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REVIEWS: CURRENT TOPICS

The potential for treatment with dietary long-chain polyunsaturated n-3 fatty acids during chemotherapy☆

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Abstract

Dietary intake of long-chain ω-3 (or n-3) polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) can affect numerous processes in the body, including cardiovascular, neurological and immune functions, as well as cancer. Studies on human cancer cell lines, animal models and preliminary trials with human subjects suggest that administration of EPA and DHA, found naturally in our diet in fatty fish, can alter toxicities and/or activity of many drugs used to treat cancer. Multiple mechanisms are proposed to explain how n-3 PUFA modulate the tumor cell response to chemotherapeutic drugs. n-3 PUFA are readily incorporated into cell membranes and lipid rafts, and their incorporation may affect membrane-associated signaling proteins such as Ras, Akt and Her-2/neu. Due to their high susceptibility to oxidation, it has also been proposed that n-3 PUFA may cause irreversible tumor cell damage through increased lipid peroxidation. n-3 PUFA may increase tumor cell susceptibility to apoptosis by altering expression or function of apoptotic proteins, or by modulating activity of survival-related transcription factors such as nuclear factor-κB. Some studies suggest n-3 PUFA may increase drug uptake or even enhance drug activation (e.g., in the case of some nucleoside analogue drugs). Further research is warranted to identify specific mechanisms by which n-3 PUFA increase chemotherapy efficacy and to determine the optimal cellular/membrane levels of n-3 PUFA required to promote these mechanisms, such that these fatty acids may be prescribed as adjuvants to chemotherapy.

Keywords: ω-3 fatty acids; Docosahexaenoic acid; Eicosapentaenoic acid; Chemotherapy; Cancer; Mechanisms

1. ω-3 (n-3) polyunsaturated fatty acids

The ω-3 (or n-3) fatty acids refer to a class of polyunsaturated fatty acids (PUFA) having the first double bond in the n-3 position (three carbons from the methyl end of the carbon chain). n-3 fatty acids are considered essential since they cannot be synthesized by mammals and so must be obtained from the diet [1]. The three main dietary n-3 fatty acids are α-linolenic acid (C18:3n-3, all-*cis*-9,12,15-octadecatrienoic acid), found in green leafy vegetables, walnuts, canola oil, soybean oil, and flaxseed; and the longer-chain eicosapentaenoic acid (C20:5n-3, all-*cis*-eicosa-5,8,11,14,17-pentaenoic acid, EPA) and docosahexaenoic acid (C22:6n-3, all-*cis*-docosa-4,7,10,13,16,19-hexaenoic acid, DHA), found primarily in cold-water fatty fish (Fig. 1). Human beings are able to produce EPA from α-linolenic acid through chain elongation and desaturation; however, the extent of this conversion is quite inefficient (~5–10% of α-linolenic acid is converted to EPA) [2,3], such that EPA and DHA are acquired mainly through consumption of fish. Amounts of n-3 fatty acids in fish vary widely depending on the type of fish and habitat in which they live, but in general, higher concentrations of EPA and DHA are found in sardines, salmon, mackerel, herring and rainbow trout [4]. Unless otherwise stated, the use of "n-3

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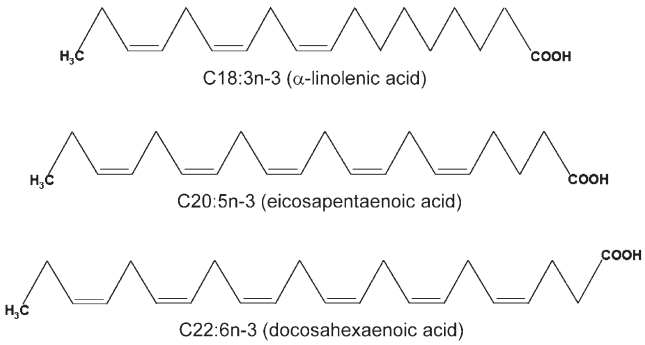

Fig. 1. Structures of the main dietary n-3 PUFA.

PUFA" or "n-3 fatty acid" in this review refers to EPA and/or DHA.

