JOURNAL OF CANCER PREVENTION
VOL. 18, NO. 3, SEPTEMBER, 2013

pISSN 2288-3649 · eISSN 2288-3657

Omega-3 Polyunsaturated Fatty Acids as Potential Chemopreventive Agent for Gastrointestinal Cancer

REVIEW

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Omega-3 polyunsaturated fatty acids (n-3 PUFAs), particularly eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), has been acknowledged as essential very long-chain fatty acids contributing to either achieving optimal health or protection against diseases, and even longevity. Recent high impact studies dealing with EPA and DHA have sparked a renewed interest in using n-3 PUFAs for cancer prevention and cancer treatment, for which n-3 PUFAs may exert their anticancer actions by influencing multiple targets implicated in various stages of cancer development, including cell proliferation, cell survival, angiogenesis, inflammation, and metastasis against various cancers. However, gastrointestinal cancers develop implicated with the close connection between inflammation and cancer and n-3 PUFAs especially imposed excellent actions of antiinflammation and antioxidation as well as their restorative actions. In detail, these beneficial lipids can restore or modify inflammation-associated lipid distorsion and alteration of lipid rafts. Although the chemopreventive effect of n-3 PUFAs has been studied in various experimental models, our understanding regarding the underlying mechanisms of n-3 PUFAs against GI cancer is still limited. In this review article, we described the in-detailed perspective and underlying mechanism of n-3 PUFAs application for GI cancers and added *in vivo* efficacy of n-3 PUFAs with *Fat*-1 transgenic mice experience. We suggest that future work should consider the n-6/n-3 FA ratio, combination treatment of other nutritions and alteration of lipid rafts to be a key element in experimental design and analysis. **(J Cancer Prev 2013;18:201-208)**

Key Words: Omega-3 polyunsaturated fatty acids, Gastrointestinal cancer, Chemoprevention, Lipid rafts, *Fat*-1 transgenic mice

INTRODUCTION

The role of diet in human health remains controversial. The contribution to human health of the specific fatty acid (FA) composition of the diet has received considerable attention in the literature. Fatty acids are key nutrients that affect early growth and development as well as the prevention of chronic disease in later life.[1] Among the FAs, Omega-3 polyunsaturated fatty acids (n-3 PUFA) and omega-6 polyunsaturated fatty acids (n-6 PUFA) have been suggested to decrease and increase several human diseases, respectively. PUFA that contains more than one carbon double bond consists of two major classes such as n-6 and n-3 (Fig. 1). Their metabolism can produce various lipids. Linoleic acid (LA) is a representative n-6 PUFA, serving as a substrate to be converted into an arachidonic acid (AA) affecting the prevalence and severity of inflammation. n-6 PUFA can induce cardiovascular disease, diabetes, cancer, and age-related disease.[1] α-linolenic acid (ALA), eicosapentaenoic acid (EPA 20 : 5) and docosahexaenoic acid (DHA 22 : 6) are important n-3 PUFA involved in human. The health benefits of n-3 PUFA have been long known. They are essential fatty acids that cannot be synthesized by mammals, by which must be obtained from dietary sources such as cold-water fish, certain seeds (flax) and nuts (walnuts). A lot of studies suggest that n-3

Received August 30, 2013, **Revised** September 11, 2013, **Accepted** September 11, 2013

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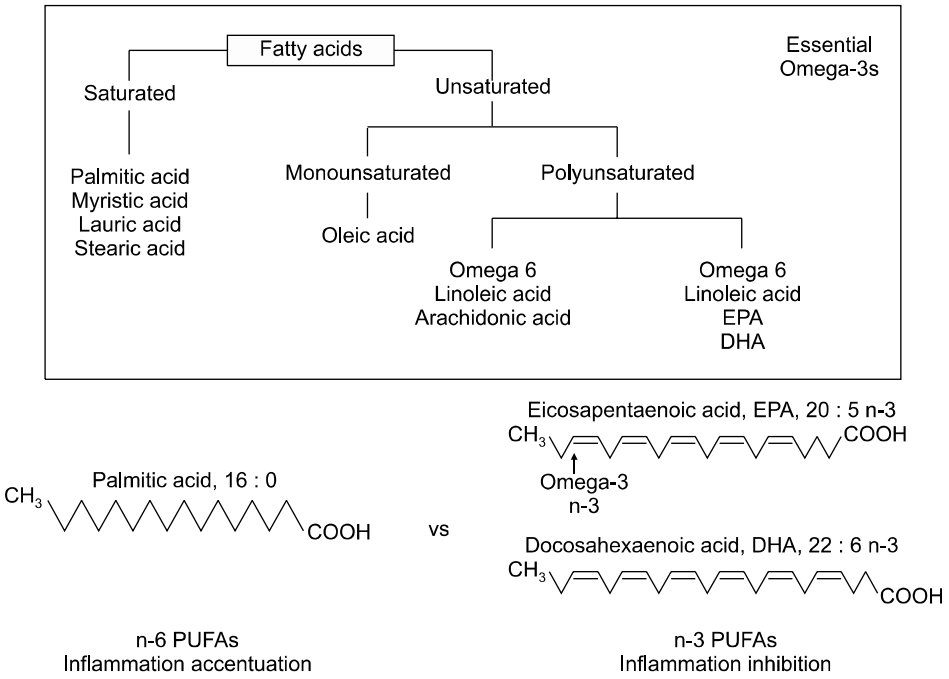

