, by 2025, chronic diseases will affect an estimated nearly half of the U.S. population[1]. In addition, the number of cancer cases diagnosed annually by 2050 is likely to double as a result of population aging. Therefore, if the healthcare community hopes to head off the coming storm, we need to expand efforts in chronic disease prevention soon[2]. Heading off this escalating burden of age-related illnesses requires an emphasis on primary (cancer) prevention research and training in cancer-related lifestyle decisions, including diet and exercise[2]. Unfortunately, less than 1.5% of total biomedical research funding is targeted to early detection and prevention of chronic disease[3]. As an example, colorectal cancer (CRC) is the third most common type of cancer in the U.S., accounting for roughly 8% of new cancer cases and 9% of cancer deaths in 2014[4]. Overall, CRC incidence and mortality rates have decreased in the past 20 years, attributed largely to use of CRC screening and polypectomy in adults over 50 years of age. However, among adults younger than 50 years, for whom screening is not recommended if at average risk, CRC incidence rates have been increasing by ~2% per year since 1994 in both men and women[4]. While genetic factors account for some of the CRC risk, environmental factors account for the majority of risk[5]. Here, we describe the future challenges to the cancer field regarding the identification of additional molecular mechanisms that can be targeted as part of novel prevention strategies.

1. Dietary chemoprevention and CRC risk

CRC risk could be greatly reduced through dietary modification, including increased dietary fiber intake and reduced fat intake[6]. With respect to dietary fat intake, in observational studies, the evidence is mixed for associations between total dietary fat, specific types of fat, and CRC[7]. Omega 3 (n-3; α-linolenic acid, ALA) and omega 6 (n-6; linoleic acid, LA) polyunsaturated fatty acids (PUFA) are essential nutrients that are incorporated into tissue membranes, and modulate a variety of physiologic roles, including production of eicosanoids[8] and pro-resolving lipid mediators[9,10]. Most noteworthy are the long-chain n-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found in fish oils[11]. Although long chain n-3 PUFA can be metabolically generated from ALA, the process is not very efficient in humans[12]. Additionally, there is competition with long-chain n-6 PUFA synthesis, di-homo-gamma-linolenic acid, and arachidonic acid (AA), which are produced from LA, and are found in much greater abundance in a typical Western diet[13]. In general, n-6 PUFA are pro-inflammatory whereas mediators produced from n-3 PUFA tend to have opposing effects and exhibit anti-inflammatory properties[14,15]. However, cases of n-3 PUFA exhibiting immune enhancing properties do exist[16,17]. Given the strong association between inflammation and CRC[18], higher intakes of n-3 PUFA provide biological plausibility for a chemoprotective effect[8,19]. Indeed, experimental preclinical models consistently show reduced CRC risk with n-3 PUFA[20–24]; however, epidemiologic data are inconsistent and the majority of studies did not include PUFA intake from supplemental fish oil[25–29]. Two meta-analyses have concluded that fish intake is

associated with decreased risk of CRC[30,31]; however, two systematic reviews of n-3 PUFA on cancer risk qualitatively concluded that there is inadequate[32] or limited[28] evidence to suggest an association between long-chain n-3 PUFA intake and CRC risk. In contrast to the epidemiologic literature, in an endoscopy-based case-control study on colorectal adenomas, serum n-3 PUFA levels were inversely associated with colorectal adenoma risk [33]. Recently, in the VITamins And Lifestyle (VITAL) cohort, it was noted that persons using fish oil supplements on 4+ days per week for 3+ years experienced 49% lower CRC risk than nonusers [34].

With respect to clinical studies, mounting evidence suggests that the consumption of fish oil may reduce colon cancer risk in humans[35–41]. EPA and DHA appear to be ideally suited to work either alone or in combination with chemoprotective drugs[42]. Recently, it was demonstrated that EPA reduced rectal polyp number and size in patients with familial adenomatous polyposis (FAP)[19,43]. Most impressive was the fact that fish oil derived n-3 PUFA suppressed FAP to a degree similar to the selective COX-2 inhibitor celecoxib. Ongoing clinical trials (Clinical Trials.gov) are currently examining the effects of EPA on subjects at high risk of CRC (NCT02069561); the combinatory role of EPA and DHA in reducing rectal cancer risk (NCT02534389), and the combinatory role of EPA and NSAIDs on polyp recurrence in the colon (NCT01070355; ISRCTN05926847)[44,45]. Collectively, these data indicate that n-3 PUFA hold promise as chemoprevention agents. Hence, 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[46], relatively inexpensive and provide additional health benefits[47–49]. In addition, the ingestion of n-3 PUFA in combination with other agents with complementary anti-tumor action, e.g., curcumin[50–55] and drugs[56],may improve their efficacy in colon cancer prevention/therapy. However, before a drug-nutrient combination approach can be adopted, it is imperative that we fully elucidate the molecular mechanisms of action.

