Lipid Inflammatory Mediators in Cancer Progression and Therapy

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10.1 Introduction

Leukocyte infiltration of tumors can have either a pro-tumorigenic or tumor-inhibitory functions. As an example tumor-associated macrophages (TAMs) have tumoricidal activity and can induce antitumor T-cells; but, can also suppress cytotoxic T-cell responses capable of inhibiting tumor growth (Fig. 10.1). Myeloid cell infiltration of tumors is associated, in part, with tumor-derived cytokines, GFs, chemokines, and expression of immune checkpoint molecules that regulate the expansion of myeloid progenitors within the marrow and at extramedullary sites and to an extent within the tumor (Fig. 10.2). Numerous studies have demonstrated that activated macrophages can kill tumor cells *in vitro*. However, macrophage infiltration of tumors is predominately, a pro-tumorigenic/tumor-progressive phenotype [1]; although, some human studies have been equivocal [2]. Indeed, most studies have found no relationship between immunogenicity, metastatic propensity and infiltrating TAM frequency [3–5]. Despite this lack of an immune correlation, TAM infiltration is associated with a poor prognosis [6] and rapid tumor progression [7, 8]. Myeloid-derived suppressor cells (MDSCs) have also been identified in the circulation of tumor bearing (TB) hosts and to infiltrate tumors [9–13]. The immunosuppressive activity of MDSCs (both murine and human) occurs through multiple mechanisms including the upregulation of reactive oxygen species (ROS), nitric oxide (NO) production and arginase levels, as well as the secretion of immunosuppressive cytokines [14]. Preclinical studies have shown that MDSCs can control tumor growth [3, 15], while immune augmenting type-1 macrophages (M1) and/or dendritic cells (DC1) cells contribute to the induction of an antitumor T-cell response, although their presence is not sufficient for tumor rejection [16]. M1 macrophage depletion or an increase in infiltrating M2 macrophages, DC2s, and MDSCs are associated with a poor prognosis and increased tumor relapse post resection.

Lymphocytes also infiltrate tumors (Fig. 10.1) and the associated adaptive immune response has a positive prognosis. However, the infiltrating lymphocytes can also be T-cell suppressive. Thus, while T-cells have the potential to kill tumor cells, frequently they are of low frequency and avidity [17], and cannot control tumor growth [18]. Nonetheless, increased T-cell infiltration of

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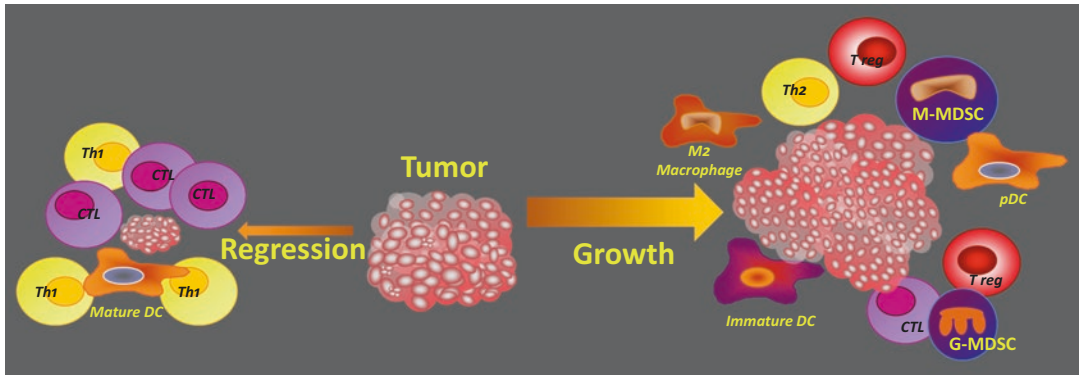