Fig. 1. Essential n-3 PUFAs contributed to anti-inflammatory action based on their unsaturated bonds.

PUFA, as diet-dependent factors, may be critical to preventing disease backed up with authentic antioxidative and anti-inflammatory actions. Especially, n-3 PUFA has been shown to exert beneficial effects on some chronic degenerative diseases such as cardiovascular disease,[2,3] rheumatoid arthritis,[4] diabetes,[5] other autoimmune diseases,[6,7] and cancer.[8,9]

Increased fat consumption by western diet has been associated with the development of cancer such as breast, colon, pancreatic, and prostate cancers with the notable exception of n-3 PUFA, which show to have multiple beneficial anti-tumor actions that affect the essential alterations that dictate malignant growth in a number of studies.[10] A diet rich in n-3 PUFA may protect from cancer, at least at certain sites. Studies on the fatty acid status of patients with several cancer types including bladder, pancreatic, lung and esophageal cancer show low concentrations of plasma phospholipid n-3 PUFA, ranging from 55 to 88% of amounts in healthy individuals.[11-13] Recent studies have found a positive association between n-6 PUFA and cancer risk, whereas in the same model, n-3 PUFA were shown to reduce the development of cancer. Epidemiological studies suggest that a high n-3 PUFA to n-6 PUFA ratio may be the optimal strategy to decrease breast cancer risk.[14] Solid epidemiological study shows that consumption of n-3 PUFA appears to protect against the development of hepatocellular carcinoma, even among patients with Hepatitis B Virus (HBV) and/or Hepatitis C Virus (HCV) infection.[15] Recently, Zhennan et al. has reviewed prospective studies investigating the possible protective effects of the dietary intake of n-3 PUFA on prostate cancer development.[16] Fasano et al also has reviewed a lot of *in vivo* and *in vitro* experimental studies providing strong indications of the anti-tumor action of n-3 PUFA against lung cancer.[17]

The purpose of this review is to discuss the potential role of n-3 PUFA in gastrointestinal (GI) cancer development. We believe that increased consumption of n-3 PUFA may lower the risk of GI cancer development via various chemopreventive activities. Future studies should include combination treatment of n-3 PUFA and nutrients with different and complementary mechanisms of chemopreventive action.

THE EFFICACIES OF N-3 PUFA IN PREVENTION OF GI CANCERS

Among various cancers, most of the GI cancers including esophageal cancer, stomach cancer, and colon cancer, have a natural history of multi-step transition from pre-

cursor lesions to malignant lesions, inflammation, adenoma formation, dysplastic changes.[18] Therefore, GI cancers usually have premalignant lesions before developing invasive cancers, for instances, Barrett's esophagus for esophageal cancer, chronic atrophic gastritis accompanied with intestinal metaplasia for gastric cancer, and adenoma or dysplasia originating from chronic ulcerative colitis for colon cancer. Because the Western diet contains disproportionally high amounts of n-6 PUFA and low amounts of n-3 PUFA, denoted as a high n-6 to n-3 PUFA ratio, n-3 PUFA may feasibly play a role in several stages of GI cancers management.

