

Mini-review

Nutritional intervention with omega-3 fatty acids enhances tumor response to anti-neoplastic agents

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Abstract

Nutritional intervention with specific fatty acids depresses tumor growth and enhances tumor responsiveness to chemotherapy. Supplementation of tumors with long chained omega-3 polyunsaturated fatty acids results in enrichment of tumor phospholipid fractions with omega-3 fatty acids resulting in an altered membrane composition and function. Tumors enriched with long chained omega-3 polyunsaturated fatty acids possess membranes with increased fluidity, an elevated unsaturation index, enhanced transport capabilities that results in accumulation of selective anti-cancer agents, increased activity of selected drug activating enzymes, and alteration of signaling pathways important for cancer progression. These nutritionally induced changes in tumor fatty acid composition result in increased sensitivity to chemotherapy, especially in tumor lines that are resistant to chemotherapy and cause specific enhancement of cytotoxicity to tumor cells and protection of normal cells.

Pre-disposing tumors to increased chemo-sensitivity through nutritional intervention with specific fatty acids has the potential to improve patient response to chemotherapy with fewer untoward side effects if these pre-clinical findings carry over into a clinical setting.

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Contents

1. Introduction	90
2. Modification of tumor cell fatty acid composition enhances chemosensitivity <i>in vitro</i>	90
3. Lipid modification leads to enhanced sensitivity to drugs in drug resistant cell lines	93
4. Lipid enrichment preferentially enhances drug cytotoxicity in tumor cells compared to normal parent cells	94
5. Enrichment of lipid pools with polyunsaturated fatty acids enhances pro-oxidant chemotherapy through oxidative stress	95
6. Targeting specific signaling pathways for nutritional intervention to enhance cytotoxicity of anti-cancer drugs	96
7. Modification of tumor fatty acid composition enhances cancer chemotherapy <i>in vivo</i>	97

Abbreviations: AA, arachidonic acid C20:4n – 6; ALA, alpha-linolenic acid C18:3n – 3; DHA, docosahexaenoic acid C22:6n – 3; DPA, docosapentaenoic acid C22:5n – 3; EPA, eicosapentaenoic acid C20:5n – 3; GLA, gamma-linolenic acid C18:3-n – 6; LA, linoleic acid C18:2n – 6; OA, oleic acid C18:1n – 9

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8. Fatty acid enhancement of radiation therapy of cancer	101
9. Fatty acid enhancement of hyperthermia therapy of cancer	101
10. Conclusion	102
References	102

1. Introduction

The potential to alter tumor lipid fatty acid composition through nutritional intervention was established from observations that tumors derive their fatty acids from the denovo synthesis pathway that generates stearic (C18:0) and oleic acid (C18:n – 9) and from the circulation which provides the essential polyunsaturated fatty acids consumed in the diet of the host.

In ascites tumors in mice, free fatty acid and triglyceride containing lipoproteins [1,2] were the source of fatty acids for tumor cells. The tumor cell phospholipid fraction undergoes rapid turnover, permitting tumor cell fatty acid composition to be altered through nutritional intervention in a relatively short period of time [3,4].

Indeed, there have been numerous reports on increasing the long chain polyunsaturated omega-3 fatty acid content of tumor cells by altering the fatty acid composition of the growth medium *in vitro* [5–15] or by feeding long chain polyunsaturated omega-3 fatty acids to laboratory animals implanted with tumors [16–20] and EPA has been reported to decrease the uptake of saturated fatty acids, monounsaturated fatty acids, and omega-6 unsaturated fatty acids including linoleic acid in MCF-7 [21] and omega-3 fatty acids decreased linoleic acid uptake in hepatoma *in vivo* [22].

The implications of altering the fatty acid composition of tumor cells on chemotherapy responsiveness are discussed below.

2. Modification of tumor cell fatty acid composition enhances chemosensitivity *in vitro*

Numerous studies on the impact of modifying tumor lipid profiles by increasing the exposure to omega-3 long chain polyunsaturated fatty acids have reported a beneficial impact on tumor cell response to chemotherapy *in vitro* (Table 1). Pre-incubation of L-1210 murine leukemia cells with docosahexaenoic acid (DHA) enhanced sensitivity to the anti-biotic anti-neoplastic agent doxorubicin in a dose dependent manner [5] with a concomitant three to four-fold increase in polyunsaturated fatty acid (PUFA) concentration in the phospholipid fractions and over a 70-fold increase in

DHA concentrations. Enriching L-1210 cells with DHA augmented the uptake of doxorubicin without altering its efflux, resulting in an increase of intracellular drug concentration by 32% [6]. Electron spin resonance showed that the resulting membrane fatty acid modification was sufficient to alter the physical properties of the plasma membrane, resulting in an increase in the uptake of methotrexate [15] at a DHA concentration that did not affect L-1210 growth rate, but enhanced the cytotoxicity of doxorubicin. Similarly, pre-incubation of L-5178Y lymphoma cells in culture with increasing concentrations of DHA decreased survival at all concentrations of mitomycin C (MMC), dexamethasone and doxorubicin [23]. Pre-incubation of human bronchial carcinoma A-427 cells and human glioblastoma A-172 and U-87MG cells with non-toxic doses of DHA enhanced the cytotoxicity of doxorubicin at all concentrations tested. DHA enhanced sensitivity to doxorubicin in the glioblastoma cells was more pronounced in cells that were resistant to DHA toxicity [24]. In A-427 lung carcinoma cells a modest potentiation of doxorubicin cytotoxicity with DHA was observed, whereas, in SK-LU-1 lung carcinoma cells the effect was marginal and not significant.

Out of six fatty acids tested DHA was the most potent enhancer of doxorubicin cytotoxicity toward the human breast tumor MDA-MB-231 cells *in vitro* in a dose and time dependent manner [25], lipid peroxidation was implicated.

5-Fluorouracil cytotoxicity was enhanced in colon cancer Caco-2 cells enriched with a fish oil emulsion containing long chained polyunsaturated omega-3 fatty acids [26]. The fish oil treatment alone inhibited Caco-2 cells growth in a dose and time dependent fashion and induced apoptosis with accumulation of cells in the G₂/M phase. Treatment with 5-FU alone resulted in accumulation of cells in the S phase and the combination treatment with fish oil emulsion and 5-FU increased accumulation of cells in the S phase. The combined treatment resulted in additive growth inhibition by depressing cell cycle progression.