2. Evidence that n-3 PUFA can improve the response to chemotherapy

Several studies demonstrated that n-3 PUFA sensitize tumor cells to effects of anticancer drugs in culture or in tumor-bearing animals. In vitro, DHA and/or EPA improve cytotoxic effects of several anticancer drugs [doxorubicin, epirubicin, paclitaxel, 5-fluorouracil, cytosine arabinoside (Ara-C), mitomycin, arsenic trioxide] toward various human cancer cell lines, including those of breast, colon, bladder, neuroblastoma, and glioblastoma [5–13]. In animal models, representing a variety of tumor sites, dietary supplementation with DHA and/or EPA in combination with chemotherapeutic drugs (Ara-C, doxorubicin, epirubicin, irinotecan, methotrexate) decreased tumor size [14–16], reduced side effects [17–20] and prolonged survival [21,22] (Table 1).

Few studies have been conducted with human cancer patients to examine effects of n-3 PUFA specifically on tumor response to chemotherapy or drug-induced side effects. In a recent study, patients with advanced colorectal cancer consumed an oral nutritional supplement (ProSure) that provided 2.18 g EPA and 0.92 g DHA per day for up to 9 weeks, prior to and during chemotherapy treatment [23]. Patients experienced significant increases in body weight and energy levels while consuming the n-3 PUFA-enriched supplement, which were maintained during the course of chemotherapy [23]. Although the sample size was too small to draw conclusions regarding effects of EPA on chemotherapy-induced side effects, there was a trend toward reduced diarrhea and fatigue among compliant patients [23]. In another study, patients with cancer cachexia who were receiving chemotherapy consumed an oral nutritional supplement enriched with EPA (median EPA intake was 1.06 g/day at Week 4 and 1.36 g/day at Week 8) for 8 weeks [24]. Patients consuming the EPA-enriched supplement had increased protein and energy

Table 1
n-3 PUFA modulation of the tumor response to chemotherapy in animal models

Animal model	Tumor type	Diet fat	Diet n-3 PUFA	Chemotherapy drug	Effect of n-3 PUFA	Reference
Female Fisher 344 rats	Ward colon tumor	20% w/w	0.64% w/w EPA+ 0.16% w/w DHA	Irinotecan (CPT-11)	CPT-11+EPA/DHA ↓ tumor volume cf. CPT-11+mixed fat control diet	[14]
Female Sprague–Dawley rats	Mammary (NMU-induced)	15% w/w	~0.7 g DHA/day per rat (~8% w/w DHASCO)	Epirubicin	Epirubicin+DHA ↓ tumor area cf. epirubicin+palm oil	[15]
Female athymic *nu/nu* mice	Mammary (MDA-MB-231)	5% w/w	3% w/w FOC (34% EPA, 24% DHA)+2% w/w CO	Doxorubicin (DOX)	DOX+FOC ↓ tumor size cf. DOX+5% CO	[16]
Male Fischer 344 rats	Fibrosarcoma	10% w/w	10% w/w DHASCO (46% DHA)	Ara-C	Ara-C+DHA ↑ granulocyte- macrophage precursors in bone marrow and ↑ intestinal crypt depth and villus height cf. Ara-C+10% SO	[17]
Male ddY mice	None	N/A	5 or 50 mg/kg BW	Methotrexate (MTX)	MTX+5 mg/kg DHA ↓ MTX-induced intestinal permeability	[18]
Male Swiss mice	None	10% w/w	1–3% w/w INCELL AAFA (55% EPA+DHA)	Irinotecan (CPT-11)	CPT-11+2–3% AAFA ↓ apoptotic intestinal cells, ↓ liver PGE_2 and ↑ RBCs cf. CPT-11+10% CO	[19]
Male Wistar rats	None	N/A	1% DHA with or without protein supplementation	5-fluorouracil (5-FU)	5-FU+DHA+protein ↑ intestinal mucosa length and crypt depth, and ↓ apoptotic cells in intestinal crypts cf. 5-FU+standard diet	[20]
Adult dogs	Lymphoblastic lymphoma	33% w/w	2.9% w/w EPA+ 2.4% w/w DHA+ 5.5% w/w arginine	DOX	DOX+n-3 PUFA+arginine ↑ disease-free interval and survival time of dogs with stage III lymphoma cf. DOX+SBO	[21]
Male BDF1 mice	L1210 leukemia	5% or 10% w/w	1.5% or 3.5% w/w DHA (3% or 8% w/w DHASCO)	Ara-C	Ara-C+1.5% DHA ↑ survival time cf. Ara-C+chow, but survival time was not different from animals fed Ara-C+5% or 10% SO	[22]