1. Esophageal cancer

Esophageal cancer is ranked as the sixth leading cause of cancer death worldwide. According to the increasing incidence of gastroesophageal reflux disease (GERD), esophageal cancer is a tumor that has increased in incidence more than 7-fold over the past several decades. Recent studies have found a positive association between n-3 PUFA and esophageal cancer prevention. Kubo et al. suggests that negative associations between n-3 PUFA intake and the risk of esophageal adenocarcinoma.[19] Higher intakes of n-3 PUFA [cases *vs.* population controls; OR=0.46, 95% CI=0.22-0.97, 4th *vs.* 1st quartiles of intake] was associated with a lower risk of Barrett's esophagus. In contrast, higher trans-fat intakes were associated with increased risk (OR=1.11; 95% CI=1.03-1.21 per g/day). Moreover, it is reported that n-3 PUFA supplemented parenteral nutrition can reduce inflammation and improve immune function in patients following esophageal cancer surgery[20] and n-3 PUFA-containing diet may be beneficial to patients with esophageal cancers who receive chemoradiation therapy (CRT) by reducing CRT toxicity.[21] In contrast to other GI cancer, little work has so far been performed on the influence of n-3 PUFA in oesophageal adenocarcinogenesis and neoplastic progression.[22] There is a need for appropriately powered randomized-controlled studies to assess the long-term benefit of n-3 PUFA.

2. Gastric cancer

Gastric cancer (GC) is the fourth most common cancer worldwide, and almost two thirds of affected individuals will die of their disease. Some studies about the association of n-3 PUFA and gastric disease suggests a protective effect of n-3 PUFA on gastric cancer. Recently, Correa et al. suggests that docosahexanoic acid (DHA) inhibits *Helicobacter pylori* (*H. pylori*) growth *in vitro* and mice gastric mucosa colonization.[23] *H. pylori* are recognized as a major etiological factor in chronic active gastritis, gastric duodenal ulcers and gastric cancer. It has been proposed that PUFA hold an inhibitory effect on bacterial growth via disruption of cell membrane leading to bacteria lysis.[24] Mohamed also shows that n-3 PUFA reduced iodoacetamide-induced gastritis in rats through decrease of malondialdehyde (MDA), gastrin, and nitric oxide (NO) and normalization of mucosal glutathione.[25] Especially, it is reported that the erythrocyte composition of DHA was found to be negatively linked to risk of gastric cancer, of well-differentiated adenocarcinoma.[26] Application of a diet enriched with n-3 PUFA delayed tumor growth in a mouse xenograft model.[27] *In vitro* studies have shown that n-3 PUFA inhibited macrophage-enhanced gastric cancer cell migration and attenuated matrix metalloproteinase (MMP)-10 expression through ERK and STAT3 phosphorylation[28] and inhibited the growth of human gastric carcinoma cell via apoptosis and combination with 5-fluorouracil has synergetic effect in inhibiting the proliferation of gastric cancer cells.[29] Moreover, n-3 PUFA are beneficial for preventing oxidative stress-induced apoptosis by inhibiting apoptotic gene expression and DNA fragmentation of gastric epithelial cells.[30] On the other hand, DHA induced apoptosis of gastric cancer cells by inducing the expression of apoptotic genes in gastric cancer cells.[31] Although a large body of literature spanning numerous cohorts from many countries and with different demographic characteristics does not provide evidence to suggest a significant association between n-3 PUFA and stomach cancer incidence,[32] further studies are needed to investigate action of n-3 PUFA relevant to antitumor effects in the stomach.

3. Colorectal cancer

Colorectal cancer (CRC) is the second leading cause of cancer-related death in men after lung cancer and third in women behind lung and breast cancers in the United