Synergistic inhibition of cell growth by the phytoestrogen isoflavonoid, genestein and EPA was observed at high EPA concentrations in MCF-7 (estrogen sensitive) and MDA-MB-231 (estrogen insensitive) human

Table 1
Fatty acid enhanced response to chemotherapy in tumor cells *in vitro*

Tumor line	Drug	Fatty acid (concentration)	Implicated mechanism	References
L-1210 mouse leukemia	Doxorubicin	DHA (32 μ M)	Increased uptake of drug	[5,6,15]
L-5178Y lymphoma	Doxorubicin Dexamethasone Mitomycin C	DHA > AA (25 μ M)	Oxidative stress Altered membrane permeability Alteration of membrane and enzymes	[23]
A-427 human lung carcinoma	Doxorubicin	DHA (10–40 μ M)	Increased uptake of drug Alteration of anti-oxidant enzyme activities	[24]
A-172, V87MG human glioblastoma	Doxorubicin	DHA (30 μ M)	Increased uptake of drug Alteration of anti-oxidant enzyme activities	[24]
P-388/DOX doxorubicin resistant mouse leukemia	Doxorubicin	DHA > GLA (50 μ M)	Increased uptake of drug Anti-oxidant enzyme alteration	[33]
Small cell lung carcinoma, doxorubicin resistant	Doxorubicin	DHA (32 μ M)	Increased uptake of the drug	[7]
MDA-MB-231 human breast carcinoma	Doxorubicin	DHA (29 μ M)	Increased oxidative stress	[25]
GLC-4-CP human small cell carcinoma <i>cis</i> -platinum-resistant	<i>Cis</i> -platin	DHA (32 μ M)	Increased uptake of the drug Increased interstrand cross-links	[8]
L-1210 murine leukemia	Doxorubicin Ara C	DHA (30 μ M)	Increased uptake of the drugs oxidative stress	[12]
Transformed rat 2 fibroblasts	Doxorubicin	DHA (20 μ M)	Alters nuclear transport proteins	[11]
L-1210 murine leukemia	BM-41440 thioether lipid analogue	DHA (32 μ M)	Oxidative stress	[10]
MDA-MB-231 human mammary carcinoma	Paclitaxel	GLA > ALA > EPA > DHA (20 μ g/ml)	Oxidative stress	[44]
NCG human neuroblastoma	Vincristine	DHA & GLA (10–50 μ g/ml)	Increased uptake of the drug	[9]
NCG/VCR-1 human neuroblastoma VCR-resistant	Vincristine	DHA & GLA (10–50 μ g/ml)	Increased uptake of the drug	[9]
KB-Ch ^R -8-5 human cervical carcinoma vincristine-resistant	Vincristine	DHA, GLA, EPA, AA (10–40 μ g/ml)	Increased uptake of the drug	[14]
Hela human cervical carcinoma	Vincristine Doxorubicin <i>Cis</i> -platinum	EPA, GLA (10–40 μ g/ml)	Increased uptake of the drug	[14]
HL-60 human leukemia As ₂ O ₃ -resistant	Arsenic trioxide	DHA (25 μ M)	Oxidative stress	[57]
HT-29 colon carcinoma As ₂ O ₃ -resistant	Arsenic trioxide	DHA (25–100 μ M)	Oxidative stress	[35]

Table 1 (Continued)

Tumor line	Drug	Fatty acid (concentration)	Implicated mechanism	References
SW-620 colon carcinoma As ₂ O ₃ -resistant	Arsenic trioxide	DHA (25–100 μM)	Oxidative stress	[35]
LS-174T colon carcinoma As ₂ O ₃ -resistant	Arsenic trioxide	DHA (25–100 μM)	Oxidative stress	[35]
SK-Br-3 mammary carcinoma As ₂ O ₃ -resistant	Arsenic trioxide	DHA (25–100 μM)	Oxidative stress	[35]
SKOV-3 ovarian carcinoma As ₂ O ₃ -resistant	Arsenic trioxide	DHA (25–100 μM)	Oxidative stress	[35]
PC-3 Prostate carcinoma As ₂ O ₃ -resistant	Arsenic trioxide	DHA (25–100 μM)	Oxidative stress	[35]
Primary melanoma As ₂ O ₃ -resistant	Arsenic trioxide	DHA (25–100 μM)	Oxidative stress	[35]
MDA-MB-468 mammary carcinoma	Arsenic trioxide	DHA (25–100 μM)	Oxidative stress	[35]
A-427 lung carcinoma	Acrolein	DHA (20 μM)	Oxidative stress	[45]
A-2780AD human ovarian carcinoma doxorubicin-resistant	Doxorubicin	EPA, GLA (40 μg/ml)	Oxidative stress	[34]
A-2780AD human ovarian carcinoma cisplatin-resistant	Doxorubicin Cisplatin	EPA, GLA (40 μg/ml)	Oxidative stress	[34]
NIH 3T3, oncogene transformed fibroblasts	Mitomycin C	EPA (2 μg/ml)	Increase in membrane fluidity	[13]
MCF-7 mammary carcinoma	Genistein	EPA	G2/M cell cycle arrest apoptosis	[27]
MDA-MB-231 mammary carcinoma				
Caco-2 colon carcinoma	1,4-Phenylenebis (methylene) selenocyanate	DHA (2.5–2.5 μM)	Decrease β-catenin Decreased activity of COX-2 Decreased activity of NFκB-65 Decreased cyclin D	[30]
Caco-2 colon carcinoma	S Fluoro-uracil	Fish oil emulsion (10–100 μM EPA + 7.5–75 μM DHA)	Inhibition of cell cycle	[26]
MCF-7 mammary carcinoma AKt-active tamoxifen resistant	Tamoxifen	EPA (40 μM)	Inhibition of AtK kinase activity	[58]
Normal rat colonic epithelial cells D/V-src (103 cells/plate)	AraC	DHA, AA (3 μM)	Inhibition of protein kinase C	[36]
MCF-7 mammary carcinoma	Vinorelbine	GLA (n – 6)	Non-oxidative stress	[64]
MDA-MB-231 mammary carcinoma	Vinorelbine	GLA (n – 6)	Non-oxidative stress	[64]
T47D mammary carcinoma	Vinorelbine	GLA (n – 6)	Non-oxidative stress	[64]
Sk-Br3 mammary carcinoma	Vinorelbine	GLA (n – 6)	Non-oxidative stress	[64]
HCA-7 human colon carcinoma	Celecoxib	DHA (75 μM)	Inhibition of COX-2 pathway	[61]
MCF-7 human mammary carcinoma (estrogen-dependent)	Docetaxel (taxotere) (synergistic)	GLA (n – 6)	Decrease in p185 Her-2/neu oncoprotein	[65]

MDA-MB-231 human mammary carcinoma (estrogen-dependent)	Docetaxel (taxotere) (synergistic)	GLA (n – 6)	Decrease in p185 Her-2/neu oncoprotein	[65]
Sk-Br3 Human Mammary Carcinoma (Estrogen-Dependent)	Docetaxel (taxotere) (additive)	GLA (n – 6)	Decrease in p185 Her-2/neu oncoprotein	[65]
BT-474 human mammary carcinoma	(Herceptin) trastuzumab	OA (n – 6)	Decrease in p185 Her-2/neu oncoprotein	[65]
LnCap prostate carcinoma	Celecoxib	DHA (2.5 μM)	Inhibition of COX-2 pathway, NFκB	[63]

DHA, docosahexaenoic acid C22:6n – 3; EPA, eicosapentaenoic acid C20:5n – 3; GLA, δ-linolenic acid C18:3n – 3; OA, oleic acid C18:1n – 9; AA, arachidonic acid C20:4n – 6.

mammary carcinoma cell lines [27] determined by median effect analysis [28,29]. These findings demonstrate a synergism of cell growth inhibition between this isoflavone and EPA that is independent of the estrogen sensitivity of the breast cancer cell line.