Polyphenolic and terpenoid phytochemicals have become increasingly popular with consumers in part because of their putative health benefits. Of these, turmeric (*Curcuma longa Linn*) extracts, including curcumin (diferuloylmethane), a yellow color pigment of turmeric, have been shown to suppress colitis and colon cancer development in experimental models and placebo controlled clinical trials[56–63]. Ongoing clinical trials (Clinical Trials.gov) are currently examining the role of curcumin with respect to aberrant crypt foci (NCT00365209), cell proliferation and apoptosis in colonic mucosa (NCT00118989), drug combination therapy (NCT00745134), and treatment of FAP (NCT00927485, NCT00641147)[64]. Recent data from one of these Phase IIa clinical trials indicates that consumption of curcumin at 4 g per day for 30 days significantly (by 40%) reduces aberrant crypt foci (ACF) number in men and women, >40 years of age with a history of smoking and 8 or more rectal ACF by magnification chromoendoscopy[65]. Importantly, several studies have confirmed that curcumin is well tolerated in humans and is directly incorporated into colonic mucosa[65,66].

n-3 PUFA and curcumin paradoxically increase injury scores in an inflamed animal model [52], while the same n-3 PUFA dose has beneficial effects in a colon cancer model [67]. Along these lines, it has been demonstrated that fish oil feeding increases colon

inflammation in a genetically susceptible mouse model [68], while exhibiting a chemo-protective tumor suppressing effect in an inflamed colon cancer animal model [69]. These findings suggest that adverse effects associated with preclinical studies may depend on the animal model and treatment dose/duration. In contrast, the majority of n-3 PUFA and curcumin associated clinical studies have reported beneficial outcomes (Table 1). For example, n-3 PUFA reduce rectal polyp number and size in FAP [43] and curcumin reduces aberrant crypt foci formation [65]. No adverse effects were reported following high dose n-3 PUFA [70] and curcumin [71] treatment.

As a major component of the Mediterranean diet[72], walnuts (*Juglans regia* L.) contain a number of nutritional bioactive compounds, including PUFA, tocopherols, and membrane targeted dietary bioactive (MTDB)-like phenolic compounds, e.g., procyanidins[73–78]. Although intact procyanidins have some systemic biological activity, they are poorly absorbed and pass into the distal intestine (colon) where they are further metabolized by gut microbes to generate monomeric catechin and epicatechin compounds along with other dimers-hexamer species[79–81]. These microbial metabolites can interact with the apical membranes of colonic epithelial cells (Figure 1). Recent evidence from our lab and others suggest that the phenolic compound (+)-catechin, and its dimeric form procyanidin B2 can alter the biophysical properties of cell plasma membranes[82,83] (Figure 2), similar to what has been observed with other MTDB's such as n-3 PUFA and curcumin[8,84–86]. Interestingly, walnuts have been shown to inhibit the growth of human cancer cell lines[87,88], and colon cancer in mouse models[89–91], similar to what has been demonstrated with other MTDB's such as n-3 PUFA[8,23,69,92–95]. In addition, a meta-analysis concluded that nut consumption is associated with decreased risk of cancer mortality, further supporting the inclusion of nut consumption for cancer prevention[96]. However, the properties of procyanidins that contribute to a reduction in cancer risk have not been well characterized. In comparison, tocopherols exert beneficial properties by promoting plasma membrane repair[97], and attenuate oxidation of polyunsaturated phospholipids[98,99].