Fig. 10.1 The leukocytes infiltrating tumors regulates their growth and progression. Tumor regression is associated with infiltration by mature dendritic cells (DCs), cytotoxic T cells (CTL) and type 1T-helper cells (Th1). Contrasting with this, tumor growth is facilitated by immune mediated immunosuppression and neoangiogenesis by immature DCs, myeloid-derived suppressor cells, (MDSCs) plasmacytoid DCs, (pDCs) M2 macrophages, as well as T regulatory (T-reg) cells and a low frequency of CD4 and CD8 effector T cells. The expansion of myeloid cell proliferation, including immunosuppressive populations, is regulated by colony stimulating factors (CSFs), chemokines and dietary w-6 PUFA

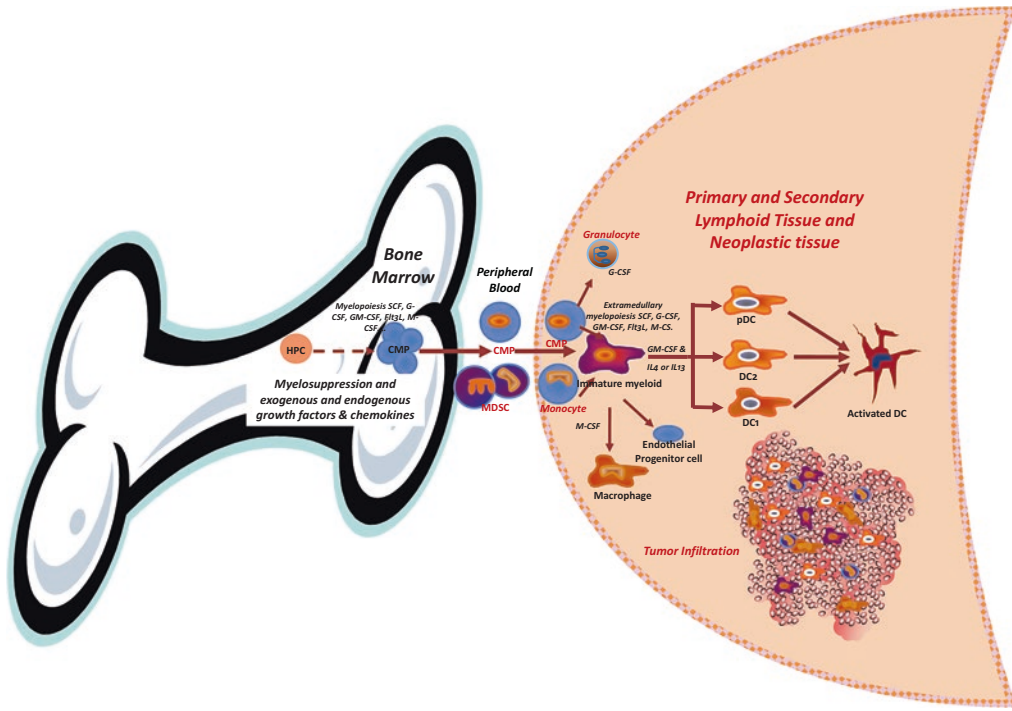

Fig. 10.2 Tumors secrete growth factors that expand and mobilize committed myeloid progenitors (CMP) and hematopoietic progenitor cells (HPC) from the marrow to extramedullary sites of myelopoiesis including the spleen, liver, lungs and primary and metastatic tumor lesions. Diets with increased levels of ω6 polyunsaturated fatty acids (PUFA) can increase myeloplasia largely as an extramedullary process. These CMPs can mature into dendritic cells (DCs), myeloid derived suppressor cells (MDSCs); both monocytic (M) and granulocytic (G), monocytes, endothelial progenitor cells and macrophages including tumor-associated macrophages (TAMs), as well as become activated, or "paralyzed", within the tumor environment. DC1 and DC2 are dendritic cell subsets that are immune augmenting and suppressive respectively. Dependent upon the infiltrating subset and extent of maturation and activation, these cells are critical components and regulators of immune suppression, angiogenesis, vasculogenesis, and tumor regression or growth

tumors is associated with an improved outcome [19–26], and an increased understanding of infiltrating T-cell phenotypes and their functions has resulted in an improved understanding of their prognostic potential. However, some tumor cells express checkpoint molecules that downregulate immune responses. Myeloid cells, including macrophages, PMNs and MDSCs, can also express immunosuppressive checkpoint mediators, such as PD-L1 [27], providing another mechanism to down regulate T-cell proliferation and function. Consequently, although anti-tumor T cells are present in the tumor microenvironment their anti-tumor activity may be limited. However, antibodies that inhibit immune checkpoints are demonstrating efficacy in reactivating anti-tumor T cell responses [28].