States. Among the GI tract cancer, CRC cancer has raised the most attention over the past decades, as they share a long precancerous stage (the adenoma in CRC) which provides a window of opportunity to intervene and prevent development of cancer. Recently, n-3 PUFA have been recognized to have anti-tumor activity in colon cancer. There is also evidence suggesting improved efficacy and/or tolerability of conventional colon cancer chemotherapy when administered with n-3 PUFA.[9] Epidemiological studies about the association of dietary fat and cancer suggests a protective effect of n-3 PUFA and a promoting effect of n-6 PUFA on cancer. Although epidemiological studies of the association between fish intake, n-3 PUFA intake or blood n-3 PUFA levels and CRC risk have not consistently suggested beneficial effects of n-3 PUFA on CRC and other GI cancer risk. Dietary administration of one or both of the main n-3 PUFA in rodent models of colorectal carcinogenesis has been demonstrated to reduce colorectal tumor size and multiplicity, compatible with CRC chemopreventive activity.[33] In meta-analyses of prospective cohort studies that evaluated the association between fish consumption or n-3 fatty acids and colorectal cancer incidence or mortality, the pooled relative risks for colorectal cancer incidence were 0.96~0.97 (95% confidence interval: 0.92, 1.00) for each extra occurrence of fish consumption per week.[34] In a population-based prospective study on the association of n-3 PUFA and cancer, there was an inverse relationship between marine n-3 PUFA intake and the risk of colorectal cancer, but this association was only statistically significant in the proximal site of the large bowel.[35] Cockbain et al.[9] has reviewed a lot of *in vitro* and *in vivo* experimental studies and epidemiological observations providing strong indications of the cancer treatment and prevention of n-3 PUFA against colorectal cancer. Kim et al.[36] provided a significant dose-dependent reduction in CRC risk for total n-3 PUFA intake (OR=0.61 for the highest vs lowest quartile), as well as for EPA and DHA intake individually in a case-control study of 1,872 patients (929 cases of distal CRC and 943 controls). Pot measured and compared serum n-3 PUFA levels in 861 patients (363 cases of colorectal adenoma and 498 controls). There was a significant reduction in colorectal adenoma risk (OR=0.67) between low level of n-3 PUFA (<1.8%) and high level of n-3 PUFA (>2.3%).[37] Recently, Sorensen et al.[38] reported that n-3 PUFA is incorporated rapidly into colonic mucosa and colonic muscular layer in patients given 3g of n-3 PUFA daily for 7 days before surgery for colorectal cancer. The preventive effect of n-3 PUFA has been demonstrated in a number of carcinogen-induced models like AOM and dimethylhydrazine (DMH)-induced rat model and $APC^{Min/+}$ mouse models relevant to prevention of CRC including ours. Studies of rodents fed an n-3 PUFA-supplemented diet versus a control diet have consistently reported a 20-50% reduction in chemically induced tumor incidence, together with a 30-70% reduction in tumor multiplicity, in both carcinogen and $APC^{Min/+}$ mouse studies. Similar findings have been reported for studies of growth of human CRC cell lines such as HCT26, HT-29, HCT116, and DLD-1 grown as xenograft tumors in immunecompromised mice.[9]

n-3 PUFAs are likely to have multifaceted roles in both prevention and treatment of CRC. The excellent tolerability and safety profile of n-3 PUFAs combined with other health benefits, particularly cardiovascular, make n-3 PUFAs an attractive candidate for prevention and treatment of CRC.[9]

MECHANISMS OF N-3 PUFAS ON CHEMOPREVENTION

n-3 PUFA, especially, EPA and DHA, have been shown to have multiple anti-tumor actions. Current knowledge of the anti-tumor activity of n-3 PUFA has been comprehensively reviewed elsewhere.[9,16,39] Recently, Stephenson et al.[10] has reviewed more recent underlying mechanisms providing strong indications of the anti-tumor actions of n-3 PUFA on the hallmarks of cancer. Firstly, n-3 PUFA inhibits growth signal transduction. n-3 PUFA appear to down-regulate epidermal growth factor receptor (EGFR), protein kinase C (PKC), *Ras*, and NF-κB, insulin like growth factor (IGF), which are important cell signaling mediators often found to be elevated in carcinogenesis. Secondly, n-3 PUFA induces cancer cell apoptosis via modulation of peroxisome proliferator-activated receptors (PPARs), the Bcl-2 family, and NF-κB cell signaling. Thirdly, n-3 PUFA decreases sprouting angiogenesis by suppressing vascular

endothelial growth factor (VEGF)- and platelet derived growth factor (PDGF)-stimulated endothelial cell proliferation, migration, and tube formation and by inhibition of MMPs via NO production and NF-κB and β-catenin cell signaling. Fourthly, n-3 PUFA also decreases cell-cell adhesion via down regulation of Rho-GTPase, which inhibits cytoskeleton reorganisation, and reduction in intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 expression.

Cockbain et al.[9] has also proposed the four main anti-tumor actions of n-3 PUFA (i) modulation of COX activity; (ii) alteration of membrane dynamics and cell surface receptor function; (iii) increased cellular oxidative stress and (iiii) derived anti-inflammatory lipid mediators. Firstly, n-3 PUFA can act as an alternative substrate for COX-2, instead of AA, leading to a reduction in formation of pro-tumorigenic '2-series' PGs (PGE_2) in several cell types. It also binds the substrate channel of COX-2 and inhibits COX-2 activity. Secondly, incorporation of n-3 PUFA into cell membranes alters the fluidity, structure and/or function of lipid rafts or calveolae. Especially, the localization of cell surface receptors, such as G protein-coupled receptors (GPCRs), toll-like receptors (TLRs), and epidermal growth factor receptor (EGFR), in lipid rafts is believed to be crucial for downstream receptor signaling, controlling proliferation and apoptosis. Thirdly, n-3 PUFA may have an anti-tumor effect through alteration in the cellular redox state. n-3 PUFA can increase reactive oxygen species (ROS) because it is highly peroxidisable. Therefore, n-3 PUFA can induce cancer cell apoptosis via elevation of intracellular ROS levels. Fourthly, n-3 PUFA can be metabolized novel anti-inflammatory lipid mediators including resolvins, protectins and maresins. It is known that resolvins exhibit antineoplastic activity via anti-inflammatory and inflammation resolution activity in animal models of acute inflammation.