2. Putative mechanisms of MTDB action: Importance of nanoclustering as a dominant feature of plasma membrane organization

One of the criticisms facing the dietary chemoprevention field is the fact the dietary bioactives, i.e., constituents in foods or dietary supplements other than those needed to meet basic human nutritional needs [100], appear to be pleiotropic and affect diverse physiological processes including cell membrane structure/function, eicosanoid signaling, nuclear receptor activation, and inflammatory responses. Investigators are challenged to explain and unify these apparently disconnected signaling nodes. We propose a unifying mechanistic hypothesis to explain the function of these bioactives. Specifically, we postulate that n-3 PUFA and curcumin / curcuminoids / procyanidins fall into a unique class of MTDB's which, because of their unique amphiphilic properties, are capable of modulating plasma membrane hierarchical organization, leading to the disruption of oncogenic signaling, and thereby ultimately reduce tumor growth.

With respect to membrane structure, the plasma membrane is 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 sub-membrane cytoskeletal elements[101]. Although, still controversial in nature, lipid rafts are believed to be dynamic and small (10–200 nm) membrane microdomains enriched in sphingolipids and/or cholesterol, which function as sorting platforms for many membrane-associated proteins[102–106]. Stabilization of these domains is generally thought to be maintained by lipid and cytoskeletal influences[107,108]. 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[109–111]. There is also evidence suggesting that disruption of lipid rafts in cancer can lead to increased responsiveness to anti-cancer therapies[112,113]. Additionally, some anti-cancer drugs have beneficial effects through alteration of the protein content of lipid rafts[114,115]. In colon cancer, lipid rafts have been shown to function in cell death-mediated signaling[116,117], cell entry/bioavailability of bioactive compounds[118], and localization of key proteins involved in immune response[119]. These findings indicate that lipids can no longer be ignored in the structures of membrane complexes, due to their ability to fine-tune and stabilize different signaling interfaces[120–122].

Highly relevant to the cancer biology field, it is now recognized that the geometry of biological membranes is tightly intertwined with signal processing capability[123]. According to this emerging picture, protein and lipid nanoclusters can be organized to form domains that are capable of facilitating signaling events[124–126]. The formation of dimers/nanoclusters is believed to be driven by cortical actin and/or proximal transmembrane proteins[124]. Currently, protein-protein, lipid-lipid and protein-lipid nanoclusters are considered a predominant feature of the plasma membrane and appear to mediate critical signaling processes[126], including signal integration and cross talk of the transduction of oncogenic Ras and the epidermal growth factor receptor (EGFR)[126–128] regulated pathways. This is noteworthy, because there is emerging evidence that drugs and MTDB's can attenuate Ras and EGFR[126,129] activity by modulating nanocluster organization. In accordance with these findings, we hypothesize that MTDB's are capable of disrupting clustering/dimerization of membrane associated proteins, leading to attenuation of downstream oncogenic signaling and the suppression of tumor growth (Figure 3).

3. Effects of MTDB's on membrane organization and signaling

There is a growing body of *in vitro* and *in vivo* evidence indicating that MTDB's reshape plasma membrane domains. For example, EPA and DHA, whose levels are readily influenced by diet in general [130], affect diverse physiological processes including cell membrane structure/function and signaling[8,84]. n–3 PUFA are rapidly incorporated into cells, primarily into membrane phospholipids at the *sn*-2 position[131,132]. Specificaly, DHA is known to influence membrane fluidity, ion permeability, fatty acid exchange, and resident protein function [133,134], including the inhibition of EGFR signaling in tumor bearing mice by reducing localization of EGFR to lipid rafts [69]. The presence of long chain n-3 PUFA in membrane phospholipids imparts unique physicochemical properties which have been linked to alterations in plasma membrane structure and function and its

pleiotropic chemoprotective effects[122,132,135–138]. Interestingly, other MTDB's known to reshape membrane domains, e.g., curcumin, capsaicin, and glycyrrhizin, exhibit similar properties[139–145]. For example, curcumin inserts deep into the membrane in a trans-bilayer orientation, anchored by hydrogen bonding to the phosphate group of lipids in a manner analogous to cholesterol. Similar to cholesterol, curcumin induces segmental ordering in the membrane [139,146,147]. These properties may explain why curcumin can suppress EGFR localization to lipid rafts decreasing EGF stimulation in cells [148]. Although intact procyanidins have some systemic biological activity, they are poorly absorbed and pass into the distal intestine (colon) where they are further metabolized by gut microbes to generate monomeric, dimers-hexamer species[79–81]. These microbial metabolites interact with the apical membranes of colonic epithelial cells (Figure 1). Recent evidence from our lab and others suggest that the phenolic compound (+)-catechin, and its dimeric form procyanidin B2 can alter the biophysical properties of cell plasma membranes[82,83] (Figure 2), similar to what has been observed with other MTDB's such as n-3 PUFA and curcumin[8,84–86]. These findings are consistent with previous observations that procyanidins have the ability to modulate membrane biophysical properties[82,83].