DHASCO, a DHA-enriched oil produced by microalgae; FOC, fish oil concentrate; CO, corn oil; SO, safflower oil; Ara-C, cytosine arabinoside; BW, body weight; N/A, not available; SBO, soybean oil; RBCs, red blood cells.

intakes and improved nutritional status and quality of life assessments compared to their baseline measures [24].

Breast cancer patients with higher concentrations of DHA in breast adipose tissue at the time of cancer diagnosis were reported to respond more favorably to chemotherapy (i.e., had greater tumor regression) than patients with lower levels of n-3 PUFA [25]. Since diet is the only source of n-3 PUFA, this suggests that patients with higher DHA intakes had a more beneficial response to chemotherapy. The same group of researchers went on to validate these promising clinical observations in a rat model of mammary cancer. Sprague–Dawley rats were randomized to a diet supplemented either with corn oil or with a microalgae-produced oil rich in DHA. Rats were subsequently treated with epirubicin; in the DHA-supplemented group, epirubicin induced a 45% decrease in tumor size, whereas tumor size continued to increase in the corn oil-supplemented group [15]. Based on their initial observational study and the encouraging animal work, the investigators initiated a study examining effects of DHA supplementation on epirubicin efficacy in breast cancer patients. Early results of this study were presented at the Annual Meeting of the American Association of Cancer Researchers in 2006 [26]. Patients were supplemented with 1.8 g of DHA provided as 4.5 g of DHASCO, and plasma phospholipid DHA levels were determined at the time of enrollment and after 10 days of supplementation. Not all patients incorporated DHA in the lipid pool equally well, suggesting an effect of cancer on n-3 metabolism, so the sample was divided into a high DHA-incorporating group and a low DHA-incorporating group. When patient survival was assessed at 42 months, median survival had not been reached in the high-DHA group (i.e., more than 50% of the high-DHA group patients were still alive), whereas the median survival in the low-DHA group was 18 months ($P<.004$). In addition, the median time to progression was significantly higher in the high-DHA group compared to the low-DHA group (8.7 vs. 4.35 months, respectively; $P<.03$). Similarly, there were improvements in toxicity with less anemia and thrombocytopenia in the high-DHA group ($P<.006$ and $P<.01$ respectively). This study suggests that successful supplementation with DHA could reduce toxic effects and perhaps improve the efficacy of epirubicin treatment.

To our knowledge, only two studies have examined the influence of other dietary components on the ability of n-3 PUFA to moderate responses to chemotherapy. Dietary DHA had a moderate protective effect against intestinal damage induced by 5-fluorouracil administration in rats, but this effect was improved by the addition of dietary protein [20]. Among tumor-bearing mice, the addition of vitamins E and/or C to a fish oil diet during cisplatin administration decreased lung tumor weight to a greater extent than consumption of the fish oil diet alone during cisplatin treatment [27]. These interesting, albeit preliminary, studies warrant further investigation into the effects of combining n-3 PUFA with other food-derived compounds believed to influence tumor growth on tumor cell chemotherapeutic sensitivity.

3. Mechanisms by which n-3 fatty acids modify cellular function

It is well established that increasing the consumption of the long-chain n-3 PUFA can impact numerous processes in the body, including cancer and cardiovascular, neurological and immune functions [28–30]. Several candidate mechanisms have been proposed to explain the varied effects of n-3 PUFA on cellular function (Table 2). Long-chain n-3 PUFA are incorporated into the phospholipids of cell membranes of many cell types, such as immune cells, following inclusion in the diet [58,59]. Alterations in membrane fatty acid composition as a result of n-3 PUFA incorporation may lead to changes in membrane fluidity, membrane-mediated functions and signals (e.g., eicosanoids, lipid second messengers, signaling proteins) as well as the composition of lipid rafts and their functions. n-3 PUFA also alter gene expression and transcription factor activity; whether or not this is related to their incorporation into membrane phospholipids remains to be elucidated.