10.2 Immune Cell Infiltration of Tumors

The hypothesis that hematological markers of systemic inflammation, in particular the neutrophil–lymphocyte ratio (NLR), can predict survival in tumor bearing patients has recently received much interest. Many groups have investigated the prognostic value of the NLR in a variety of tumors and at different disease stages. To date, over 60 studies (>37,000 patients) have examined the clinical utility of the NLR to predict outcomes [29]. There is also an emerging relationship between proinflammatory cytokines in the plasma of patients with elevated NLR (>5) and the tumor microenvironment. A number of studies have measured circulating cytokines together with the NLR [30, 31] providing insight into the mechanisms underlying the NLR, including one study that documented an elevated NLR associated with an increased peritumoral infiltration of macrophages [30]. Together, these observations suggest that the NLR reflects, at least in part, the up-regulation of innate immunity providing easily measurable biomarkers that can predict OS and PFS in cancer patients.

The interactions between tumor infiltrating immune effector cells takes place primarily around the tumor. Thus, while the NLR may have prognostic significance, specific subsets of infiltrating cells, as discussed above, may prove more informative. Specifically, cytotoxic $CD8^+$ lymphocytes, as a component of tumor-specific adaptive immunity, may constitute a critical mediator. Further, the T-cell suppressive nature of myeloid cells, including MDSCs, M2 macrophages, and DC2s suggests the potential sensitivity and criticality of the myeloid cell-to-$CD8^+$ lymphocyte ratio in tumor tissue. A few studies have undertaken such analyses observing, for example, that $CD66^+$ myeloid cells provide an independent prognostic factor for poor disease free survival (DFS) and overall survival (OS) [32]. This observation has been extended by the analysis of infiltrating NLR (iNLR) as a CD66b:CD8 cell ratio with the observation of a relationship with a cumulative incidence of relapse, OS and tumor stage [33]. As discussed below, a patient's lifestyle, both preceding and following diagnosis, can contribute to not only cancer initiation and progressions but also outcome. Thus, hosts eating a high-fat diet, or one with a high level of saturated fat or ω-6 PUFAs generally have an inflammatory phenotype with neutrophilia, which may contribute to cancer development and poor outcomes. Conversely, and with little data to date, diets with a high ω-3 PUFA content have been associated with decreased inflammation and extramedullary myelopoiesis, and potentially improved clinical outcomes. We posit, herein, that dietary ω3 PUFA may also increase infiltrating T-cells thereby contributing to improved clinical outcomes.

10.3 PUFA Regulation of Inflammatory Cells in Rodents

Several lines of evidence suggest that the dietary PUFA composite can influence inflammatory or anti-inflammatory cellular responses. Fatty acids