To further evaluate the effects of procyanidins compounds on plasma membrane organization, we utilized the polarity sensitive dye di-4-ANEPPDHQ (Di4). We utilized Di4 over the commonly used dye, laurdan, because Di4 exhibits slower internalization kinetics in live cells[149,150]. This provides a more representative measure of plasma membrane organization. In addition, although laurdan and Di4 are both used to quantify membrane order[151], recent findings highlight the fact that they probe different properties of the membrane, with Di4 being more sensitive to cholesterol status[152]. Di4 excites at 488 nm and its emission maximum emits at 565 nm or 605 nm in ordered and disordered membranes, respectively[149,151]. We chose the *in vitro* young adult mouse colonocyte (YAMC) cell model expressing oncogenic HRasG12V[153] as a representation of normal colonocytes on the route to malignancy[154]. These cells typically exhibit upregulated macropinocytosis[155], which is the cellular process of nonselective endocytic uptake of extracellular lipids and proteins driven by membrane-lipid and cytoskeletal remodeling[156,157]. Macropinocytosis provides the fuel for Ras-driven tumor growth resulting in altered metabolism[155,158], which creates a dependency that can be exploited as a pharmacological target[159,160]. To specifically further assess how exogenous treatments affect the plasma membrane, we generated giant plasma membrane vesicles (GPMVs) which retain most of the full diversity of native membrane components, but lack cytoskeletal attachment[161]. This type of reductionist approach allows us to probe specific questions regarding the interaction between diet-derived bioactives and lipid membranes without the complication of compensatory mechanisms imparted by the live cell such as membrane tension[162] and cytoskeletal remodeling[108]. GPMVs were isolated from YAMC-HRasG12V cells pre-labeled with Di4 and incubated with varying doses of (+)-catechin and procyanidin B2 (Figure 2C&D). We utilized a wide range of doses (1–10 uM) which have been associated with circulating levels in vivo (0.1–3 uM) [163–168], and higher doses (100 uM) that are present in the lumen of the colon[79,169–172]. A short incubation time frame (30 min) was used to mimic the passage of these compounds through the colon,

and avoid large changes in gene or protein expression that may occur during longer incubation periods. Membrane order of GPMVs was then determined using imaging based flow-cytometry. Interestingly, (+)-catechin dose-dependently reduced membrane rigidity while procyanidin B2 increased rigidity. We subsequently used confocal microscopy to determine if the same effects would occur in live cells (Figures 2E&F), which are generally more refractive to plasma membrane changes[173,174]. Surprisingly, both compounds decreased membrane rigidity in intact cells. Internalization of fluorescently labeled dextran was used as an indicator of macropinocytosis[175], which was quantified by image based flow-cytometry. Incubation of cells with low doses of these compounds attenuated epidermal growth factor (EGF) stimulated macropinocytosis, with procyanidin B2 exhibiting greater inhibitory activity (Figure 2G&H). These proof of principle experiments demonstrate how procyanidin based MTDBs can modulate cellular processes including plasma membrane organization in both a cytoskeletal dependent and independent manner.

4. Potential effects of MTDB's on cancer development and stem cells

It is noteworthy that many proteins involved in colon cancer cell signaling, including transmembrane receptors and G proteins, localize to lipid rafts[109] and nanocluster[176]. For example, the EGFR, a tyrosine kinase that plays a critical role in cell proliferation and resistance to cancer therapy[177], requires lipid raft localization and nanoclustering for efficient signaling[112,176,178]. n-3 PUFA in part through a reduction in lipid raft cholesterol composition, displace EGFR from rafts, leading to an altered phosphorylated state[179–181]. This in turn has been linked to the suppression of colonocyte downstream signaling events involving EGFR, such as phosphorylation of ERK1/2, STAT3, Akt and activation of H- and KRas[92,136,181].

