 doi:10.20944/preprints201804.0115.v1

Concept Paper

Omega-3 fatty acid supplementation – A possible dietary adjunct to enhance immune therapy in cancer?

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Abstract

Omega-3 polyunsaturated fatty acids (n-3 PUFA) have been found to be modulators of immune function. Additionally, they may affect the growth of colorectal cancer (CRC). With the advent of novel treatment approaches in oncology targeting immune checkpoint inhibition and aiming to boost the immune response against tumors the exact role of n-3 and n-6 PUFA in inflammation as well as in CRC needs to be re-evaluated in order to understand potential interactions with these new treatment paradigms. Interestingly, for the cyclooxygenase (COX) inhibitor aspirin a possible synergistic effect together with a PD1-Ligand antibody has been shown. However, could n-3 PUFA be disadvantageous in the context of immune tumor therapy due to an immune suppressive effect that has been described for these fatty acids in the past, or could they also enhance the effect of immune checkpoint inhibition?

In this paper, we discuss the current data regarding the immune modulatory as well as the anti-CRC effect of n-3 PUFA. Arguing towards an immune-activating effect of n-3 PUFA, we demonstrate the results of a pilot study. Here, we show that incubation of human peripheral blood mononuclear cells (PBMCs) with the n-3 PUFA docosahexaenoic acid (DHA) significantly decreases CRC-cell supernatant-triggered secretion of IL-10 and increases secretion of TNF-α, while the omega-6 polyunsaturated fatty acid (n-6 PUFA) arachidonic acid (AA) reduced TNF-α secretion. These changes in cytokine secretion upon incubation with DHA demonstrate a possible enhancing effect of n-3 PUFA on an anti-tumor immune response.

Keywords: Omega-3 and omega-6 polyunsaturated fatty acids; colorectal cancer; Cancer Immune Therapy.

Abbreviations: AA, arachidonic acid; ASA, acetylsalicylic acid; CM, conditioned media derived from human colorectal adenocarcinoma HT-29 cells; COX, cyclooxygenase; CRC, colorectal cancer; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LX, lipoxin; n-3, omega-3; n-6, omega-6; NSAID, nonsteroidal anti-inflammatory drug; PBMC, peripheral blood mononuclear cell; PG, prostaglandin; PUFA, polyunsaturated fatty acid; SEM, standard error of the mean; TX, thromboxane.

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1. Introduction

Aspirin and Cancer Immune Therapy in Colorectal Cancer

Colorectal cancer (CRC) is one of the most common cancers worldwide [1, 2]. Several epidemiological studies show that the long-term intake of acetylsalicylic acid (ASA, aspirin) and other NSAIDs reduces the incidence of CRC [3-7]. Moreover, aspirin-intake is associated with improved overall survival in CRC-patients [8, 9]. These beneficial effects are associated with the reduced conversion of the omega-6 polyunsaturated fatty acid (n-6 PUFA) arachidonic acid (AA) into biologically active eicosanoids [5, 10]. Experimental evidence suggests that CRC and other tumors evade immune surveillance and T-cell mediated immune response by an overproduction of prostaglandin E2 (PGE_2) [11, 12]. PGE_2 is established as pro-tumorigenic eicosanoid in CRC development [13-15] and oral treatment with aspirin decreases colonic tissue PGE_2 levels in healthy individuals [7] and in those suffering from CRC [16]. Thus, NSAIDs, such as aspirin, can help reverse immune evasion by reducing high levels of anti-inflammatory, tumor-promoting lipid mediators, such as PGE_2 [15, 17-21].