Low doses of the chemopreventative agent, organoselenium 1,4-phenylenebis (methylene) selenocyanate (p-XSC) and DHA synergistically inhibited Caco-2 colon carcinoma cell growth and induced apoptosis [30]. The combined treatment was shown to decrease levels of the transcriptionally active beta-catenin, an important element of the cadherin-mediated cell–cell adhesion system and associated with the Wnt-APC signal transduction pathway [31,32]. In addition, COX-2, iNOS, cyclin D and the nuclear transcription factor NF-κB-65 concentrations were lowered by the combination therapy, which could account for the observed inhibition of cell growth.

3. Lipid modification leads to enhanced sensitivity to drugs in drug resistant cell lines

Several studies have demonstrated that altering tumor lipid fatty acid composition changes drug-resistant phenotypes to drug-sensitive ones as summarized below.

Treatment of P-388/DOX doxorubicin resistant murine leukemia cells with DHA or gamma linolenic acid (GLA) increased accumulation of doxorubicin and decreased the I_{50} value for doxorubicin when either fatty acid was added individually [33]. The sensitivity of doxorubicin-sensitive P-388 leukemia cells towards doxorubicin was not improved by pre-incubating with either fatty acid. However, both P-388/DOX and P-388 cells became more sensitive to doxorubicin when treatment was combined with GLA or DHA and anti-oxidants. The increased responsiveness to doxorubicin was attributed to increased drug uptake and altered anti-oxidant enzyme activities.

Incubation of doxorubicin-sensitive and resistant small cell lung carcinoma cells with DHA resulted in a significant increase in phospholipid DHA-concentration without loss of cell viability [7]. Only the doxorubicin resistant cell line pre-incubated with DHA showed increased sensitivity to doxorubicin. Similarly, culturing cisplatin resistant (GLC-4-CP) and sensitive (GLC-4) human small cell lung carcinoma cells with non-toxic levels of DHA resulted in increased accumulation of DHA in the tumor membranes and a three-fold decrease in the resistance of GLC-4-CP cells toward cisplatin (CP) but had no influence on the cytotoxicity of CP towards the susceptible GLC-4 small cell carcinoma cells [8]. DHA enrichment preferentially sensitized drug resistant tumor

cells to chemotherapy with CP by increasing the intracellular platinum levels. DHA treatment increased cellular platinum, total platinum bound to DNA, and inter-strand cross-linking (platinum-GG, platinum-AG, G-platinum-G and platinum-GMP adducts) in both cell lines. In most subclasses of phospholipids and sphingomyelin an increase in the fatty acid chain length and double bond index was observed in the cells treated with DHA. The increased interstrand cross-linking observed in the DHA-treated cells was related to the decreased resistance toward CP in the resistant cell line. The increased sensitivity towards doxorubicin was related to the concentration of DHA in the ovarian carcinoma cells and a modest increase in lipid peroxidation. The synergism was found to be complex and cell specific.

The sensitivities of the ovarian carcinoma cell lines resistant to doxorubicin (2780-AD) and *cis*-platinum (2780-CP) were increased towards doxorubicin by pre-loading the tumor cells with GLA or EPA, whereas, the parent ovarian cell line (2780) sensitivity to doxorubicin was not changed by lipid modification [34]. In addition, the sensitivity of the *cis*-platinum resistant ovarian cell line 2780-CP was sensitized to CP by pre-incubation with GLA and EPA, whereas, the parent ovarian line 2780 and the doxorubicin resistant cell line 2780-ADR were not. Isobologram analysis revealed that the interactions between cytotoxic drugs and the PUFAs were additive. All additivity preferentially occurred with the drug-resistant sub-lines.

If the tumor fatty acid concentration of long chained polyunsaturated omega-3 fatty acids is important for drug sensitivity, then the drug resistant tumor cells would be expected to possess lower levels of the long chained omega-3 polyunsaturated fatty acids. Indeed, the fatty acid composition of KB-3-1 human cervical carcinoma-vincristine sensitive and KB-Ch^R-8-5 human cervical carcinoma-vincristine resistant was quite different [14]. The resistant (KB-Ch^R-8-5) tumor cell phospholipids were low in GLA, ALA, DPA and DHA compared to the vincristine susceptible cells (KB-3-1). When the KB-Ch^R-8-5 (resistant) cells were incubated with GLA and DHA, their concentrations were increased 10–15-fold in the phospholipid, free fatty acid and ether lipid pools and the tumoricidal activities of vincristine, *cis*-platinum and doxorubicin were potentiated. The enhanced sensitivity of the KB-Ch^R-8-5 vincristine resistant cell line towards vincristine was associated with an increased concentration of the respective fatty acid into the tumor membrane lipids. Thus, specific fatty acid concentrations of tumor phospholipids may be an important biomarker for determining the responsiveness to specific anti-cancer drugs.

Similarly, enrichment of human neuroblastoma cells (NCG) and the vincristine resistant subline (NCG/VCR) with polyunsaturated fatty acids enhanced the cytotoxicity of vincristine by increasing intracellular accumulation of drug in both cell lines [9]. DHA was the most potent “drug enhancing” fatty acid, but the vincristine resistance was not totally overcome by DHA supplementation. The incomplete reversal of vincristine resistance was attributed to the modest increase in accumulation of DHA into cellular membranes.

Pre-incubation of Hela cells with DHA, GLA, EPA and ALA enhanced the uptake of vincristine (3H) *in vitro* [14]. DHA enriched cells contained the highest intracellular concentration of vincristine as a result of increased VCR uptake and decreased efflux. The increased intracellular concentration of vincristine in PUFA (DHA, GLA) loaded cervical carcinoma cells led to the increased responsiveness to chemotherapy in both sensitive and resistant tumor cells *in vitro*.

Seven of 10 arsenic trioxide resistant tumor cells became susceptible to the pro-oxidant arsenic trioxide following combination treatment with DHA [35]. Collectively, these findings show that increased accumulation of long chained omega-3 fatty acids into tumor cell phospholipids results in increased drug uptake and chemo-sensitivity towards anti-cancer agents in drug resistant tumor cells *in vitro*.