As mentioned above, since changes in plasma membrane structure alter receptor-mediated cell signaling[182], there is mounting interest regarding the use MTDB's to modulate membrane-mediated signaling pathways and their target genes. Consistent with the fact that DHA has been shown to significantly alter plasma membrane lipid raft composition [136,138], our lab recently demonstrated that n-3 PUFA alter EGFR lipid raft localization and HRAS, KRAS and NRAS activation [181]. These findings can be attributed to the fact that prolonged intake of dietary lipids modifies membrane order[183]. Interestingly, other MTDB's, e.g., curcumin, have been shown to alter localization of α6β4/EGFR to lipid rafts[184]. It is noteworthy that the ordering effect of curcumin is strongest in the head group region of the phospholipid bilayer[139,185], whereas n-3 PUFA acyl chains impact the organization of the tail group region within rafts[186–188] implying that these bioactives may act synergistically at the membrane.

Wnt signaling is important for the maintenance of stem cells of various lineages. A classic example is in the digestive tract, where in the crypt of the colon the loss of transcription factor TCF4 leads to depletion of stem cells[189]. Dysregulation of Wnt/β-catenin signaling via genetic alterations of APC, β-catenin or Axin2 drives stem cell hyperproliferation which promotes colorectal cancer[190–192]. In contrast, neonatal mice lacking TCF4 exhibit reduced proliferation in the crypt [189]. These findings are consistent with the fact that chronic upregulation of Wnt signaling in Lgr5 positive stem cells drives colon cancer[193].

Interestingly, from a membrane perspective, Wnt signaling components, e.g., Lypd6 and CK1γ, have been shown to mainly localize to lipid rafts in the plasma membrane[194]. This is noteworthy, because lipid rafts play a fundamental role in mediating multiple cell functions, including signal transduction[195]. LRP6 is localized to both raft and non-raft fractions but its phosphorylation by GSK-3 and CK1γ, essential for the Wnt-dependent accumulation of β-catenin, resides primarily in lipid rafts, not in the non-lipid raft[196]. Thus, it has been proposed that the localization of these proteins to lipid rafts actively contributes to the stabilization of β-catenin[197,198]. Based on these findings, we propose that MTDB's may modulate the Wnt signaling pathway.

5. Summary

With respect to all human malignancies, 35% are linked directly to diet and an additional 14-20% to obesity[5]. Consistent with these data, cancer risk can be lowered by 36% when humans adhere to healthy dietary principles, e.g., high intake of fruits, vegetables, and whole grains and low meat consumption[199]. Therefore, it is imperative that health professionals make sound dietary/lifestyle recommendations. However, even though there are many observational / epidemiological studies linking diet and cancer risk, the association cannot be easily explained mechanistically. Therefore, establishing a causal role for cancer dietary chemoprevention approaches that are generally free of safety problems intrinsic to drugs administered over long periods of time would have a major translational impact in cancer prevention and patient survivorship[46,199]. In view of this need, our long-term goal is to better understand the molecular mechanisms modulating cell responses to MTDB's. Specifically, we propose that by altering cell membrane nanoscale assemblies, and possibly protein spatial localization and signaling, that select amphiphilic dietary agents, e.g., n-3 PUFA, curcumin, procyanidins, will reduce oncogenic signaling and cancer risk.

Acknowledgments

This work was supported by NIH grants R35CA197707, CA168312, and P30ES023512, the Cancer Prevention Research Institute of Texas (CPRIT) and the American Institute for Cancer Research (AICR). Natividad Fuentes is a recipient of a Predoctoral Fellowship in Pharmacology/Toxicology from the PhRMA Foundation. Natividad Fuentes and Michael Salinas are recipients of the National Science Foundation Texas A&M University System Louis Stokes Alliance for Minority Participation (TAMUS LSAMP) Bridge to the Doctorate Fellowship.