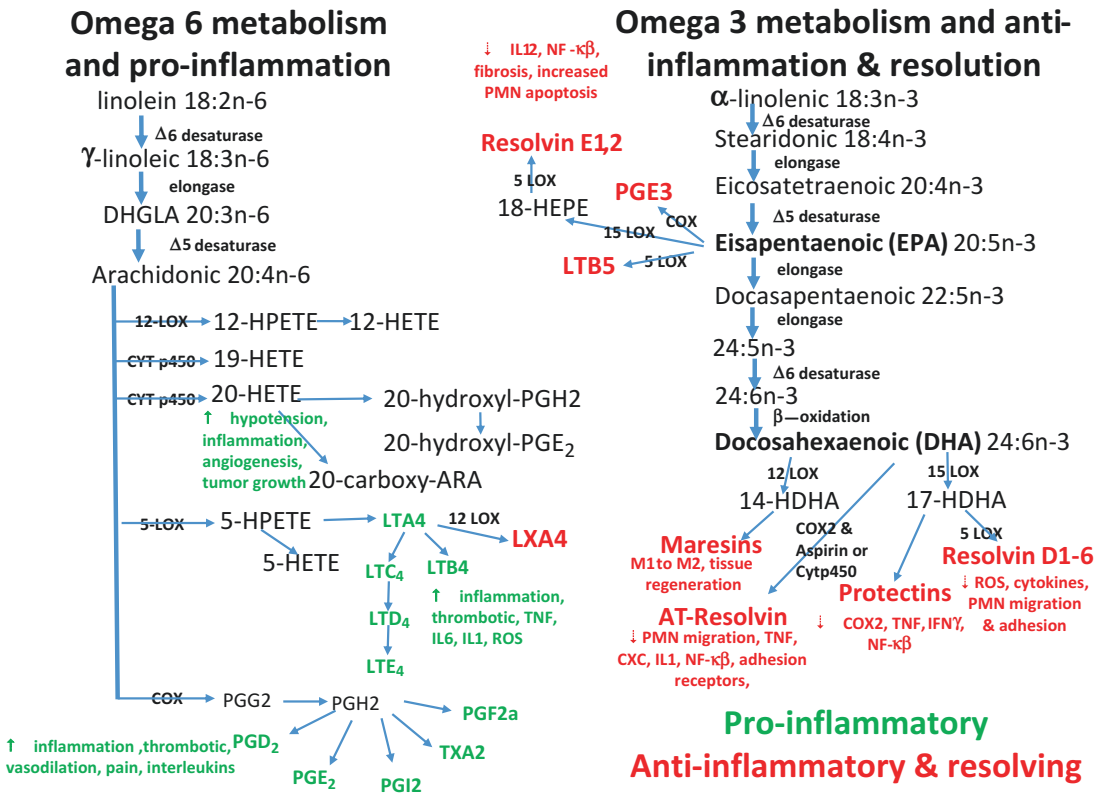

Fig. 10.3 Outline of the eicosanoid and resolvin related mediator synthesis pathways from arachidonic acid (AA) and alpha linolenic acid and their inflammatory and anti-inflammatory activities. *COX* cyclooxygenase, *CYT p450* cytochrome p450, *CXC* CXC chemokines, *HETE* hydroxyeicosatetraenoic acid, *HDHA* hydroxyldocosahexaenoic acid, *HPETE* hydroperoxyeicosatetraenoic acid, *HPDHA,* hydroperoxydocosahexaenoic acid, *HPEPE* hydroperoxyeicosapentaenoic acid, *IL* interleukin, *IFN* interferon, *LOX* lipoxygenase, *LT* leukotriene, *LX* lipoxin, *PG* prostaglandin, *PMN* polymorphonuclear leukocytes, *ROS* reactive oxygen synthetase, *TNF* tumor necrosis factor, *TX* thromboxane

from animal sources, mainly contain saturated fatty acids (SFAs) or ω6 PUFA. In contrast, fatty acids derived from some plant-based oils, and certain types of fatty fish consist mainly of ω3 PUFA. Recent studies have suggested that diets rich in ω6 PUFAs increase the risk of inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease, and asthma [34]. In contrast, diets rich in ω3 PUFAs have anti-inflammatory effects as supported by a decreased risk and control of these diseases [34]. PUFAs can be oxidized to generate either pro-inflammatory or pro-resolving lipid mediators (Fig. 10.3). These mediators have potent immune modulatory capacities and are generated rapidly during an inflammatory response [35]. Pro-inflammatory mediators, including prostaglandin (PG)s and leukotrienes (LTs), are induced in response to "foreign" materials and when they are cleared, pro-resolving lipid mediators restore normal tissue homeostasis [36]. Diets rich in ω3-PUFAs such as α linolenic acid (ALA, **18:3n-3**), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are associated with a decreased incidence and severity of inflammatory diseases [37]. The beneficial effects of these dietary FAs include anti-inflammatory metabolites such as a subset of PGs, LTs, thromboxanes, resolvins and lowered levels of inflammatory cytokines. However, the activities of ω3-PUFA contrast with other FAs that differ mainly in the position of their double bonds in the acyl chain, such as linoleic acid (LA) and arachidonic acid (AA) found with ω6-PUFA containing diets and their corresponding metabolites (Fig. 10.3).