4. Mechanisms of n-3 PUFA-induced modulation of tumor cell response to chemotherapy

Although animal studies, and the few clinical trials that have been conducted, support a beneficial role for supplementation with n-3 PUFA before or during chemotherapy, the mechanism(s) have not been clearly established. Elucidation of these mechanisms is essential to ensure both optimal efficacy of chemotherapy drugs and to develop target levels at which to modify the diet or supplement with

Table 2
Potential mechanisms by which n-3 PUFA affect cellular function

Mechanism	References
Modulation of eicosanoid production by: • Displacing arachidonic acid from cell membranes • Competing with n-6 PUFA for desaturase and elongase enzymes, as well as cyclooxygenase and lipoxygenase enzymes • Decreasing expression of cyclooxygenase enzymes	[30–32]
Alteration of membrane fluidity or permeability	[33–36]
Changes in the production of lipid second messengers (e.g., diacylglycerol, ceramide)	[37–39]
Incorporation into signaling molecules (e.g., diacylglycerol, acylated proteins)	[37,40,41]
Incorporation into lipid rafts, leading to changes in the distribution and/or activity of raft-related signaling proteins	[42–48]
Modulation of gene expression	[31,49]
Modulation of gene expression via activation of peroxisome proliferator-activated receptors	[50,51]
Modulation of transcription factor activity (e.g., NFκB)	[52–57]

Table 3
Potential mechanisms by which n-3 PUFA modulate the tumor response to chemotherapy

Mechanism	References
Altered membrane-associated signal transduction	[7,47,48,57,60]
Increased lipid peroxidation causing irreversible cell damage	[15,61,62]
Decreased NFκB activity	[54,56,57,63]
Enhanced drug uptake or intracellular accumulation	[64–66]
Altered expression or function of apoptotic or antiapoptotic proteins	[8,67–70]
Enhanced nucleoside analogue drug activity	[71]

n-3 PUFA so as to optimize the benefits to the patient. Potential mechanisms by which n-3 PUFA may improve the efficacy of chemotherapeutic drugs are presented in Table 3.

4.1. Effects on membrane-associated signal transduction

Diet significantly influences cell membrane lipid composition. Inclusion of n-3 PUFA in the diet leads to their incorporation into membrane phospholipids [58,59]. EPA and DHA may alter the distribution or function of membrane-associated signaling molecules by inducing changes in physical properties of membranes due to their long-chain, highly unsaturated nature, or perhaps by some other mechanism. The following discussion provides some examples of n-3 PUFA effects on membrane-related signal transduction, and how these might affect tumor cell susceptibility to chemotherapy.

4.1.1. Ras signaling

Constitutive activation of Ras signaling pathways plays a key role in tumor development and progression, since Ras proteins (H-ras, K-ras, N-ras) promote cell growth, differentiation, survival and resistance to apoptosis [72]. These guanine-nucleotide binding proteins cycle between inactive GDP-bound and active GTP-bound forms, a process that is dependent on interactions with plasma membranes [73]. Ras proteins must undergo posttranslational modification (farnesylation, palmitoylation) to interact with membrane lipids, where they are activated and bind GTP [74]. Given the importance of elevated Ras signaling in tumor growth promotion, Ras pathway inhibitors (e.g., farnesyl transferase inhibitors) are being developed and tested singly and in combination with other anticancer therapies [72,74–76].