4. Lipid enrichment preferentially enhances drug cytotoxicity in tumor cells compared to normal parent cells

The ability to alter chemosensitivity of tumor cells through alteration of the tumor fatty acid composition is of potential benefit for the treatment of cancer. Of critical importance is the impact of altering fatty acid composition on the chemo-sensitivity of non-malignant normal cells. If chemosensitivity of normal non-malignant cells is increased by altering the fatty acid composition to the same extent as malignant cells, then the potential advantage of nutritional manipulation of fatty acid composition to enhance cancer chemotherapy would potentially be lost. In this section, the impact of modifying the fatty acid composition on drug cytotoxicity in tumor cells will be compared to normal parent cells.

In NIH3T3 non-malignant mouse fibroblasts and NIH3T3-SIC, sigmoidal colon cancer oncogene transformed cells [13] EPA supplementation preferentially decreased saturated and increased PUFA concentrations in SIC transformed cells compared to the non-malignant parent cells, thereby increasing membrane

fluidity specifically in the malignant cells and increasing chemosensitivity of the NIH3T3-SIC transformed cells to mitomycin C(MMC) treatment. In the NIH3T3 non-malignant parent cell line, EPA enrichment lowered MMC toxicity. This suggests that the increased therapeutic index for MMC in cells treated with EPA, is related to preferential incorporation of the long chained PUFA into the tumor cell membrane.

Enriching murine leukemia L-1210 cells with DHA increased sensitivity to Ara-C and doxorubicin with I_{50} values of 1/10 and 1/4 of the unmodified cells. The addition of DHA specifically exacerbated the cytotoxicity of doxorubicin in L-1210 cells, and both EPA and DHA enhanced the cytotoxicity towards Ara-C. There were no observable differences in the cytotoxicity of doxorubicin or AraC in unsupplemented murine SI macrophages grown in culture with those supplemented with DHA, EPA or LA, thus, treatment with PUFA omega-3 fatty acids preferentially increased susceptibility of leukemic but not normal macrophage to the chemotherapeutic agents AraC and doxorubicin [12].

Similarly, EPA and DHA preferentially enhanced the cytotoxicity of doxorubicin to v-fps oncogene transformed rat fibroblast cells (C10) compared to normal rat fibroblasts (R2) [11]. In these two model systems, omega-3 fatty acid enrichment resulted in selective enhancement of cytotoxic drug action towards oncogene transformed compared to normal cells in culture.

Normal colonic epithelial cells (4D/WT) and v-src transformed cells (D/v-src) supplemented with a low dose (3 mM) of DHA or AA resulted in a 30-fold increase in the cytotoxicity of AraC in the transformed cell line [36]. The toxicity of AraC was increased only 1.7-fold in normal cells supplemented with DHA. There was a three-fold increase in the therapeutic index in DHA versus AA supplemented cells, indicating that DHA is a more potent fatty acid in reversing drug resistance in v-src transformed colonic cells. Protein kinase C signaling pathways may be implicated in the DHA enhancement of AraC chemotherapy since PKC has been shown to be an important modulator of AraC toxicity [37,38] and a decrease in overall PKC activity was associated with increased AraC cytotoxicity [38]. Omega-3 long chained PUFAs have been shown to inhibit certain protein kinase C activities [39–41], so the enhanced responsiveness of DHA enriched transformed cells toward AraC may be related to the DHA induced depression of PKC activity and its associated signaling pathways. More research on the association of PKC alteration in normal versus tumor cells in relation to their sensitivity to anticancer treatment is required to clarify this potential relationship.

5. Enrichment of lipid pools with polyunsaturated fatty acids enhances pro-oxidant chemotherapy through oxidative stress

Enriching tumor cells with the highly unsaturated long chained fatty acids from marine sources significantly increases the degree of unsaturation and methylene carbon content of the tumor lipids which would be expected to increase the susceptibility to lipid peroxidation and pro-oxidant therapy. Literature describing the omega-3 fatty acid induced enhancement of tumor responsiveness to chemotherapy through an oxidative stress mechanism will be reviewed in this section.

In MDA-MB-231 human mammary cells in culture, DHA decreased cell viability from 54% with doxorubicin alone to 21% with doxorubicin plus DHA to 12% with doxorubicin plus DHA plus oxidants. The cytotoxicity of the low peroxidizing drug mitoxantrone was not influenced by DHA treatment and DHA stimulated cytotoxicity was inhibited by the addition of anti-oxidants [25]. These findings show that DHA increases the efficacy of oxyradical-generating anti-cancer agents like doxorubicin [42,43] by incorporating highly peroxidizable fatty acids into the tumor lipids and support the conclusion that synergism was mediated by oxidative stress.

GLA, ALA, EPA and DHA synergistically increased chemosensitivity to paclitaxel in breast cancer cell lines MDA-MB-231, SK-Br3, T47-D and MCF-7 when fatty acid and drug were added concurrently. The addition of alpha-tocopherol partially reversed the lipid-induced synergism with paclitaxel and the synergism with oleic acid a non-oxidizable, mono-unsaturated fatty acid was markedly lower than with GLA [44].

Enriching L5178Y lymphoma cells with DHA enhanced the cytotoxicity of doxorubicin, dexamethasone and mitomycin C. Increasing DHA concentrations in the media generally decreased survival at all drug concentrations [23]. Survival of L5178Y lymphoma cells was reduced with increasing unsaturation and chain length of exogenous fatty acid.

Enriching murine leukemia L-1210 cells with DHA increases the sensitivity to the membrane active thioether lipid BM41.400 compared to oleic acid (C18:1n-9) [10]. Incorporation of DHA into L-1210 membranes increased the cellular/membrane fatty acid degree of unsaturation and did not enhance transport or accumulation of drug. The enhanced drug sensitivity was attributed to an alteration in oxidative stress events.

Acrolein was reported to be cytotoxic to two lung carcinoma cell lines A-427 and SK-LU-1 and the glioblastoma cell line A-172 [45] by reducing the levels of

glutathione in the cell lines. Lung carcinoma A-427 was the most sensitive to acrolein, possessed the lowest level of endogenous GSH and became the most depleted upon acrolein treatment. The cytotoxic action of acrolein was enhanced by DHA and reversed by anti-oxidants. Acrolein, an alpha beta-unsaturated aldehyde is a product of lipid peroxidation, and is capable of inducing lipid peroxidation [46]. It can also be formed by the metabolism of threonine by myeloperoxidase in neutrophils [47] and is produced during the bioactivation of the cytostatic drug, cyclophosphamide [48–50]. This synergism between DHA and acrolein could be expected to translate to synergism between cyclophosphamide and omega-3 fatty acids, and in fact, synergism between menhaden oil feeding and cyclophosphamide therapy was observed in athymic mice implanted with human mammary carcinoma MX-1 [20]. The sensitivity of the cell lines to acrolein was related to depressed levels of the antioxidant glutathione and subsequent lipid peroxidation, a conclusion supported by the observation that the addition of antioxidants reversed acrolein's toxicity. In contrast, SK-LU-1 bronchial carcinoma and glioblastoma A-172 cells containing higher levels of GSH showed less synergism between acrolein and DHA indicating that the glutathione redox status of a particular tumor line is important for the responsiveness to pro-oxidants. This conclusion is consistent with the observations of others that tumor glutathione level is important in responsiveness to chemotherapy [51–56].