Recently, several studies have been published, which offer the prospect to a novel treatment of CRC by targeting cancer-immune checkpoints, such as the programmed cell death 1 (PD-1) pathway, in the tumor microenvironment [12]. The PD-1 pathway is a negative feedback system that suppresses the Th1 cytotoxic immune response. Its blockade with the PD-1 monoclonal antibody pembrolizumab can overcome immune resistance of tumor cells [22, 23]. In this context, Le *et al.* and others demonstrated that especially CRC with high-level microsatellite instability (MSI) are susceptible to the blockade of the PD-1 pathway [12, 22, 24, 25]. Indeed, the U.S. Food and Drug Administration (FDA) approved the anti-PDCD1 (PD-1) antibody pembrolizumab for treating solid tumors with high-level microsatellite instability (MSI) or mismatch repair deficiency, including MSI-high CRC. These have a large number of neoantigens due to a high number of (frameshift) mutations. Particularly for this CRC subgroup the concept of an anti-tumor immune response is well represented [26, 27]. The inflammatory reaction in the tumor-microenvironment is thought to represent the host's local immune response against infiltrating tumor cells [28]. Numerous studies concluded that the inflammatory infiltrate in and around the tumor correlates with improved survival [28-30]. Especially tumor infiltrating T lymphocytes and their subsets show strong associations with disease outcome. Thus, a pronounced infiltrate of cells positive for T-cell markers such as CD3, CD45RO (memory T-lymphocytes), and CD8 (cytotoxic T-lymphocytes) correlates with improved survival and a reduced recurrence of CRC [28, 31-33]. Galon *et al.* were able to demonstrate that type, density, and location of T-lymphocytes represent a more accurate predictor of survival than the widely used UICC-TNM classification [32]. Moreover, tumor associated macrophages, which are associated with poorer outcome in several other tumor entities, predominantly correlate with improved survival in CRC [28, 34-36].

Evidence for a synergistic effect of PD-1 immune checkpoint blockade and NSAID-mediated antitumor pathways has been provided by Zelenay *et al.* [21, 24]. These data show a pivotal role of PGE_2 in the context of antitumor immune surveillance (Figure 1). It was also shown that association of aspirin use with CRC survival is stronger in patients with tumors expressing a low PD1 ligand

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expression level. These findings suggest a differential antitumor effect of aspirin according to immune checkpoint status [11].

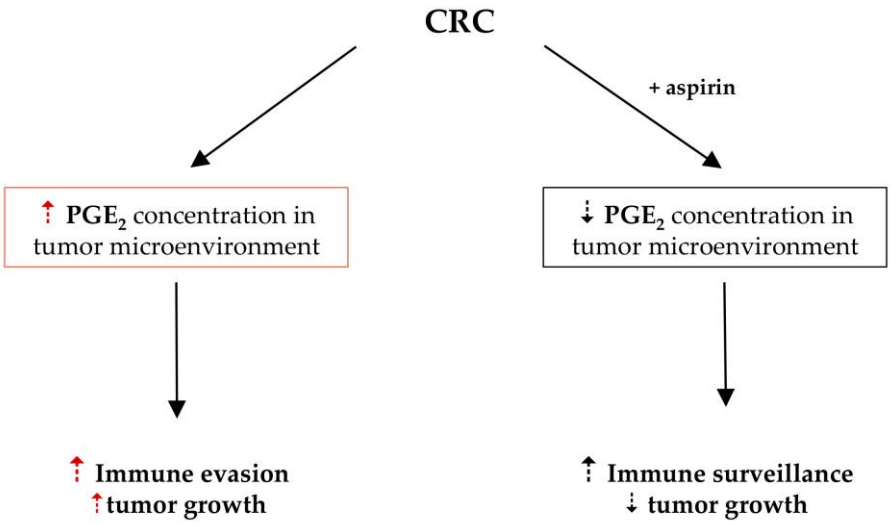

Figure 1. Effect of PGE_2 in colorectal cancer. Figure adapted and modified from [21].

2. Hypothesis

Omega-3 polyunsaturated fatty acids to enhance immune therapy in colorectal cancer?