Several studies have reported effects of n-3 PUFA on Ras signaling. A high-fat fish oil diet fed to rats decreased protein levels of total and membrane bound Ras, increased protein levels of cytosolic Ras and decreased farnesyl protein transferase activity in colonic mucosa and colon tumors compared to rats fed a high-fat corn oil diet [77,78], suggesting that n-3 PUFA may interfere with Ras activation by decreasing its membrane localization. In vitro, DHA treatment of mouse colon cells decreased Ras GTP binding and Ras localization to the plasma membrane compared to cells treated with linoleic acid [79]. In this study, there was no effect of DHA on farnesyl transferase activity or Ras palmitoylation [79]. However, DHA enrichment in cell membranes was found to be necessary for inhibiting Ras localization to the plasma membrane [80]. Recent findings have demonstrated a partitioning of Ras into lipid rafts and caveolae [81], and effects of n-3 PUFA on Ras membrane compartmentalization have been examined in vivo. Ma et al. [47] reported decreased levels of H-Ras protein in colonic caveolae of mice fed n-3 PUFA vs. n-6 PUFA. This was accompanied by decreased H-ras activity in colonic epithelium of the n-3 PUFA-fed mice. n-3 PUFA were significantly incorporated into caveolae phospholipids, suggesting that alterations in the caveolae lipid environment can lead to protein displacement from these membrane microdomains [47]. Taken together, these studies suggest that n-3 PUFA may affect Ras signaling and could potentiate effects of anti-Ras therapies.

4.1.2. PI3K/Akt signaling

The PI3K/Akt signaling pathway plays an important role in cell cycle progression. PI3K regulates a number of cellular functions including proliferation, apoptosis, differentiation and chemotaxis [82]. PI3K catalyzes phosphorylation on position-3 of phosphatidylinositides, mainly PIP_2, to form PIP_3. Akt (also known as protein kinase B) is a primary mediator of PI3K signaling. Akt is a serine/threonine kinase that is recruited to the plasma membrane through direct contact with PIP_3, where it is phosphorylated and activated [82]. Akt functions directly to promote cell survival and protect cells from apoptotic cell death, by phosphorylating and inactivating components of the cell death machinery (e.g., caspase-9, BAD). In addition, Akt can indirectly promote cell survival by activating prosurvival transcription factors such as nuclear factor-κB (NFκB) [82].

Recent evidence suggests that the PI3K/Akt signaling pathway is constitutively active in many types of human cancer [83]. Overexpression of Akt has also been linked to resistance to chemotherapy [84]. Stably transfecting breast cancer cells with constitutively active Akt attenuated doxorubicin-induced apoptosis [85]. Ovarian cancer cells overexpressing constitutively active Akt, or containing Akt gene amplification, were highly resistant to paclitaxel treatment, as compared to cells expressing low levels of Akt [86]. Activation of Akt has also been implicated as one of the mechanisms involved in mammary tumor resistance to tamoxifen [60].

Inhibition of PI3K/Akt signaling by various compounds (PI3K inhibitors, dominant-negative Akt mutants) enhances chemosensitivity [85,87–90]. EPA and/or DHA decrease both Akt phosphorylation [57] and activity [60] in mammary tumor cells in vitro. Consequently, EPA enhanced sensitivity to tamoxifen in breast cancer cells that expressed constitutively active Akt [60]. EPA and/or DHA have also been reported to prevent Akt phosphorylation or activation in several other cell types [53,91–93]. Thus, changes in cellular concentrations of long-chain n-3

PUFA may affect the chemotherapeutic response by altering Akt activity in tumor cells.

4.1.3. Her-2/neu signaling

Her-2/neu is a transmembrane tyrosine kinase receptor belonging to the epidermal growth factor receptor (EGFR) family. Overexpression of Her-2/neu confers resistance to taxane-based chemotherapy; down-regulation of this protein sensitizes cells to taxanes [94–96]. DHA treatment of Her-2/neu-overexpressing breast cancer cells reduced Her-2/neu protein expression and enhanced cytotoxic effects of paclitaxel and docetaxel [7]. Although the authors did not specifically measure DHA incorporation into membrane lipids, the 24-h incubation of breast cancer cells with fatty acid is presumed adequate for incorporation into cellular phospholipids [97,98].