DHA supplementation increased the sensitivity of HL-60 leukemia cells resistant to the pro-oxidant arsenic trioxide with cell viability decreasing from 85% in arsenic trioxide treated cells alone to 13% in treated cells that had been supplemented with DHA [57]. The combined effect of arsenic trioxide and DHA was attributed to increased apoptosis induced by production of reactive oxygen species (ROS) and lipid peroxidation.

These synergistic studies were extended to 10 arsenic trioxide resistant-solid tumor cell lines [35], where the combined treatment with DHA and arsenic trioxide reduced the viability in 7 of 10 arsenic trioxide resistant cell lines including HT-29, SW-620, LS-174T colon carcinoma, SK-BR-3 breast carcinoma, SKOV-3 ovarian carcinoma, PC-3 prostate carcinoma and skin melanoma. In addition, synergism between arsenic trioxide and DHA was apparent in MDA-MB-468 breast carcinoma although this tumor line was not resistant to arsenic trioxide [35]. These effects were specific to tumor cells as normal endothelium; blood (PBMcs) and skin fibroblast cells did not show increased sensitivity to dual treatment. Furthermore, they observed that dual treatment resulted in increased lipid peroxi-

dation only in the susceptible cells, whereas, no lipid peroxidation was observed in cells resistant to the combination. Anti-oxidants and incorporation with non-peroxidizable fatty acids prevented lipid peroxidation, apoptosis and decreased cell viability. These findings show that enriching tumor phospholipid with the highly peroxidizable long chained omega-3 polyunsaturated fatty acids increased the tumor sensitivity to pro-oxidant chemotherapy through the generation of oxidative stress and damage. The PUFA enhanced response to redox-active chemicals was reversed by the addition of anti-oxidants and exacerbated in tumors possessing low endogenous anti-oxidants.

Developing strategies that decrease tumor anti-oxidant capacity while increasing oxidant potential through nutritional intervention may prove useful for enhancing pro-oxidant chemotherapy.

6. Targeting specific signaling pathways for nutritional intervention to enhance cytotoxicity of anti-cancer drugs

Alteration of cell signaling pathways by fatty acid treatment has been shown to modulate chemo-sensitivity towards specific anti-cancer agents, and these interactions are reviewed in this section.

MCF-7 human mammary carcinoma cells that are transfected with the Akt plasmid express constitutively active myristoylated Akt-1 kinase resulting in resistance to the anti-estrogen agent, tamoxifen [58]. Activation of Akt activity has been reported to promote tamoxifen resistance in breast cancer cells [59,60] but enrichment of the Akt transfected MCF-7 cells with DHA and EPA resulted in a decrease in Akt-1 activity by 20% and 67%, respectively, whereas, linoleic acid (LA) treatment resulted in increased Akt activity [58]. Co-treatment of the transfected cells with EPA increased sensitivity towards tamoxifen in a dose dependent manner which was attributed to inhibition of the Akt protein kinase, reported to phosphorylate the estrogen receptor alpha (ER α) [58]. Indeed, omega-3 fatty acids have been reported to inhibit a variety of protein kinases [39–41] and phosphorylation of the ER α was shown to prevent responsiveness of tumor cells to tamoxifen [59,60]. Thus the partial reversal of the resistance of MCF-7 transformed cells to tamoxifen is attributed to the inhibition of Akt kinase by EPA. High Akt activity was also associated with resistance to the cytotoxic agents paclitaxel and doxorubicin indicating that the omega-3 fatty acid inhibition of Akt kinase may also be responsible for the enhancement of chemosensitivity to paclitaxel and doxorubicin.

Synergistic stimulation of apoptosis and inhibition of proliferation occurred when HCA-7 human colon carcinoma cells were incubated with low levels of celecoxib and DHA [61]. In fact, greater growth inhibition was observed with lower concentrations of celecoxib and DHA together than higher concentration of each individually. Cyclo-oxygenase-2 expression and PGE-2 levels reacted similarly to co-treatment with celecoxib and DHA. Modification of the COX-2 pathway by omega-3 fatty acids, a well known phenomenon [62] pre-disposes the HCA-7 colon carcinoma cells to the action of celecoxib [61], and decreases the dose of celecoxib required to inhibit growth of colon tumor cells. Synergism between DHA and celecoxib was reported in LnCap, DU-145 and PC-3 prostate carcinoma cells *in vitro* and implicated the COX-2, NFkB pathway [63]. Thus, depression of the COX-2 pathway through nutritional alteration pre-disposes breast and prostate carcinoma to therapy with the COX-2 inhibitor celecoxib.

Synergistic anti-proliferative activity between the omega-6 fatty acid GLA and vinorelbine was observed in MCF-7, MDA-MB-231, T47D and SK-BR3 cells, and Vitamin E only partially protected the cells from synergistic activity [64]. When GLA was added concurrently with the microtubule-disrupting anti-cancer agent docetaxel, synergistic cytotoxicity towards the estrogen-dependent MCF-7 and estrogen-independent MDA-MB-231 human mammary carcinoma cell lines and additive cytotoxicity towards the estrogen independent SK-Br3 human mammary carcinoma line was observed [65]. Synergistic cytotoxicity was observed in MDA-MB-231 and SK-Br3 estrogen independent lines when GLA exposure was followed sequentially by docetaxel, whereas, this treatment schedule resulted in additive cytotoxicity towards MCF-7 cells. Lipid peroxidation as a mechanism of synergistic activity was ruled out because the addition of Vitamin E only marginally abolished the synergistic cytotoxicity. GLA induced depression of p185Her-2/neu oncoprotein and the dose dependent decrease in Her-2/neu mRNA was suggested as a mechanism of the synergistic cytotoxicity.

In both BT-474 and SK-Br-3 human breast carcinoma cells that over express Her-2/neu oncogene, exposure to oleic acid reduced the cell surface-associated p185 (Her-2/neu) [66]. The combination treatment with OA and trastuzumab a drug that targets the Her-2/neu oncogene resulted in a synergistic decrease in Her-2/neu expression, a decrease in cell viability and an increase in nuclear concentrations of p27 (kip1), a cyclin dependent kinase inhibitor that regulates the cell cycle and progression of Her-2/neu stimulated breast cancer. The combined treat-

ment also was shown to inhibit production of phosphorylated Akt and MAPK, signaling systems downstream of Her-2/neu oncogene.

Since oleic acid is a non-peroxidizable fatty acid, lipid peroxidation was ruled out as a mechanism. It is more likely that the synergistic inhibition of the Her 2/neu signalling cascade by OA and herceptin is responsible for the synergistic decrease in tumor cell viability.