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Highlights

- Diet dynamically shapes plasma membrane organization
- Intrinsic adaptation of plasma membrane organization mediates cell signaling
- Diet-derived polyphenolic compounds disrupt oncogenic Ras-driven dependencies

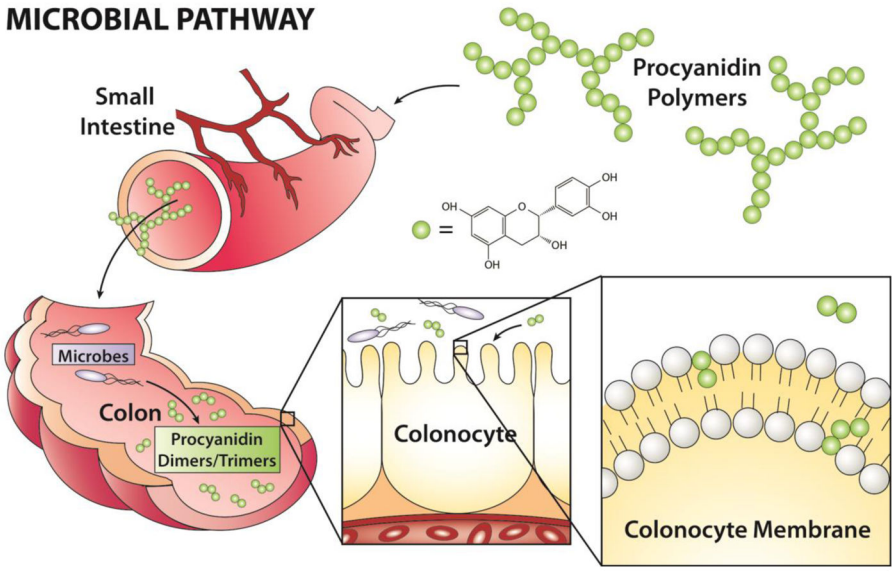

Figure 1. Putative role of microbial metabolites of procyanidin based dietary bioactives as modulators of colonocyte membrane-dependent oncogene signaling and cancer risk

We hypothesize that select poorly digestible dietary-microbial derived bioactives can promote a chemoprotective cell membrane microenvironment in the colon.