Based on their systematic literature review, the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) expert panel concluded that high fish or marine n-3 PUFA (EPA and DHA) consumption possibly decreases CRC risk [37, 38]. PUFA are fatty acids, characterized by at least two carbon-to-carbon double bonds. Their classification is based on the position of the first double bond, counting from the methyl (omega) end [39, 40]. Thus, in omega-3 (n-3) PUFA, the first double bond is located at the third carbon atom, whereas in n-6 PUFA it is at the sixth carbon atom [41, 42]. Eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) are essential members of the n-3 series [43, 44]. Likewise, arachidonic acid (AA; C20:4n-6) has been found to be a crucial member of the n-6 series [45, 46]. A recently published meta-analysis including 19 case-control and 22 prospective cohort studies was able to conclude that intake of fish high in n-3 PUFA may significantly reduce the risk of CRC by as much as 12% [47]. Additionally, Hall and Kojima *et al.* observed an inverse correlation between n-3 PUFA blood levels and risk of CRC [48, 49].

Several clinical, animal, and *in vitro* studies have demonstrated the possible preventive and therapeutic role of n-3 PUFA against CRC [50] Thus, it was demonstrated that dietary supplementation with fish oil or EPA significantly reduced crypt cell proliferation and increased apoptosis in patients with colorectal adenomas [51, 52]. Additionally, the *fat-1* transgenic mice model provides evidence that n-3 PUFA and their metabolites are likely to suppress colitis-associated

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cancer development [53-56]. These mice carry a transferred gene, which encodes for a fatty-acid desaturase enzyme that converts n-6 to n-3 PUFA, resulting in a low n-6 to n-3 ratio of almost 1 [53]. In a study with dextran-sodium-sulfate-induced colitis-associated colon cancer, *fat-1* mice showed a reduced number of colonic adenocarcinomas, elevated apoptosis, and enhanced ability to resolve chronic colitis [55]. An animal model using mice with an APC gene defect ($Apc^{Min/+}$ mice) reported similar results for EPA [57]. Moreover, several *in vitro* experiments conducted with CRC cell lines demonstrated anti-proliferative effects of EPA and DHA [58-61].

These studies have also elucidated the multiple molecular pathways by how n-3 PUFA may modulate cancer development. Previous reviews by us and others summarized and critically analyzed these pathways [50, 62-64]. Several of the effects associated with n-3 PUFA, such as EPA and DHA, as well as n-6 PUFA (e.g. AA) are believed to be mediated through action of lipid mediators [44, 65-69]. AA-derived PGE_2, in particular, has shown to be pro-tumorigenic as well as instrumental in tumor immune evasion [13, 14, 21]. Moreover, in a previous study we demonstrated the ability of DHA to reduce proliferation as well as formation of PGE_2 in CRC cells [70].

Many observations point towards polyunsaturated fatty acids (PUFA) modulating the immune response in the context of colon cancer, possibly mediated by their lipid mediators. These are synthesized through several enzymatic pathways, including COX, lipoxygenase (LOX), and cytochrome P-450 (CYP) monooxygenase pathways [71-73]. Past decades have seen a great number of studies on the functions of AA-derived prostaglandins (PGs), leukotrienes (LTs), lipoxins (LXs), and thromboxanes (TXs) [74]. This interest is largely due to the well-established role of these metabolites in several pathological processes, including inflammatory disorders, cellular proliferation and thrombosis [69, 75].

The n-6 PUFA AA promotes a predominantly pro-inflammatory state, whereas EPA and DHA exert a modulating influence on immune cells [75, 76]. Indeed, AA-derived LTs and PGs can act as potent pro-inflammatory lipid metabolites (depending on cell type and receptor) [75, 77-81]. EPA and DHA, on the other hand, inhibit synthesis of AA-derived, pro-inflammatory eicosanoids such as PGE_2 [82]. Moreover, n-3 PUFA are also precursors to anti-inflammatory lipid mediators, such as resolvins (RVs) and protectins (PDs) (Figure 2) [42, 76, 77, 83]. In this context, n-3 PUFA and their derivatives have shown to decrease activation of nuclear factor kappa B (NFκB), a major transcription factor for the upregulation of genes involved in the inflammatory process [46, 84-86]. Activation of NF-κB results in the secretion of pro-inflammatory cytokines (e.g., IL-1, IL-2, IL-6, IL-12, and TNF-α), adhesion molecules, and the expression of inducible enzymes, such as COX-2 [76]. N-3-PUFA-supplementation and *in vitro* studies demonstrated that n-3 PUFA can suppress the secretion of IL-1, IL-2, IL-6, and TNF-α from immune cells [87-92]. EPA- and DHA-derived lipid mediators, such as RVs, PDs, and maresins (MaRs) as well as 18-HEPE and 17-HDHA can reduce inflammatory parameters in animal and *in vitro* studies [66, 93-98].