4.1.4. Lipid raft-associated signaling

Lipid rafts are membrane microdomains rich in saturated fatty acids, sphingolipids, cholesterol and several signaling proteins [99]. A subset of specialized rafts termed *caveolae* has also been described, which are flask-shaped invaginations in the membrane that are enriched in the integral membrane protein caveolin [99]. n-3 PUFA incorporation into rafts or caveolae can alter the distribution or function of raft-associated signaling proteins. Studies from our own laboratory show that EPA and DHA are incorporated into whole membranes and lipid rafts of breast cancer cells in vitro, and this is associated with reduced EGFR levels in the rafts and increased whole cell levels of phosphorylated EGFR [48]. In this case, the increased EGFR phosphorylation did not appear to promote cell growth but rather was associated with p38 MAPK phosphorylation and possibly induction of apoptosis. In vivo, mice fed a diet enriched with n-3 PUFA had increased levels of EPA and DHA and decreased levels of H-ras and eNOS in colonic caveolae [47]. Alterations in raft lipid composition by polyunsaturated fatty acids have also been shown to displace signaling proteins from rafts in immune cells [43,44,100]. The study of lipid rafts is still a relatively young science, and whether or not effects of n-3 PUFA on raft-associated signal transduction affect the tumor cell response to chemotherapy remains to be elucidated.

4.2. Lipid peroxidation

PUFA, particularly the long-chain n-3 PUFA, are susceptible to free radical attack, ultimately leading to formation of lipid hydroperoxides. Lipid peroxidation is initiated by hydrogen abstraction from an unsaturated fatty acid by reactive oxygen species. The resulting lipid radical reacts with oxygen to form a fatty acid peroxyl radical, which can attack adjacent fatty acid chains in cell membranes, and thus propagate lipid peroxidation [101]. The major effects of the products of lipid peroxidation are inhibition of DNA synthesis, cell division and tumor growth, and induction of tumor cell death [102,103].

Drugs belonging to the anthracycline family of chemotherapeutic compounds (e.g., doxorubicin, epirubicin and daunorubicin) are thought to induce tumor cell death in part by stimulating formation of oxygen free radicals and ultimately causing irreversible cell damage [104]. The long-chain n-3 PUFA may potentiate the peroxidizing effects of such drugs. Germain et al. [5] reported that DHA (29 μM) increased doxorubicin cytotoxicity toward MDA-MB-231 human breast cancer cells in culture, and this effect was increased by addition of an oxidant system and decreased by addition of α-tocopherol, an antioxidant. In rats, DHA fed at approximately 0.7 g/day, for several weeks prior to and 6 weeks during chemotherapy, enhanced epirubicin cytotoxicity toward mammary tumors; the effect was countered by adding α-tocopherol to the diet [15].

Arsenic trioxide is an antineoplastic agent that has been used to treat acute promyelocytic leukemia since the 1970s [61]. Arsenic trioxide is believed to induce apoptosis by a reactive oxygen species-dependent pathway [61]. DHA (25 μM) enhanced the efficacy of arsenic trioxide and increased reactive oxygen species levels in leukemia cells in vitro [61]; this effect was abrogated by addition of vitamin E [61]. Similarly, addition of vitamin E abolished the sensitization of neuroblastoma cells by DHA (25–150 μM) to arsenic trioxide in vitro [12].

Further support for this mechanism comes from a study reporting that 30 μM DHA induced an increase in doxorubicin efficacy toward human breast cancer cells in culture. This was accompanied by an increase in cellular malondialdehyde and glutathione concentrations, markers of oxidative stress [6]. In mice fed a 3% w/w fish oil concentrate (34% EPA, 24% DHA) prior to, and for 5 weeks during chemotherapy, there was increased doxorubicin efficacy toward mammary tumors. This was accompanied by increased lipid peroxidation and a decreased ratio of GPX: SOD activity, a putative indicator of increased oxidative stress [16,105].

4.3. Effects on NFκB signaling

Nuclear factor-κB is a transcription factor that plays a key role in cellular survival, growth, differentiation, adhesion and inflammation [106]. Active NFκB promotes cellular survival and inhibits apoptosis by regulating the expression of a number of genes involved in cell transformation, proliferation, invasion and apoptosis (e.g., cyclin D1, Akt, bcl-2, bcl-xl, matrix metalloproteinases) [106]. As a result, NFκB has been implicated as a promoter of tumorigenesis. Indeed, several tumor types express constitutively active NFκB [106–108], which may confer a selective growth advantage.

