Review

Lipid peroxidation, oxidative stress genes and dietary factors in breast cancer protection: a hypothesis

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Abstract

We have recently proposed that lipid peroxidation may be a common mechanistic pathway by which obesity and hypertension lead to increased renal cell cancer risk. During this exercise, we noted a risk factor swap between breast and kidney cancer (oophorectomy and increased parity, detrimental for kidney, beneficial for breast; high blood pressure, detrimental for kidney, beneficial for breast when it occurs during pregnancy; alcohol, beneficial for kidney, detrimental for breast, and so on). We have subsequently proposed the hypothesis that lipid peroxidation represents a protective mechanism in breast cancer, and reviewed the evidence of the role of lipid peroxidation on established hormonal and non-hormonal factors for breast cancer. Here, we review the evidence in support of lipid peroxidation playing a role in the relationships between dietary factors and breast cancer. Available evidence implicates increased lipid peroxidation products in the anti-carcinogenic effect of suspected protective factors for breast cancer, including soy, marine n-3 fatty acids, green tea, isothiocyanates, and vitamin D and calcium. We also review the epidemiological evidence supporting a modifying effect of oxidative stress genes in dietary factor-breast cancer relationships.

Introduction

Lipid peroxidation is a natural metabolic process under normal conditions. It can be divided into three stages: initiation, propagation and termination [1]. The initiation phase includes activation of oxygen and is rate limiting. Polyunsaturated fatty acids (the main component of membrane lipids) are susceptible to peroxidation. Lipid peroxidation is one of the most investigated consequences of reactive oxygen species' (ROS) actions on membrane structure and function. The idea of lipid peroxidation as a solely destructive process has changed during the past decade. It has been shown that lipid hydroperoxides and oxygenated products of lipid peroxidation degradation as well as lipid peroxidation initiators (that is, ROS) can participate in the signal transduction cascade [2,3], the control of cell proliferation, and the induction of differentiation, maturation, and apoptosis [4-6]. It has been shown that lipid peroxidation and ROS are triggers and essential mediators of apoptosis, which eliminates pre-cancerous and cancerous, virus-infected and otherwise damaged cells that threaten our health [7-10]. In addition, although the essential n-6 fatty acid linoleic acid has been shown to increase breast cancer in experimental studies, other n-6 fatty acids (such as conjugated linoleic acid, and gamma linolenic acid) and n-3 fatty acids (such as eicosapentaenoic acid, docosahexaenoic acid (DHA), and alpha-linolenic acid) have been shown to inhibit the growth of breast cancer *in vivo* and *in vitro* and this inhibition is correlated with the extent of lipid peroxidation generated in tumor cells [11]. This suppression of cancer growth is enhanced by pro-oxidants and eliminated by antioxidants, and this elimination is proportional to the inhibition of lipid peroxidation products by antioxidants [11].

We have recently proposed the hypothesis that lipid peroxidation represents a protective mechanism in breast cancer, and reviewed the evidence of the role of this process on established reproductive, hormonal, and non-hormonal factors for breast cancer [11]. There is some supporting evidence that lipid peroxidation may play a role in the potential anticarcinogenic effects of other breast cancer factors, including soy [12-18], marine n-3 fatty acids [19], isothiocyanates (ITCs) [20-23], green tea [24-28], and vitamin D [29] and calcium [30,31]. Here, we review the evidence in favor of lipid peroxidation playing a role in these relationships between dietary factors and breast cancer. We also review the evidence favoring a modifying effect of oxidative stress genes in these dietary factor-cancer associations.

ALA = alpha linolenic acid; CCND1 = cyclin D1; CI = confidence interval; COMT = catechol-O-methyl transferase; DHA = docosahexaenoic acid; EGCG = epigallocatechin gallate; GST = glutathione *S*-transferase; ITC = isothiocyanate; MDA = malondialdehyde; MnSOD = manganese superoxide dismutase; ROS = reactive oxygen species.

Lipid peroxidation: beneficial, detrimental or both?