Modulating specific oncogene signalling through nutritional manipulation that can potentiate the inhibition of selective anti-cancer agents acting on that pathway represents a novel and important anti-cancer strategy that requires further exploration.

7. Modification of tumor fatty acid composition enhances cancer chemotherapy *in vivo*

The level and type of fat in the diet has been shown to influence the chemosensitivity of experimental tumors to chemotherapy (Table 2) and these *in vivo* studies are reviewed in this section.

Feeding low fat diets containing 10% fish oil decreased the growth of human mammary carcinoma MX-1 in athymic mice and increased the responsiveness of the tumors to mitomycin C and doxorubicin compared to tumors from mice fed a low fat 10% corn oil diet [16]. The responsiveness of MX-1 carcinoma in athymic mice to mitomycin C chemotherapy was enhanced when host animals were fed a high fat diet of corn oil compared to a group fed a low fat corn oil diet [67]. This suggests that the level of fat in the diet is an important factor in chemosensitivity of anti-neoplastic therapy with a high fat diet improving the responsiveness to chemotherapy [67]. The mechanism of the high fat diet enhanced chemotherapy response is presently unexplained although tumors from the high fat corn oil fed mice were subjected to increased oxidative stress with and without chemotherapy as measured by increased lipid peroxidation [67].

Feeding a high fat menhaden oil diet suppressed tumor growth and enhanced responsiveness to mitomycin C therapy [17]. Tumors from animals fed a high fat fish oil diet were 10-fold more sensitive to mitomycin C than tumors from low fat corn oil fed mice, and five-fold more sensitive than tumors from high fat corn oil fed mice. During chemotherapy with MMC the high fat diet rich in long chained omega-3 PUFAs supported a superior anti-cancer response compared to both high and low corn oil fed groups supporting the conclusion that both the level of fat in the diet as well as the type of fat in the diet can influence the chemosensitivity of anti-neoplastic therapy with mitomycin C.

Table 2
Fatty acids enhance tumor response to chemotherapy *in vivo*

Tumor system	Drug/dietary regime	References
MX-1 human mammary carcinoma in athymic mice	Mitomycin C 5% CO 5% CO + 20% MO	[17]
MX-1 human mammary carcinoma in athymic mice	Cyclophosphamide 5% CO 25% CO 5% CO + 20% MO	[20]
MX-1 human mammary carcinoma in athymic mice	Doxorubicin Mitomycin C 5% CO 25% CO 5% CO + 20% MO	[16]
MCF-7 human breast carcinoma	CPT-11 (irinotecan) 7% CO 4% CO + 3% FO 1% CO + 6% FO	[71]
A-549 human lung carcinoma	Doxorubicin 20% CO 19% FO	[73]
MDA-MB-231 human mammary carcinoma	Edelfosine 5% CO 1% CO + 119% FO	[69]
L-1210 murine leukemia cells	AraC 1.5% DHA 3.5% DHA	[68]
Transformed rat-2 fibroblasts (C-10)	AraC 10% DHA No change in therapeutic response but lowered drug toxicity. Increased therapeutic index	[18]
NMU-induced rat mammary tumors	Epirubicin	[75]
MDA-MB-435 human mammary carcinoma	Indomethacin 18% MO (trend for synergism but not significant)	[62]
3LL Lewis lung carcinoma	Cisplatin 5% soy oil + E 4% FO + 1% CO 4% FO + 1% CO + E	[79]
MDA-MB-231 human mammary carcinoma	Doxorubicin 5% CO 2% CO + 3% FOC	[74]
MAC16 colon adenocarcinoma	Epithilone, DHA 2.4 g/kg; cyclophosphamide, EPA 2.0 g/kg 5-FU, EPA 2.0 g/kg	[80]

CO = corn oil; MO = menhaden oil; FO = fish oil; FOC = fish oil concentrate; DHASCO = algae oil containing 44.6% DHA as the single long-chained omega-3 fatty acid; E = Vitamin E (alpha-tocopherol).

Tumors from the menhaden oil fed group possessed an elevated level of the long chained omega-3 PUFAs, an increased unsaturation index in their phospholipids, enhanced activity of NAD(P):quinone oxidoreductase, an enzyme reported to activate MMC, and elevated activities of the anti-oxidant enzymes SOD,

catalase glutathione peroxidase and glutathione reductase. The NAD(P): quinone oxidoreductase and several anti-oxidant enzymes were further enhanced following MMC treatments, and all were significantly elevated over tumors from both corn oil fed groups and corn oil fed mice treated with MMC. Lipid peroxidation and protein

aldehyde products (an indicator of oxidative damage to proteins) were elevated in the tumors from the menhaden oil fed animals and further increased when treated with MMC. A depressed ratio of GSH/GSSG in the tumors from menhaden oil fed mice treated with MMC further implicated oxidative stress in the omega-3 fatty acid enhanced sensitivity to MMC.

MMC is a bioreductive alkylating naphthoquinone which requires bioreduction with NAD(P)⁺: quinone oxidoreductase and subsequent formation of a quinone-methide-intermediate capable of alkylating DNA and proteins within tumor cells. Treatment with MMC decreased the activity of tumor glutathione reductase, an anti-oxidant enzyme critical for maintaining glutathione in a reduced state [17] for participation in the glutathione peroxidase reaction.

Dietary fish oil induced increased sensitivity of MX-1 human mammary carcinomas towards MMC was attributed to the enhanced activity of the MMC “activating” enzymes as well as the increased unsaturation index of the tumor lipids thereby predisposing them to pro-oxidant chemotherapy. These *in vivo* results are consistent with the *in vitro* studies showing synergism between omega-3 fatty acid enrichment and pro-oxidant chemotherapy [10,25,35,44,45,57].

Synergism was also observed between dietary long-chained omega-3 fatty acids and the anti-neoplastic agent cyclophosphamide in athymic mice inoculated with human mammary carcinoma MX-1 [20]. At a low concentration of cyclophosphamide, feeding high levels of menhaden oil increased the anti-neoplastic effect compared to mice fed a low fat diet of corn oil.

The high fat menhaden oil diet increased the activity of several cytochrome P-450 isoenzymes in the liver and tumor, especially CYP2B1 an important enzyme for bioactivation of cyclophosphamide. Thus, dietary menhaden oil induced the *iso*-enzyme that produces pharmacologically active metabolites of cyclophosphamide including the alkylating phosphoramidate mustard and the pro-oxidant acrolein. Enhanced formation of phosphoramidate mustard would be expected to increase the anti-cancer activity and could account for the synergistic anti-neoplastic activity observed between low levels of cyclophosphamide and dietary menhaden oil. In addition, increased intracellular levels of acrolein would also be expected to initiate lipid peroxidation. Synergistic cytotoxicity was observed between acrolein and DHA enriched A-427 lung carcinoma *in vitro* [45]. Therefore, multiple mechanisms may well be responsible for the lipid enhanced anti-neoplastic activity of cyclophosphamide.