Omega-3 PUFAs are anti-inflammatory in part by modulating the metabolism of inflammatory eicosanoids, cytokines, ROS and the expression of adhesion molecules [38]. EPA and DHA dietary supplementation has proven effective in decreasing intestinal damage and improving gut histology in inflammatory bowel disease [39], as well as, decreased joint pain, number of tender and swollen joints, and duration of morning stiffness in patients with arthritis [40]. Due to these responses, the effects on the immune response in various organs has been the subject of recent review articles [41].

10.4 PUFA and Immune Function

Studies using both ω6 and ω3 PUFA in rodent dietary studies, have documented different effects depending on the type of study (*in vitro* or *in vivo*), and the response measured. *In vitro* studies with ALA have shown an enhanced secretion of superoxides from neutrophils and macrophages [42], resulting in neutrophil adhesion to endothelial cells [43] promoting pro-inflammatory effects. However, ALA has also been shown to inhibit the proliferation of rodent and human lymphocytes following mitogen stimulation [44] suggesting that ALA may also be immunosuppressive. Studies where rodents were fed a high-fat diet, rich in ALA resulted in decreased mitogen-stimulated lymphocyte proliferation and NK cell activity [45].

In vitro studies using the ω6 PUFA; AA, have documented inflammatory properties including enhanced superoxide release [42], neutrophil adhesion to endothelial cells [43], and IL-1β production by macrophages [46]. Feeding mice a diet with high levels of ω-6 PUFA has been shown, in a dose dependant manner, to result in increased levels of LTE_4 and PGE_2 following in vivo stimulation with zymosan [47]. In a recent study, diets high in AA were shown to increase angiotensinogen, IL6 and MCP-1 levels in response to the proinflammatory transcription factor; nuclear factor κβ (NFκβ) stimulation [48].

A number of studies have shown that the ω3 PUFA, ALA inhibits the proliferation of rodent and human lymphocytes in vitro [44, 49, 50]. Studies where rats were fed an oil with a high ALA composition (linseed oil, 100 g/kg diet) for 8 weeks, a decrease in superoxide production by peritoneal macrophages in response to phorbol esters, was observed [51]. However, rodents fed linseed oil also had an increase in TNF secretion by resident macrophages, but no effect on TNF production by inflammatory macrophages [52]. Thus, the precise effect of the w-3 PUFA, ALA on lymphocyte functions appears to depend on the levels of ALA and the total PUFA content of the diet [53].

Because dietary fish oil leads to decreased PGE_2 production, it has been suggested that ω3 PUFAs should have anti-inflammatory activities, enhance the production of Th1-type cytokines, increase MHC II expression, lymphocyte proliferation and NK cell activity, as well as, decrease IgE production. Culture of human neutrophils with EPA or DHA has been shown to inhibit superoxide production and phagocytosis [54]. Similarly, the incubation of murine peritoneal macrophages with EPA or DHA inhibits expression of MHC II [55]. In a study, in which human monocytes were incubated with either EPA or DHA, both were shown to decrease the proportion of HLA-DR or -DP positive monocytes in response to IFN-γ [56] resulting in a reduced ability to present antigen [57]. The addition of fish oil to rodent diets can also decrease superoxide and hydrogen peroxide production by macrophages [58]. As compared to safflower oil, the addition of fish oil to murine diets results in lower peak plasma levels of TNF-α, IL-1β, and IL-6 following endotoxin injection [59]. Furthermore, parenteral nutrition that includes fish oil can decrease serum TNF-α, IL-6, and IL-8 levels in rats with burns compared with animal given ω6 PUFA–rich parenteral nutrition [54]. However, the majority of rodent studies with dietary fish oil use a diet in which EPA plus DHA comprise up to 30% of dietary fatty acids and up to 12% of dietary energy. The conclusions from these studies have been refined by studies in rats and mice that have indicated that relatively low levels of