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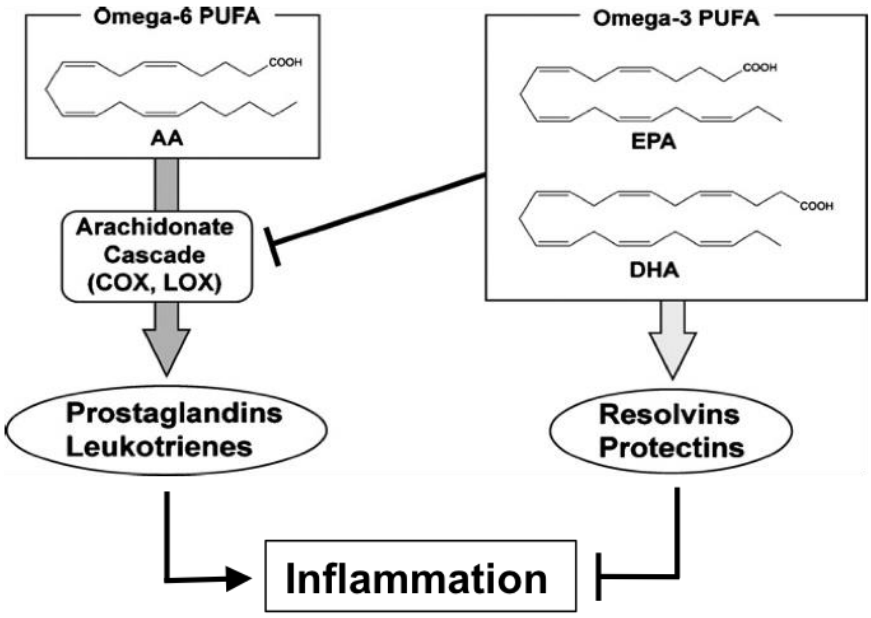

Figure 2. Possible mechanisms of PUFA pro- and anti-inflammatory actions. N-3 PUFA prevent the conversion of AA into pro-inflammatory eicosanoids, such as AA-derived PGs and LTs. In addition, EPA and DHA are precursors to potent anti-inflammatory lipid mediators, such as resolvins and protectins. Figure adapted and modified from [78, 83].

Due to the demonstrated effects of n-3 and n-6 PUFA on the immune system, these fatty acids could affect the immune response to tumors. Particularly in light of the previously published findings of immune-based therapies in CRC patients, and the paradigm of immune-activation as anti-cancer treatment approach, it is now pertinent to reassess the possible effects of n-3 and n-6 PUFA on immune cell activity in this context: Do these fatty acids suppress immune function including anti-tumor immune reactions, or could they even have immune-stimulatory effects in the tumor microenvironment, supporting Cancer Immune Therapy?

As outlined above, high levels of PGE_2 play an important role in mediating effects of AA [80, 99-101]. PGE_2 inhibits phagocytosis and the TLR-dependent activation of TNF-α secretion via the IL-1R–associated kinase-M [100-106]. A decrease in PGE_2 therefore seems to be a plausible mechanism for immune-modulating effects of DHA and EPA (Figure 2). In line with the possibility to transfer the anti-tumor paradigm stated by Zelenay *et al.* [21] for aspirin to the n-3 PUFA, an increase in the dietary n-3 to n-6 PUFA ratio was found to not only correlate with higher TNF-α secretion but also with significantly lower levels of AA-derived PGE_2 in immune cells [102] as well as colon cancer cells [70]. Indeed, several studies with n-3 PUFA emphasize their inhibitory action on the synthesis of PGE_2 [102, 103, 107-109], supporting the hypothesis of increased tumor immune surveillance due to this PGE_2-suppressive n-3 PUFA effect.