There is ample evidence supporting a causative role of lipid peroxidation in selected human cancers, including kidney, liver and skin, and in degenerative diseases. In experimental models, estrogen treatment induces lipid peroxidation and subsequently increases the incidence of renal cell cancer [32,33]. Because estrogen is a risk factor for breast cancer, it has been hypothesized, based on this model, that lipid peroxidation may be one mechanism by which estrogen increases breast cancer risk [11]. But estrogen induces renal cancer or liver cancer in this experimental model, not breast cancer. Indeed, lipid peroxidation may be a relevant mechanism for renal carcinogenesis, a concept that we have proposed and that is strongly supported by experimental and epidemiological data [32-34], but there is a lack of experimental models in support of estrogens increasing lipid peroxidation and subsequently inducing breast cancer development [11]. In contrast, there is evidence favoring lipid peroxidation as an anticarcinogenic mechanism in breast cancer. A consideration of the literature on animal and *in vitro* studies suggests that an influence on breast cancer protection relates to the generation of lipid peroxidation products [11].

We believe that the beneficial or detrimental effects of lipid peroxidation on cellular structures may depend on several factors [11], such as baseline levels of ROS (inducers of lipid peroxidation), and the type of tissue, that is, slowly versus rapidly proliferating tissue, in which ROS exert their action [11], as we have discussed in detail in our prior communication [11].

Role of lipid peroxidation on the relationships between dietary factors and breast cancer

In general, the associations between dietary factors and breast cancer remain controversial in the epidemiological literature, but there is some support for a lipid peroxidation pathway and a protective effect from *in vitro* studies [11]. It is possible that the lack of consideration of the lipid peroxidation pathway and the implied modifying effects of related gene polymorphisms may account for some of the lack of consistency in previous epidemiological studies. We believe that many dietary factors have both antioxidant and pro-oxidant properties, but what is important is which property is responsible for their anti-cancer effect. We raise the possibility that a deficiency in lipid peroxidation, not an increased antioxidant potential, may be detrimental for breast cancer. This does not imply that antioxidants are detrimental for breast cancer, as different antioxidants may inhibit different cell structures with different affinity and intensity, such as lipids, proteins or other cell constituents. Thus, the lipid peroxidation theory does not contradict the fact that, in studies investigating blood levels of antioxidants and breast cancer risk, for example, the evidence of a benefit for protection is not clear but neither is evidence of harm.

In the following sections we consider the dietary factors n-3 fatty acids, n-6 fatty acids, soy, ITCs, tea, and vitamin D and calcium in light of the lipid peroxidation mechanism.

Marine n-3 fatty acids

Eight cohort studies, conducted in Norway [35], Japan [36], Europe [37], the US [38-41], and Singapore [42], have examined fish or marine n-3 fatty acids intake in relation to breast cancer; only one of them, the study from Singapore, found an association [42].

Results from case-control studies of marine n-3 fatty acids (eicosapentaenoic acid and DHA) measured in adipose tissue are mixed [43-47]. Maillard and colleagues [45] found strong inverse associations for DHA, and for the marine n-3/total n-6 fatty acids ratio. In a case-control study conducted in Finland, DHA level in breast adipose tissue, along with its dietary intake, were significantly lower in breast cancer patients compared to control patients, suggesting a protective effect of DHA in the risk of breast cancer [43]. Also, a case-control study conducted across five European countries found a decreased risk of breast cancer with an elevated ratio of marine n-3 fatty acids to total n-6 fatty acids in four of five centers [44]. In contrast to these findings, two case-control studies conducted in the US found no consistent association between n-3 fatty acid levels in adipose tissue and breast cancer risk [46,47].

The reason for the discrepancy between the US and European studies remains unresolved. One hypothesis is these discrepancies may be due to differences in ranges of fish intake across the various populations [42]. Another hypothesis is that the inconsistencies may be due in part to interactions between n-3 fatty acids and antioxidant compounds in the diet, affecting their roles in breast cancer risk [45,48,49]. As pointed out above, data from experimental studies suggest that the strength of the association with marine n-3 fatty acids may be reduced in the presence of high antioxidant intake because the latter inhibit the formation of lipid peroxidation products, which have been proposed as the proximal anti-carcinogens [48,50]. In fact, several experimental studies showed that the suppression in cancer growth with n-3 fatty acids is enhanced by pro-oxidants [51] and eliminated by antioxidants such as vitamin E [11,18,51,52]. This suggests that the anti-cancer effect of n-3 fatty acids is mediated, at least in part, by lipid peroxidation products and emphasizes the importance of the potential interactions of anti- and pro-oxidant compounds with marine n-3 fatty acids [45]. Maillard and colleagues [45] suggested that the lack of an association between marine n-3 fatty acids and breast cancer risk in US studies [39,46,47,53] may be due, in part, to the interfering role of antioxidant vitamins, which are commonly taken as supplements in the US [45,54].