EPA or DHA at a level of 4.4% of total fatty acids or 1.7% of dietary energy are sufficient to provide anti-inflammatory activities [60].

10.5 Clinical Anti-Inflammatory Activity of ω3 PUFA

There have been a number of clinical trials assessing the benefits of dietary supplementation with fish oil for the treatment of inflammatory diseases in humans, including rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, lupus, and multiple sclerosis [61]. Many of the placebo-controlled, double-blind trials of fish oil in chronic inflammatory diseases have shown significant benefits, including decreased disease activity and a lowered use of anti-inflammatory drugs. The evidence for a beneficial effect of fish oil is strongest in rheumatoid arthritis, where ω3 PUFA has been found to cause a concentration-dependent decrease in enzymes that degrade cartilage, expression of COX-2, but not COX-1, and TNF-α and IL-1β expression in cultured articular cartilage chondrocytes [62]. The mechanisms by which ω3 PUFAs have a beneficial effect in patients with arthritis has been postulated to be a competition with the canonical ω6 substrate AA resulting in eicosanoids that are less potent at inducing inflammation [63]. Recent observations have shown that ω3 PUFAs can be enzymatically converted to novel bioactive lipid mediators, termed resolvins, protectins and maresins, which promote the resolution of inflammation and that are log- orders more potent than their lipid precursors [64]. These observations have generated a paradigm shift documenting that the resolving phase of inflammation is not a passive process, but is actively 'switched-off' via endogenous anti-inflammatory mediators [65]. This contrasts with ω-6 PUFA associated metabolites, PGD_2, LTC_4, LTD_4, and LTE_4, which mediate pulmonary inflammation in asthma and are major mediators of asthmatic bronchoconstriction. AA is a precursor to LTs, which promote allergic inflammation, PGE_2 also regulates macrophage and lymphocyte function. Thus, it has been suggested that increased dietary intake of the w-6 PUFA LA, as the precursor of AA, is causally linked to allergic diseases and suggests a potential treatment focus for ω3 fatty acids [66].

10.6 PUFA Modulated Inflammation and Neoplasia in Rodent Tumor Models

As discussed above, clinically there have been varying associations between PUFA consumption/composition and inflammation; but there are many confounding factors including genetic susceptibility, tissue microenvironments, stress, obesity, age and duration. Murine models have identified a number of mechanisms in the association of dietary PUFA and tumor initiation and progression focused on systemic and tissue inflammation. Inflammation at tumor initiation can be regulated by risk factors, including hormones, obesity and age. However, following tumor initiation, inflammation is modulated by tumor growth in addition to existing risk factors. Thus, inflammatory microenvironments are created by cross talk between tumor-secreted GFs and host immunity.

Using mammary tumors as an example, the cellular microenvironment of mammary glands incorporate hormonal responsive epithelial cells, stromal cells, as well as, immune cells, in association with adipose tissue, that can result in an endocrine as well as an inflammatory organ [67]. The role of inflammation in tumorigenesis is supported by the evidence of a progressive increase in infiltrating inflammatory cells, which include activated macrophages and granulocytes, during the progression from normal tissue to dysplastic cells, which are believed to support tumor initiation [68].