Similarly, incubation of murine peritoneal macrophages with AA potently inhibited LPS-induced TNF-α production [110]. It was observed that concomitant treatment of these macrophages with AA and indomethacin (inhibiting the synthesis of PGs) restored 90% of the TNF-α concentration, which indicates that AA exerts an inhibitory effect on TNF-α secretion via increased PG-levels.

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In our hands, to directly test for effects of n-3 and n-6 PUFA on differentially induced cytokine secretion by human PBMCs we incubated cells derived from healthy donors with two different stimuli, (1) LPS to mimic activation by bacterial products and (2) colon tumor cell conditioned media to mimic activation by tumor cells (Figure 3). This small study showed that DHA significantly reduced LPS-triggered IL-10 secretion by PBMCs (Figure 3A). Interestingly, DHA had a more pronounced effect on cytokine secretion that was induced by conditioned media derived from human colorectal adenocarcinoma HT-29 cells (CM): CM reduced secretion of IL-10 while increasing TNF-α levels (Figure 3B). The n-6 PUFA AA, on the other hand, reduced TNF-α secretion by PBMCs stimulated with LPS as well as with CM (Figure 3A and B, respectively). In analysis of variance (ANOVA), when compared to PBMCs treated with AA, LPS- as well as CM-induced TNF-α secretion was significantly higher in cells incubated with EPA or DHA ($p < 0.05$). TNF-α is a typical pro-inflammatory cytokine, while IL-10 has been shown to exert effects limiting cytotoxic T-cell action [111-118]. Considering these findings, our results suggest that incubation of PBMCs with DHA results in a more aggressive immunological response against tumor cells, while AA could be associated with an immunosuppressive effect in the context of tumor immunity.

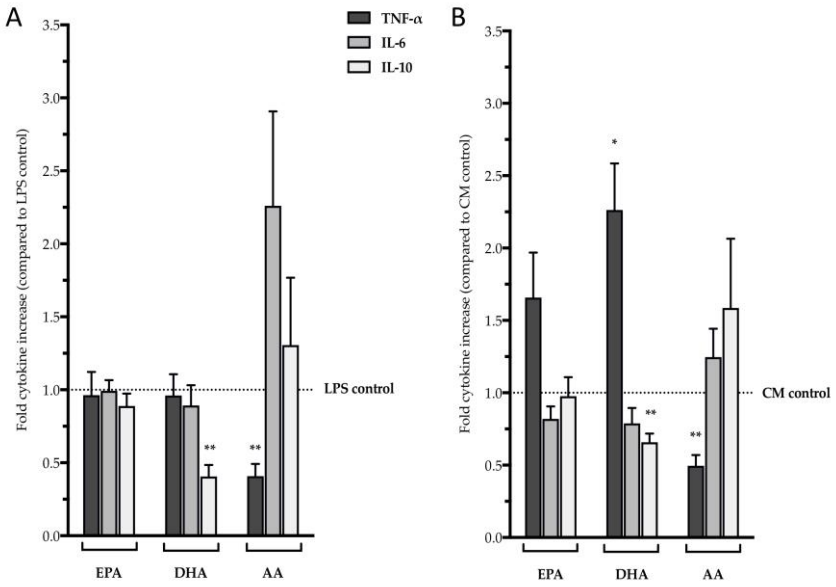

Figure 3. Effect of three major n-3 and n-6 PUFA (EPA, DHA, and AA) on cytokine secretion by PBMCs. (a) LPS-induced cytokine secretion; **(b)** Cytokine secretion induced by conditioned media derived from human colorectal adenocarcinoma HT-29 cells (CM). This small experimental series was established to explore the immunomodulatory effects of n-3 and n-6 PUFA with regard to a possible effect of CRC tumor cells. For this, PBMCs were isolated from leukocyte depletion filters, acquired from adult blood bank donors. After incubation with EPA, DHA, or AA, PBMCs were stimulated with LPS (a) or CM (b). Subsequently TNF-α, IL-6, and IL-10 secretion was measured using ELISA. For controls PBMCs stimulated with LPS or CM, without prior incubation with PUFA, were used (for a detailed description of materials and methods used, refer to supplementary data). Data is expressed as the relative mean + SEM of 5 PBMC donors as compared to LPS and CM control, respectively. *$p < 0.05$, **$p < 0.01$.