Most chemotherapeutic agents increase NFκB expression [106]. This may be an adaptive mechanism by tumor cells to prevent apoptosis induced by the DNA-damaging effects of chemotherapy. It was hypothesized that drug-induced NFκB activation contributes to resistance to chemotherapy-induced apoptosis [106,107,109,110]. More

recently it was reported that patients with breast tumors that expressed activated NFκB (i.e., nuclear staining in tumor specimens) prior to chemotherapy treatment had a significantly lower clinical response to chemotherapy than patients with undetectable NFκB staining [111]. Moreover, the number of patients with activated NFκB in tumor tissue increased after chemotherapy exposure [111], consistent with reports of chemotherapy-induced activation of NFκB.

Several studies now support that inhibition of NFκB can sensitize tumor cells to chemotherapeutic drugs [106,107,112–114]. Natural and synthetic inhibitors of NFκB are currently being studied as potential adjuvants to traditional anticancer therapies [115]. We and others have shown that the long-chain n-3 PUFA decrease NFκB activity or expression in human breast tumor cells [57,63]. Several other studies indicate that n-3 PUFA down-regulate NFκB activation in monocytes, macrophages and T cells [53–56,116]. These data suggest that the long-chain n-3 PUFA may be able to sensitize tumor cells to the effects of chemotherapy by decreasing NFκB activity or expression. It is not clear how n-3 PUFA modulate NFκB activity; however, it has been suggested that these fatty acids modulate upstream signaling involved in the activation of NFκB, such as Akt activation [53,57], TNF-α signaling [52,54], phospholipase C activation [52] and IκBα phosphorylation [54,55].

4.4. Effect on drug uptake

n-3 PUFA are readily incorporated into tumor cell membranes when provided in the diet or in cell culture media [48,97,117,118]. n-3 PUFA enrichment can affect physical properties of cell membranes, altering membrane fluidity in vivo [33] and in vitro [34], and increasing the permeability of tumor cells ex vivo [35,36]. It has been suggested that this alteration of membrane permeability may modify the influx and efflux of drugs into and/or out of tumor cells [65], particularly hydrophobic drugs that pass through the membrane by diffusion. In support of this, enrichment of cell membranes with DHA and/or EPA correlates with an increase in the intracellular accumulation and/or retention of chemotherapeutic drugs (doxorubicin, vincristine, mitoxantrone) and enhanced drug cytotoxicity in a variety of tumor cell types [62,64–66,119,120].

Increased drug uptake may also be related to n-3 PUFA effects on transport proteins within the membrane. Most nucleoside analogue drugs are hydrophilic and their cellular uptake is mediated by specific membrane transporters. EPA and DHA treatment increased the rate of purine uptake by a nucleoside transport protein in lymphoblastic leukemia cells in vitro [121]. There were no changes in the affinity or number of binding sites of the transporter, suggesting that fatty acids somehow directly interact with the transporter and affect its function (perhaps through their incorporation into the membrane, or altering the tertiary or quaternary structure of the protein) [121]. Several chemotherapy drugs are nucleoside analogues, and the action of n-3 PUFA in increasing their uptake by tumor cells could have a significant impact on their effectiveness.

Alternatively, expression of the multidrug transporter P-glycoprotein (Pgp) is under the control of NFκB [122,123]. Increased tumor cell expression of Pgp (which can be acquired over time with repeated exposure to chemotherapy) can increase efflux of drugs from cells, and this is believed to contribute to the multidrug resistant phenotype of many tumors [109]. As NFκB activity itself can be regulated by n-3 PUFA, it is possible that this modulation of NFκB expression in a tumor could decrease Pgp expression, leading to decreased efflux of chemotherapy drugs from tumor cells.

4.5. Effects on mediators of apoptosis

The majority of chemotherapeutic drugs are believed to kill tumor cells by inducing or increasing apoptosis [124,125]. Resistance to apoptosis can result in a decrease in tumor cell sensitivity to many chemotherapies [124]. Agents that can increase the expression of proapoptotic proteins and down-regulate antiapoptotic proteins may hold promise as effective adjuvants to standard chemotherapies.