The effect of dietary PUFA in tumor progression and metastasis has been studied in animal, and xenograft models of mammary cancer. In a xenograft model using MDA-MB-435 injected athymic nude mice given diets of either LA, EPA or DHA, significant retardation of tumor growth and metastasis was observed in the mice given EPA or DHA including a reduction in AA levels in tumor membrane phospholipids [69]. Further

when EPA and DHA were given as a neoadjuvant therapy, prior to tumor excision, pulmonary metastases were significantly suppressed compared to mice maintained on a LA diet [70]. Similar immune-augmenting and therapeutic activities were observed in R3230RC and MCF-7 mammary adenocarcinoma models [71, 72]. These anti-inflammatory activities may also include the regulation of MDSCs that can inhibit both non-antigen specific and antigen-specific $CD4^+$ and $CD8^+$ T-cell responses. The mechanisms of MDSC immunosuppression are diverse, including up-regulation of ROS, NO, and L-arginine metabolism, as well as immunosuppressive cytokines. In one tumor survival study, mice were switched from an 8% corn oil (1% ALA) diet to an 8% canola oil (10% ALA) diet, when the mice had an average primary tumor volume of 60 mm^3. In these studies tumor growth was significantly lower in mice fed the ω-3 based canola oil diet compared to the ω-6 based, corn oil cohort [73].

Interventions using ω-3 PUFA in chemically induced mammary tumor models support the results from xenograft tumor models. In a 7, 12-dimethylbenz (α) anthracene (DMBA) induced mammary tumor model, a fish oil diet significantly reduced tumor incidence, growth and metastasis [74, 75]. The effect of an ω-3 diet on tumor induction and growth correlated with reduced AA serum levels, protection against DNA single strand breaks, suppressed tumor cell proliferation; c-Myc and HER-2/neu expression and an increase in the apoptosis markers Bcl-2 and Bax [75–77]. Similarly, in a model of N-methyl-N-nitrosourea (MNU)-induced rat mammary tumors, the activity of dietary fat compositions including, saturated fatty acid (SFA), monounsaturated fat (MUFA), ω−6 PUFA alone or different ratios of ω−6:ω−3 PUFA were studied. It was found that a 1:1 ratio of ω−6:ω−3 PUFA was more effective in the prevention of mammary tumor development as compared to the other dietary cohorts, by decreasing mRNA expressions of fatty acid synthase, cyclooxygenase-2 (COX-2), and 5-lipoxygenase (5-LOX) in mammary tissues and decreasing peroxisome proliferator-activated receptor gamma (PPAR-γ) levels [78]. Together, these studies directly support a role for ω-3 PUFA in modulating an inflammatory tumor microenvironment by the up regulation of PPAR-γ [77, 78]. When the ω-3 PUFA content was significantly increased to a ω−6:ω3 ratio of 1:14.6 compared to 1:0.7, a 60% reduction in tumor growth was observed. This was associated with decreased cyclin-D1 and phospho-retinoblastoma protein expression and increased levels of cyclin-dependent kinase inhibitors, CIP1 (p21) and KIP1 (p27), an increased apoptotic index, reduced inflammation and *mammalian target of rapamycin* (mTOR) activity [79]. In an orthotopic 4 T1 mammary tumor model, 5% fish oil was used as therapy beginning when hosts had primary tumors that were 8–10 mm^3 and documented a significant reduced tumor growth and metastasis, which was correlated with inhibition of cancer cell proliferation [80].

The ability of ω3 PUFA to downregulate inflammatory mediators and increase apoptotic proteins emphasizes the importance of exogenous regulation of the tumor microenvironment. However the mechanism of regulation is not clear. In-vitro studies, have focused on cellular phenotypes and the effect of ω3 PUFA on inflammatory cells in both LPS and tumor induced inflammation. The majority of these studies have focused on inflammatory pathway factors. Although ω3 PUFA has anti-inflammatory effects in inflammatory diseases including cancer, its regulation of MDSCs, which is a critical regulator of the tumor microenvironments is understudied. Further, the majority of murine models, involve diets that are isocaloric but fully equivalent, raising the question of obesity verses dietary constituents. Since obesity itself is an inflammatory disorder, ruling out the effects of obesity associated inflammation as a confounding factor, is crucial to determine the actual effects of dietary components such as fatty acids in tumor initiation, progression and metastasis.