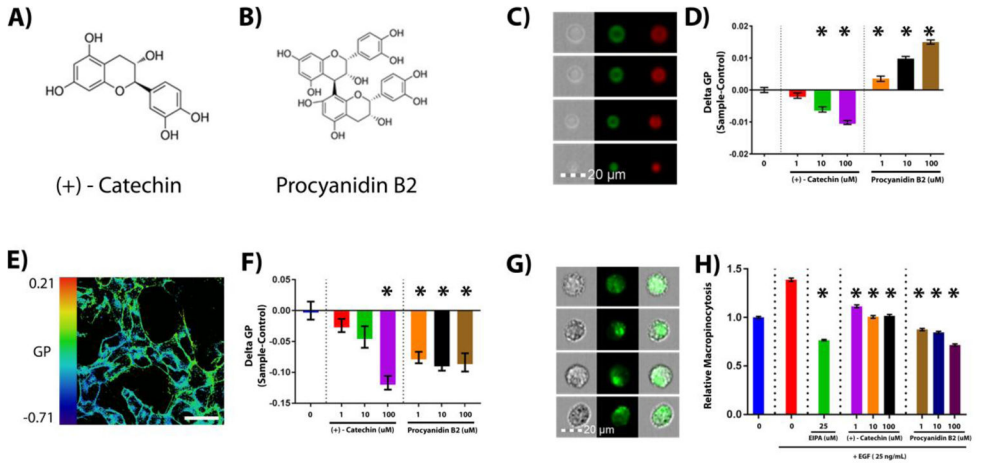

Figure 2. Procyanidins modulate colonocyte plasma membrane organization

In order to determine if microbial metabolites of procyanidins directly modulate plasma membrane biophysical properties (membrane order), immortalized young adult mouse colonocyte (YAMC) cells expressing oncogenic HRasG12V were incubated with a membrane order sensitive dye Di-4-ANEPPDHQ (5 uM) for 30 min prior to generation of giant plasma membrane vesicles (GPMVs). GPMVs were incubated with, (A) (+)-Catechin, or (B) Procyanidin B2, for at least 30 min, followed by determination of GPMV generalized polarization (GP) by (C) imaged based flow cytometry using an Amnis FlowSight system. Emission wavelengths of 480-560 and 640–745 were used for ordered (Green) and disordered (Red) channels, respectively. (D) GP was defined as the integrated fluorescence intensity from the ordered channel minus that of the disordered channel normalized by the total intensity (sum of the two channels). Quantification of membrane order is represented as mean GP, normalized to the untreated control for at least 4000 individual vesicles from two separate experiments. Statistical significance between untreated control and treatments (*P<0.05) was determined using 1-way ANOVA and Dunnett's multiple comparisons test. (E) To determine if microbe derived metabolites indirectly modify plasma membrane biophysical properties, membrane order was also determined in live YAMC-HRasG12V cells, where cytoskeletal influences contribute to membrane biophysical properties. Experiments were performed by confocal microscopy using a Zeiss 780 system, after incubation with compounds for at least 30 min. Emission wavelengths of 508–544 and 651–695 were used for ordered and disordered channels, respectively. Scale bar, 50 µM. (F) Quantification of membrane order is represented as mean GP normalized to the untreated control for at least 10 fields of view containing approximately 100 cells. Statistical significance between untreated control and treatments (*P<0.05) was determined using 1-way ANOVA and Dunnett's multiple comparisons test. (G) To assess effects on cytoskeletal-membrane dependent macropinocytosis, YAMC-HRasG12V cells were serum starved (0.5% FBS) for 18 h, then incubated with a macropinocytosis inhibitor (EIPA) or procyanidin metabolites for 30 min prior to EGF (25 ng/mL) stimulation for 5 min in the presence of fluorescently (FITC) labeled dextran (70 kDa, 1 mg/ml). (H) Quantification of macropinocytosis, normalized to non-stimulated control, for at least 13,000 cells from two separate experiments. Statistical significance between EGF stimulated control and

treatments (*P<0.05) was determined using 1-way ANOVA and Dunnett's multiple comparisons test.

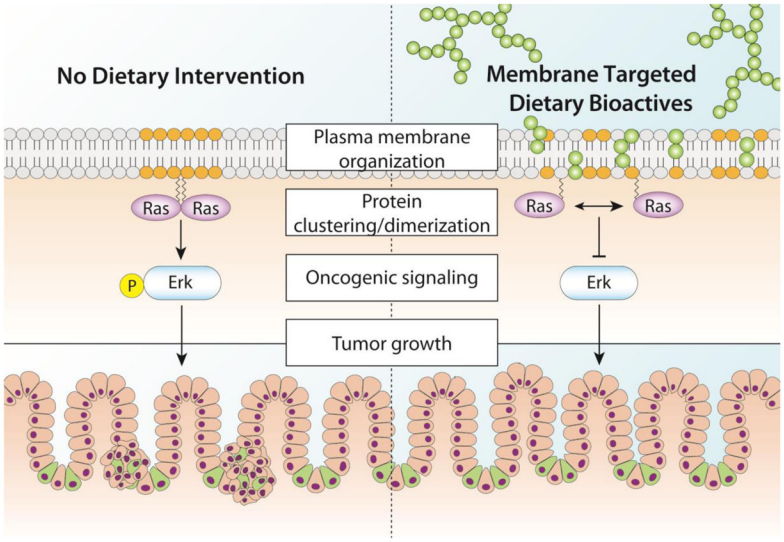

Figure 3.

Proposed mechanism describing the role of MTDB's as modulators of colonocyte membrane-dependent oncogene signaling and cancer risk. 1) Procyanidins remodel plasma membrane organization. 2) Membrane remodeling disrupts Ras nanocluster/dimerization. 3) MTDB's suppress Ras nanocluster/dimerization and attenuate oncogenic signaling. 4) This results in a reduction in tumor initiation/growth.

Table 1

Adverse and beneficial effects of marine-derived n-3 PUFA and curcumin in animal and clinical studies.