Besides these multiple anti-tumor actions, it is also reported recently that n-3 PUFA can activate Nrf2 and induced Nrf2-directed gene expression[40,41] and can suppress lipopolysaccharide-induced inflammation through induction of Nrf2 expression.[42] Nuclear factor erythroid 2-related factor 2 (Nrf2) is a redox-sensitive master regulatory transcriptional factor that plays an important protective role in cells by regulating cellular redox balance.[43] Moreover, n-3 PUFA significantly reduces oxidative stress-induced endothelial cell Ca^{++} influx. This effect might be associated, at least in part, with altered lipid composition in membrane lipid rafts.[44]

1. Useful animal model to study chemopreventive mechanism of n-3 PUFA: Fat-1 transgenic mice model

Recently, the engineered n-3 PUFA desaturase transgenic mice (*Fat*-1 mice), which can endogenously synthesize n-3 PUFA in their tissues, allows carefully controlled studies to be performed in the absence of potential confounding dietary factors.[45] The synthesis of n-3 PUFA is achieved through the expression of the *Fat*-1 transgene encoding for an n-3 desaturase, which utilizes n-6 PUFA as substrate. This allows production of high n-3/n-6 ratios in the animals, thus eliminating the potential diet variations. Hence, the *Fat*-1 transgenic mouse is a valuable *in vivo* system for elucidating the role of n-3 PUFA in carcinogenesis. Since *Fat*-1 mice were generated, Xia et al.[46] showed that melanoma formation and growth are reduced in *Fat*-1 transgenic mice. Nowak and Jia[47,48] reported that lower incidence and growth rate of colon tumors induced by DSS (dextrane sodium sulfate) plus AOM (azoxymethane) in the Fat-1 transgenic mice via its anti-inflammatory properties. Song et al.[49] also reported that the growth of pancreatic cancer *in vivo* was significantly reduced when mouse pancreatic cancer cells, PANC02 cells, were inoculated into the *Fat*-1 transgenic mice. Recently, Mohammed et al.[50] suggested the beneficial effects of n-3 PUFA for chemoprevention of pancreatic cancer using *Fat*-1 mice. They generated compound *Fat*-$1^{+/+}$-$Kras^{+/+}$ transgenic mice and showed a dramatic reduction in incidence of pancreatic ductal adenocarcinoma (84%; $P < 0.02$) in *Fat*-$1^{+/+}$-$Kras^{+/+}$ mice compared to $Kras^{+}$ mice. Besides of GI cancers, the growth of hepatocellular carcinoma (HCC) *in vivo* was significantly reduced in the *Fat*-1 transgenic mice.[51-53] Mice expressing MMTV-neu(ndl)-YD5 and *Fat*-1, which were bred with mouse mammary tumor virus (MMTV)-neu(ndl)-YD5 mice (an aggressive breast cancer model), displayed significant ($P < 0.05$) reductions in tumor volume (~30%) and multiplicity (~33%).[54] Recently, in our laboratory an *in vivo* study has shown a suppressive effect of