10.7 PUFA Regulation of Immune Cells: Consequences for Clinical Outcomes in Cancer

Epidemiological studies of the incidence and progression of breast cancer in populations of women of Japanese descent in the USA compared to women in Japan, have indicated a significantly higher incidence in the USA compared to Japan [81]. This observation was supported by the finding that offspring of Japanese immigrants to the United States, but not the immigrants themselves, had breast cancer rates similar to the general American population [82]. In the 1990s, dietary components that were implicated in these different incidences were identified [83]. These relatively weak and sometimes contradictory correlative epidemiologic data were considered plausible, given experiments demonstrating ω3 PUFAs had the potential to reduce pro-inflammatory cytokines, inflammation and development of cancer [84]. Similarly, there are indications that high fat diets increase breast cancer risk and are associated with an increased incidence of aggressive prostate cancer [85].

In an epidemiological study of 56,007 French women over 8 years, it was noted that breast cancer risk was not related to dietary PUFA overall, but a significant risk was associated with ω6 vs. ω3 PUFAs that was inversely related to ω3 PUFAs in women with the highest intake of ω6 PUFAs indicating interactions between PUFA consumption [86]. The decreased risk of breast cancer with ω3 PUFA intake (from fish) was confirmed in a case controlled study [87]. A population based study showed all-cause mortality was reduced 16–34% in women with a high intake of ω3 PUFAs [88]. Overall, during the past 20 years, data has accumulated to indicate that a high ω6 PUFA intake is pro-inflammatory, likely involving COX-2 and NFκβ activation leading to increased breast cancer incidence and all-cause mortality whereas high ω3 PUFA intake is protective, against high ω6 PUFA consumption downregulating NFκβ and decreasing breast cancer incidence and all-cause mortality.

Recent studies have shed additional light on the mechanisms involved in these clinical effects, as well as their relationship to the previously discussed innate and adaptive immune cells in the tumor microenvironment. The regulation by ω3 PUFA of macrophage function, has been documented with the use of antagonists to GPR120 (free fatty acid receptor 4 (FFA4R)) which is expressed by some myeloid cell populations [89]. It is noted that ω3 PUFAs mediate anti-inflammatory effects via this receptor. However, the nuclear receptor PPAR-γ is also a receptor for PUFAs and the regulatory mechanisms of ω3 and ω6 PUFA on obesity [90], postmenopausal breast mammary cancer [91] and microenvironmental inflammation [41] require additional study. Changes in the lipid content of cell membranes associated with ω3 and ω6 PUFA intake have effects on oncogenic signalling through modulation of lipid raft profiles and a reduction in cytokine production [92]. In addition, PUFAs contribute to the regulation of hematopoiesis in the BM, at extramedullary sites such as the spleen [93, 94] and have been suggested to induce the expansion of myeloid derived suppressor cells [95].

In summary, dietary intake of PUFAs have shown significant effects on clinical outcomes in cancer patients. In general ω6 PUFAs are associated with increased risk due to both direct effects on the mammary gland and promotion of a pro-inflammatory tumor microenvironment. In contrast, ω3 PUFAs have protective effects and counter tumor and ω6 PUFA associated inflammation. A general recommendation can be made that individuals should decrease dietary ω−6 PUFA intake and increase their ω3 PUFA consumption such that a dietary ratio of no more than 1–3 to 1 is consumed to support cancer prevention. PPAR-γ and GPR120 agonists also have potential use as neoplastic chemopreventive drugs; although both these drugs and dietary PUFA regulation have yet to definitively document anti-cancer activity. In contrast, long-term use of anti-inflammatory drugs has a clearly documented cancer preventive activity associated with inflammatory cell infiltration of tumors [96].

However, these benefits need to be weighed against the risks associated with the long-term use of anti-inflammatory drugs, which highlights the potential for dietary PUFA regulation of inflammation.

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