Bioactive compound	**Daily dose (n, duration of feeding)-mouse**	**Treatment**	**Amount of bioactive found in colon**	**Adverse biological effect**	**Human Equivalent Dose**	**Reference (PMID number)**
n-3 PUFA+ curcumin	46 mg n-3 PUFA and 90 mg curcumin (n=15, 3 wks)	5 days of 2.5 % DSS followed by 16 days recovery period+3 days of 1.5 % DSS followed by a 14 days recovery period	N.A.	n-3 PUFA±curcumin increase DSS-induced injury score	8.6 g n-3 PUFA and 17 g curcumin	21401974
n-3 PUFA	Approximately ~80 mg (n=20–30, 5 wks)	SMAD3 $^{/}$ mice	N.A.	Increased colon inflammation by increasing numbers of local neutrophils and inflammatory cytokine gene expression in the colon	8.6 g n-3 PUFA	26297475
Bioactive compound	**Daily dose (n, duration of feeding)-mouse**	**Treatment**	**Amount of bioactive found in colon**	**Beneficial biological effect**	**Human Equivalent Dose**	**Reference (PMID number)**
n-3 PUFA+ curcumin	46 mg n-3 PUFA and 45 mg curcumin (n=7–8, 3 wks)	Azoxymethane (AOM)	29–64 ug/g crypt wet weight 1-4 ug/g crypt wet weight	Remove DNA damaged Lgr5 stem cells and reduce nuclear β-catenin in ACF	8.6 g n-3 PUFA and 8.5 g curcumin	27831561
n-3 PUFA	130 mg n-3 PUFA (n=22–25, 15 wks)	One week after the AOM injection, mice were exposed to 3 cycles of 1% dextran sulfate sodium (DSS) for 4 days followed by 17 days of recovery	N.A.	fish oil fed animals developed fewer tumors	24.7 g n-3 PUFA	22761867
Bioactive compound	**Treatment dose (in vivo/ex vivo, time)**	**Treatment**	**Amount of bioactive found in colon**	**Beneficial biological effect**		**Reference (PMID number)**
Curcumin	5–20 uM (ex vivo, 0.5–24 h)	Inflammatory disease: Inflammatory bowel disease	N.A.	Suppressed p38 MAPK activation, reduced IL-1beta, and enhanced IL-10 levels in mucosal biopsies; suppressed MMP-3 in colonic myofibroblasts	Not appliable	19878610
Bioactive compound	**Daily dose (n, duration of treatment)-Human**	**Patient type**	**Amount of bioactive found in tissue or plasma**	**Beneficial biological effect**	**Mouse Equivalent Dose**	**Reference (PMID number)**
Curcumin	4 g (n=5, 3 months) 6 g (n=4, 3 months)	bladder cancer, oral leucoplakia, patiesnts with intestinal metaplasia of the stomach, CIN or Bowen's disease	0.51 uM in serum 0.63 uM in serum	Histologic improvement in 28% patients with various high-risk and pre-malignant lesions. No treatment-related toxicity up to 8 g/day	21.2 mg curcumin 31.8 mg curcumin	11712783

Bioactive compound	Daily dose (n, duration of feeding)-mouse	Treatment	Amount of bioactive found in colon	Adverse biological effect	Human Equivalent Dose	Reference (PMID number)
	8 g (n=2, 3 months)		1.77 uM in serum		42.4 mg curcumin	
	2 g (n=22, 1 month)	Phase II colon cancer	not detactable in serum, 8.2 ug/g protein in rectal mucosa	no effect on ACF reduction	10.6 mg curcumin	21372035
	4 g (n=19, 1 month)		0.01 nM in serum, 3.8 ug/g protein in rectal mucosa	40% reduction in ACF number in smokers	21.2 mg curcumin	
EPA	2 g (n=55, 6 months)	Familial adenomatous polyposis (FAP)	Significant increase in rectal mucosal EPA (2.6–fold) and DPA (1.8-fold) content after EPA treatment compared with placebo. No significant change in mucosal AA and DHA content.	22.4% reduction in polyp number	10.6 mg n-3 PUFA	20348368
LOVAZA	3.36 g (n=180, 6months)	Acute myocardial infarction	N.A.	Reduction of adverse left ventricular remodeling, noninfarct myocardial fibrosis, and serum biomarkers of systemic inflammation	17.8 mg n-3 PUFA	27482002
LOVAZA	17.6 g (n=12, 3 wks)	Healthy volunteers administered LPS	Lipid ratios of EPA and DHA in red blood cells (RBC) membranes is increased whereas, AA is decreased by n-3 PUFA supplementation	Anti-inflammatory actions in vivo remains speculative	93.3 mg n-3 PUFA	26180051

*N.A., not available / did not measure