An important question arising from observations that nutritional intervention enhances the sensitivity of tumors to chemotherapy is what impact dietary lipids may have on the toxicity to the host. Serious side effects to chemotherapy can limit the usefulness of a particular anti-cancer drug, so the effect of diet on host toxicity is an important consideration in any nutritional intervention strategy.

Feeding a high fat in menhaden oil resulted in significant protection from the acute toxicity of cyclophosphamide in the host mice [20]. Dietary menhaden oil was found to modulate aldehyde dehydrogenase and cytochrome P-450 enzyme activities in such a way that could increase the therapeutic index of cyclophosphamide towards mammary carcinoma by increasing its therapeutic effectiveness at low doses and decreasing host toxicity [20]. These findings could have important implications for the treatment of human breast cancer with cyclophosphamide.

In MDA-MB-435 human breast carcinoma grown in athymic mice, feeding a diet high in menhaden oil decreased tumor growth rates and resulted in possible synergism with indomethacin, a COX-2 inhibitor [62] (synergism was not significant but reported as a trend). Omega-3 fatty acids have been shown to inhibit COX-2 activity and decrease production of prostaglandin E₂. The nutritional depression of COX-2 activity could well predispose tumors to chemotherapy with indomethacin, a drug that also acts on COX-2. These findings *in vivo* are consistent with the observed synergism between the COX-2 inhibitor celecoxib and DHA in colon cancer cells HCA-2 *in vitro* [61].

Conversely, feeding a diet of 10% DHASCO containing 44.6% DHA as the only long chained omega-3 PUFA to rats resulted in no change in the therapeutic response of transformed rat-2 fibroblasts (C-10) to AraC; consumption of a diet rich in DHA did retard tumor growth, prevent hyperlipidemia, enhance bone marrow cellularity and promote intestinal growth compared to a similar diet containing omega-6 fatty acids. By lowering AraC toxicity, DHASCO feeding resulted in increased therapeutic index for AraC chemotherapy [18]. Similarly, in mice containing L-1210 leukemia, modest levels of dietary DHA improved the anti-neoplastic efficacy of AraC, and the DHA fed mice were protected from AraC toxicity evidenced by circulating neutrophil counts, increased platelet levels and less frequent death due to drug toxicity [68]. Thus, a diet containing DHA as the single long chained omega-3 PUFA increased the therapeutic index of AraC towards mice bearing L-1210 leukemia [18] and rats bearing transformed rat-2 fibroblasts [68].

The combination of dietary long chained omega-3 fatty acids and treatment with the lipid soluble ether-lipid drug edelfosine Et-18-OCH₃ and the pro-oxidant FeCl₃ resulted in the greatest suppression of tumor growth, the lowest mitotic index, the highest level of lipid peroxidation and an increased cytotoxic index [69]. No detectable untoward events in the host animals were observed. Indeed, the ether class of anti-neoplastic agents has been shown to enhance peroxidative damage to membranes [70], a mechanism consistent with an omega-3 PUFA enhancement of pro-oxidant chemosensitivity through a predisposition of tumor lipids to peroxidation. Feeding fish oil caused a significant regression of human mammary carcinoma in athymic mice when treated with the topoisomerase inhibitor CPT-11 (irinotecan) [71]. The same treatment in the corn oil fed group stopped tumor growth but did not cause tumor regression. In addition, dietary fish oil decreased untoward structural changes in the mucosal architecture following irinotecan therapy, whereas, in the corn oil fed group, structure changes in the intestinal mucosa were observed and persisted for 5 days. Feeding an omega-3 fatty acid rich fish oil concentrate modulated host toxicity in mice receiving irinotecan therapy as decreased apoptotic figures in duodenal crypts, suppressed levels of hepatic inflammatory eicosanoids decreased liver hypertrophy, improved white blood cell counts, increased RBCs, decreased irinotecan induced immature RBC formation and RBC micro-nuclei, increased EPA and DHA levels in hepatic cellular membranes and normal grooming behavior of the host mice was observed [72]. Thus, consumption of fish oil concentrate mediates irinotecan toxicity. In a separate study the consumption of a 19% fish oil diet resulted in significant regression of A549 lung xenograph in athymic mice treated with the pro-oxidant chemotherapeutic agent doxorubicin, whereas, doxorubicin only stopped lung tumor growth in the group fed 20% corn oil [73]. Similarly, enhanced anti-cancer responsiveness of MBA-MB-231 human breast carcinoma to doxorubicin was observed when the host athymic mice were fed fish oil concentrate [74] and the resulting altered redox state of the tumors was significantly correlated to the depressed rate of tumor growth. Doxorubicin toxicity was decreased through feeding fish oil concentrate as determined by body weight change, blood cell counts and number of micro-nuclei in peripheral blood RBCs. Again, feeding fish oil has increased the therapeutic index of doxorubicin towards mammary carcinoma by increasing tumor responsiveness to doxorubicin and decreasing host toxicity. Nutritional intervention with fish oil in rats implanted with NMU-induced rat mammary tumor,

resulted in increased responsiveness to the anthracycline anti-neoplastic agent, epirubicin known to redox cycle with oxygen, generate reactive oxygen species (ROS), and induce oxidative stress within the tumors without causing toxic side effects in the host rats [75]. Omega-3 fatty acid supplementation in rats did not prevent or aggravate epirubicin-induced cardiotoxicity, but since omega-3 fatty acid supplementation decreased tumor growth and enhanced chemotherapy, the therapeutic index was improved [76].

Consistent with these *in vitro* and *in vivo* findings are the clinical findings that breast adipose tissue levels of the omega-3 fatty acids of patients with locally advanced breast carcinoma were related to tumor response to the combination therapy involving mitoxantrone, vindesine, cyclophosphamide and 5-fluorouracil [77]. The level of omega-3 fatty acids in the breast adipose tissue was higher in the patients exhibiting a partial or complete regression of tumor compared to the patients with no response or with tumor progression. In addition, among the fatty acids tested, only DHA was significantly associated with tumor response and DHA proved to be an independent predictor of chemosensitivity to anti-cancer therapy [77]. In addition, a recent clinical trial demonstrates that DHA intake improves first line chemotherapy with epirubicin, cyclophosphamide and 5-FU and improves survival in metastatic breast cancer [78].

Feeding fish oil to mice bearing the highly metastatic clone (D-122) of 3LL Lewis lung carcinoma significantly depressed tumor growth rate and lung metastatic load compared to soybean oil fed controls [79]. Addition of anti-oxidants significantly increased tumor growth rate and metastatic load implicating oxidative stress in the tumor suppressing activity of dietary fish oil. However, in the mice fed fish oil, treatment with cisplatin following tumor resection resulted in a decreased metastatic load when anti-oxidants were present compared to the fish oil fed group without added anti-oxidants. Thus, dietary fish oil depresses metastasis during cisplatin treatment following tumor resection, and this effect did not apparently involve oxidative stress.