A few studies provide evidence that n-3 PUFA can regulate expression or activity of apoptotic proteins in tumor cells. DHA or EPA treatment of breast or colon cancer cells or leukemia cells in vitro decrease the expression of the antiapoptotic protein bcl-2 [67,68,126,127]. In addition, DHA treatment increased expression of the proapoptotic protein bax in HL-60 human leukemia cells [69]. In human pancreatic cell lines, EPA treatment induced apoptosis and increased caspase-3 activation [70]. Studies from our own laboratory showed that EPA and DHA induced apoptosis and increased caspase activity in human breast cancer cells [57]. These data support the hypothesis that n-3 PUFA enhance the proapoptotic effect of anticancer drugs. This is also supported by a study by Calviello et al. [8], who showed that DHA can act in a synergistic manner with 5-fluorouracil to inhibit cell growth and induce apoptosis of colon cancer cells in vitro. The combination of DHA and 5-fluorouracil decreased expression of the antiapoptotic proteins bcl-2 and bcl-X_L more than either agent did alone, suggesting that DHA was able to enhance 5-fluorouracil's apoptotic effect.

4.6. Effects on nucleoside analogue metabolism

Deoxycytidine kinase (dCK) and deoxycytidine deaminase (dCDA) are key enzymes involved in activation and inactivation, respectively, of a variety of nucleoside analogue chemotherapeutic drugs like Ara-C, cladribine and gemcitabine [128]. Reduced tumor cell levels of dCK, and/or increased dCDA levels, have been linked to resistance to nucleoside analogue drugs [129–132]. In a study comparing effects of DHA on dCK and dCDA activities in normal vs. transformed rat fibroblasts, DHA increased dCK and decreased dCDA activities in transformed cells, whereas the opposite effect was seen in normal

cells [71]. Mechanisms for the selective effect of DHA on normal vs. transformed cells are not known. However, these results are consistent with reports suggesting that n-3 PUFA can enhance the effectiveness of chemotherapy drugs toward tumor cells while at the same time reduce toxicity to normal cells.

5. Summary

Animal studies have clearly demonstrated that feeding n-3 fatty acids can prevent tumor development and growth by many different mechanisms. Epidemiological population studies have suggested an inverse relationship between n-3 PUFA consumption and risk of cancers of the breast and colon [133], but prospective cohort studies examining the effects of n-3 PUFA on cancer incidence have yielded mixed results [134]. To date, no human intervention trials have been conducted to specifically investigate the effects of n-3 PUFA consumption on cancer risk, although many have examined effects of n-3 PUFA on cancer cachexia, and a few have reported effects on biomarkers of cancer development.

Based on animal and epidemiological evidence, there are currently guidelines aimed at both individuals and the population at large to prevent cancer. Currently, there are no specific nutrition/nutrient guidelines for those undergoing chemotherapy. This review has illustrated that there are a growing number of studies on human cancer cell lines and in animal models of cancer that show incorporation of, or metabolism of, long-chain n-3 fatty acids DHA and/or EPA, found naturally in our diet in fatty fish, can alter the activity and/or toxicity of many chemotherapy drugs used to treat cancer. Studies suggest not one but multiple mechanisms within the cell may contribute to the effects of these fatty acids. Despite this evidence, few clinical studies have been conducted that test the hypothesis that feeding n-3 fatty acids in the short period prior to and/or during chemotherapy could improve the efficacy of treatment. Unlike many chemotherapy agents used, n-3 fatty acid administration does not appear to have toxic effects to noncarcinogenic cells; rather, n-3 PUFA may improve immune function, including the anticancer response [59,118]. Just how n-3 fatty acids selectively modify the response of tumor cells, but not normal host tissues, to chemotherapeutic agents remains to be elucidated. However, some evidence suggests that fatty acid uptake and/or metabolism by tumor cells differs from that of nontransformed cells [135–137].

Future research holds the key to enabling us to prescribe n-3 PUFA as adjuvants to chemotherapy. Although promising, there are still several questions that need to be answered before we can prescribe a specific dose of n-3 fatty acids to act as adjuvants to chemotherapy. Specifically, we need to identify specific mechanisms by which n-3 fatty acids act and then determine optimal cellular/membrane levels of n-3 PUFA required to promote these mechanisms and increase drug efficacy while decreasing toxicity to the host.

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