However, the implications of the observed changes in cytokine secretion are not entirely clear for several reasons. For one, TNF-α has shown to exert ambivalent effects on cancer cells, depending on the activation of intracellular pathways [119, 120]. Also the role of IL-10 in the context of cancer is

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controversial: While many data show that Il-10 can reduce antigen-specific T-cell activation and induce T-cell anergy [118, 121] and might thus be a pro-tumorigenic inflammatory mediator [122], recent data demonstrate an important role for IL-10 in effective immune surveillance of tumor cells [123, 124].

3. Conclusion and Outlook

The primary prevention of CRC by long-term NSAID-intake, in particular aspirin, is believed to be caused by a reduced conversion of AA into biologically active eicosanoids such as PGE_2, which has been shown to contribute to immune evasion by tumor cells [15, 17-21]. Additionally, the recently published studies on immune checkpoint inhibitors demonstrate the clinical effectiveness of increasing an anti-tumor immune response as a novel treatment approach in CRC [123-126]. Indeed, the data reviewed above indicate a possible supporting effect of aspirin in the context of immune checkpoint inhibitor use for cancer therapy [21].

Our summary presented here raises the possibility of a pro-immunogenic effect of n-3 PUFA in the context of the immune system's response to cancer (Figure 4). This could be of particular interest with the advent of immune checkpoint inhibitor therapy in oncology as this implies the possibility of an enhancing effect of n-3 PUFA in the context of these therapeutic interventions.

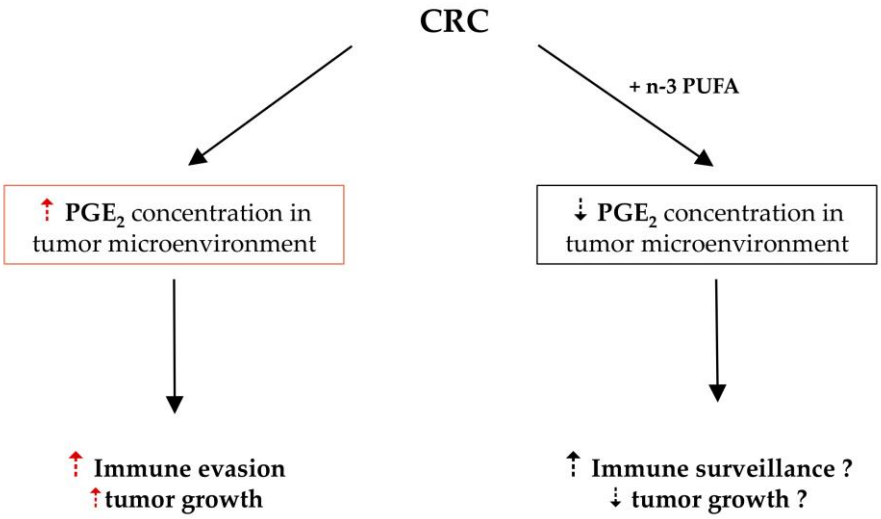

Figure 4. Possible effect of n-3 PUFA in colorectal cancer. In analogy to the aspirin effect increasing the anti-tumor immune response [21] n-3 PUFA might have a similar effect.

We therefore propose future studies, in experimental (animal) models as well as in the clinical setting, to test for an enhanced anti-tumor effect of the combination of high n-3 PUFA supplementation with cancer immunotherapy as compared to immunotherapy in the context of a high n-6 PUFA environment.

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