In MAC16 colon adenocarcinoma tumors, DHA potentiated growth inhibition of epothilone, a mitotic spindle poison, *in vivo* and EPA enhanced the anti-cancer effects of cyclophosphamide and 5-FU [80].

Collectively these *in vitro*, *in vivo* and clinical findings demonstrate that the level and type of fat in the diet impacts the chemo sensitivity of various tumors with high fat diets containing long chained omega-3 PUFAs preferentially decreasing tumor growth and increasing responsiveness to chemotherapy.

Table 3
Enrichment of tumors with long chained PUFAs results in enhanced radiosensitivity, hyperthermia and photodynamic therapy

Tumor line	Diet	References
<i>N</i> -methylnitrosourea (NMU) (<i>in vivo</i>)	7% Peanut oil + 8% DHASCO oil	[81]
36BIO rat astrocytoma	15–45 μ M GLA, EPA, DHA (<i>in vitro</i>) OA no effect	[19]
MDA-MB-231 human mammary carcinoma	10% CO 10% CO + 5% n – 3 fatty acid supplement	[82]
Rat AH 109A hepatic carcinoma (<i>in vivo</i>)	GLA (hyperthermia)	[83]
Rat AH 109A hepatic carcinoma (<i>in vivo</i>)	EPA 1 g/kg/day (hyperthermia)	[84]
L-1210 murine leukemia	DHA 32 μ M <i>in vitro</i> (hyperthermia)	[15]
A-172 glioblastoma	DHA, SS	
A-427 lung Adenocarcinoma	10 μ M <i>in vitro</i> 48 h 70 μ M <i>in vitro</i> 72 h 5-ALA-PDT	[89]

EMT-6 rat mammary carcinoma; 5-ALA-PDT 5-aminolevulinic acid, photodynamic therapy.

8. Fatty acid enhancement of radiation therapy of cancer

There are several reports that dietary fat influences the responsiveness of tumors to radiation therapy (Table 3).

Supplementation of 36BIO rat astrocytoma cells with GLA, EPA or DHA increased the methylene bridge index of the tumor lipids, thereby increasing their susceptibility toward lipid peroxidation, of their lipids through changes in the fatty acid profiles [19]. All three PUFAs increased radiation-induced astrocytoma cell kill which was attenuated by the addition of alpha-tocopherol implicating oxidative stress in the lipid-enhanced susceptibility to radiation. Supplementation with the mono-unsaturated fatty acid, oleic acid possessing a low methylene bridge index and therefore low propensity of lipid peroxidation, did not affect cell survival following radiation. *N*-methylnitrosourea (NMU) induced mammary tumors that were nutritionally enriched with DHA were two-fold more sensitive to radiation therapy than tumors from palm oil fed controls [81]. The addition of Vitamin E reversed the DHA stimulated response to radiation treatment further implicating a free radical mechanism in the enhancement of radiation therapy.

In contrast, feeding a diet supplemented with a fish oil concentrate to athymic mice orthotopically implanted with MD-MBA-231 mammary carcinoma resulted in decreases in tumor growth rate, cell proliferation and tumor blood vessel volume density, but did not increase radiation response compared to the fish oil-non-irradiated and corn oil radiated tumors [82]. There was no evidence that the side effects of irradiation was

either increased or prevented by feeding the omega-3 fatty acid diet.

9. Fatty acid enhancement of hyperthermia therapy of cancer

Injecting GLA in the arteries feeding rat hepatic carcinoma AH109A resulted in increased tumor lipid peroxidation and decreased tumor growth rate [83]. When GLA was combined with hyperthermia they observed a higher level of lipid peroxidation and a significantly enhanced anti-tumor effect. In this same system, when EPA was orally administered, they observed increases in tumor EPA levels, decreases in glutathione content and the combination of EPA consumption with hyperthermia enhanced the anti-tumor effect and lipid peroxidation [84]. EPA consumption specifically increases the susceptibility of liver tumors to lipid peroxidation, enhances the anti-tumor effect of hyperthermia and prolongs host survival. That lipid peroxidation can inhibit tumor growth *in vivo* was demonstrated when the infusion of the superoxide generating xanthine and xanthine oxidase into rabbit legs implanted with VX2 carcinoma enhanced lipid peroxidation in the tumor and was associated with significantly depressed tumor growth [85]. Co-administration of SOD and catalase significantly decreased the anti-tumor effect implicating superoxide and hydrogen peroxide known reactive oxygen species (ROS) in generating lipid peroxidation, oxidative stress, and producing an anti-neoplastic response. Furthermore, ROS produced from infusing xanthine and xanthine oxidase were more destructive to the

carcinoma than the surrounding tissue indicating a preferential and selective anti-cancer effect of pro-oxidant treatment.

Hyperthermia treatment of VX-2 carcinoma implanted in the hind leg of rabbits resulted in a significantly reduced rate of tumor growth, increased lipid peroxidation (TBARS) and decreased alpha-tocopherol levels [86]. The anti-tumor response to hyperthermia was reversed by co-administration of SOD or catalase, demonstrating that the generation of superoxide *in vivo* is involved in the hyperthermia induced depression of tumor growth in VX-2 carcinoma [87]. Taken together, these findings indicate that predisposing tumor phospholipids to superoxide mediated oxidative stress through nutritional intervention promises to be an important adjuvant to hyperthermia treatment.

Pre-treating MKN45 human gastric cancer cells in culture to hyperthermia (42 °C) significantly inhibited the TNF α -induced binding of NF κ B to nuclear DNA and decreased TNF α -induced apoptosis. Since omega-3 fatty acids have been implicated in inhibiting activation of NF κ B, their similar actions on NF κ B activation is a possible mechanism for the increased anti-tumor effect of the combination of omega-3 PUFAs and hyperthermia [88].

Photodynamic therapy of 5-aminolevulinic acid was enhanced towards A-427 lung adenocarcinoma and A-172 glioblastoma when cells were pre-treated with AA or DHA [89]. Lipid peroxidation was ruled out as a general mechanism for enhancing the cytotoxicity of 5-aminolevulinic acid and PDT, although it may be important to cell lines that are sensitive to lipid peroxidation.

10. Conclusion

Predisposing tumor cells to oxidative stress by feeding or infusing long chained PUFAs and enriching the tumor lipids with excellent substrates for lipid peroxidation results in increased response to pro-oxidant therapy including hyperthermia, radiation, photodynamic therapy and pro-oxidant chemotherapy. PUFA enrichment of tumor phospholipids also increase membrane fluidity and uptake of anti-cancer drugs resulting in enhanced accumulation of anti-cancer agents within the tumors. These nutrition-induced changes in tumor membrane composition have the potential to improve cancer chemotherapeutic response and reduce untoward side effects resulting in improved therapeutic index and patient survival and well being. Clinical trials will be required to translate these *in vitro* and *in vivo* findings into clinical usefulness.

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