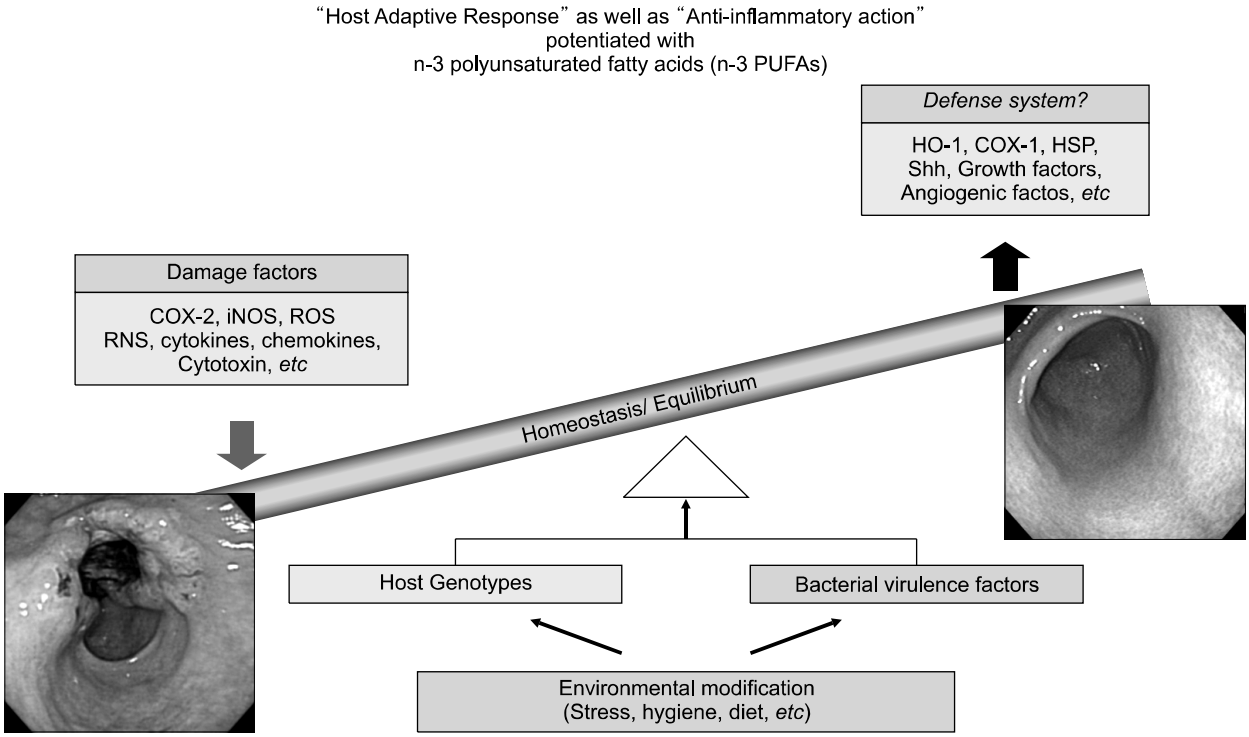

Fig. 2. Host adoptive and anti-mutagenic response enhanced with n-3 PUFAs.

Fat-1 mice in GI cancer development (unpublished data). *Fat*-1 mice were bred with the $APC^{Min/+}$ mouse colon cancer transgenic mice to generate compound *Fat*-1-$Apc^{Min/+}$ transgenic mice. A dramatic reduction in incidence of AOM-DSS induced colon adenocarcinoma in *Fat*-1-$APC^{Min/+}$ mice compared to $APC^{Min/+}$ mice was shown. Moreover, in the case of stomach cancer development, *H. pylori* initiated-, salt diet-promoted-gastric tumor was also reduced in *Fat*-1 mice. In conclusion, several studies using *Fat*-1 mice model indicate that balancing the tissue n-6/n-3 ratio could exert a significant effect on GI cancer development. The fat-1 mouse model allows carefully controlled studies to be performed in the absence of restricted diets, which can create confounding factors that limit studies of this nature.[55]

CONCLUSIONS

GI Cancer incidence and mortality are increasing in the Eastern world and a high n-6 to n-3 PUFA ratio in the Western style diet may be a contributing factor. There is much evidence to suggest that higher consumption of dietary n-3 PUFA is associated with a lower risk of GI cancer in animal models and humans. Especially, recent studies suggest that endogenous n-3 PUFA delay the progression of colon and stomach cancer and elevating n-3 PUFA may be an important strategy to delay/prevent gastrointestinal cancer in high-risk patients via various mechanisms mediating cancer prevention by n-3 PUFA (Fig. 2). In addition, using n-3 PUFA in combination with other agents with complementary antitumor action may improve their efficacy in GI cancer prevention. Recently, Manson et al. has started the vitamin D and Omega-3 Trial (VITAL), a large randomized, double-blind, placebo-controlled, 2×2 factorial trial of vitamin D and n-3 PUFA supplements in the primary prevention of cancer among a multi-ethnic population of 20,000 U.S. men aged ≥50 and women aged ≥55.[56] We expect that new findings in combination consumption of n-3 PUFA with other nutrients will provide new approaches to public health implications with regard to prevention of GI cancer through dietary and lifestyle interventions.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Ministry of Education and Science Technology (2010-0002052) and High Value-added Food Technology Development Program, iPET (Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries), Republic of Korea.

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