As mentioned above, we recently published the first set of prospective results linking intake of marine n-3 fatty acids to

breast cancer protection in Singapore Chinese women [42]. In our study, relative to the lowest quartile of marine n-3 fatty acid intake, individuals in the higher 3 quartiles exhibited a 26% reduction in risk of breast cancer (relative risk = 0.74, 95% confidence interval (CI) = 0.58, 0.94). We also have published the first set of results in humans implicating the peroxidation products of marine n-3 fatty acids as the proximal anticarcinogens [19], a notion strongly supported by experimental evidence [18,49,50,52,55-57]. Glutathione-associated metabolism is a major mechanism for cellular protection against agents that generate oxidative stress, acting by eliminating products of lipid peroxidation [58,59]. Therefore, we hypothesized that individuals possessing the low activity genotypes of *GSTM1*, *GSTT1* and/or *GSTP1* (that is, *GSTM1*-null, *GSTT1*-null and *GSTP1 AB/BB* genotypes, respectively) [60,61] may exhibit a stronger inverse association between marine n-3 fatty acids and breast cancer than their high activity counterparts. This hypothesis was supported by study results [19].

N-6 fatty acids

Meta-analysis of case-control and prospective cohort studies on breast cancer have failed to demonstrate a convincing link between n-6 fatty acids and breast cancer development [62-64]. In contrast, diets containing n-6 fatty acids have been shown to induce breast cancer in experimental studies [65]. N-6 fatty acids act as competitive inhibitors of n-3 fatty acids in fat metabolism, and it has been shown that the stimulatory or inhibitory effect of n-6 or n-3 fatty acids in experimental mammary carcinogenesis is abrogated by the addition of the other type of fatty acid [66-68]. Experimental studies have shown that a diet high in n-6 fatty acids decreases the concentrations of marine n-3 fatty acid-induced lipid peroxidation products in breast cancers to the lowest levels, and that the lower this concentration, the bigger the tumor volumes resulting from n-6 fatty acid administration [52,55]. This experimental evidence is consistent with our results [42] showing that n-6 fatty acids increase breast cancer risk only among women with a low intake of marine n-3 fatty acids; that is, when concentrations of n-3 fatty acid-induced beneficial lipid peroxidation byproducts are low. So one possible mechanism whereby n-6 fatty acids increase breast cancer growth is the decreasing of the beneficial lipid peroxidation products derived from n-3 fats.

Soy

At least 13 epidemiological studies have assessed the direct relationship between the individual dietary intake of soy products and the risk of breast cancer (reviewed by Peeters and colleagues [69]). Overall, results do not show protective effects, with the exception maybe for women who consume phytoestrogens at adolescence or at very high doses [70-72]. Only 4 of these 13 studies were prospective, and none of them found statistically significant breast cancer reductions. The only prospective study with urinary measurements taken before breast cancer occurrence was done in a Dutch postmenopausal population and showed a non-significant breast cancer risk reduction for high excretion [73]. Soy phytoestrogen levels in the Dutch study were very low, as were those in another recent study [74]. In conclusion, none of the five prospective studies assessing the effects of phytoestrogens on breast cancer risk found protective effects [69]. However, none of these studies took into account mechanistic pathways by which soy can operate, including the lipid peroxidation pathway, for example, analyzing soy oil, where lipid peroxidation may be higher.

Soybean and canola oils are the primary sources of alpha linolenic acid (ALA; an oxidizable n-3 fatty acid) in the diet [75]. Soybeans contain lipoxygenase, which is an oxidizing enzyme [76] that catalyzes lipid peroxidation [77]. It has been shown that soybean lipooxygenase increases peroxidation of membrane lipids [78] and oxidizes low-density lipoproteins [12,13,79]. A soybean oil diet fed to rabbits and rats caused an increase in lipid peroxidation compared to controls [14-16]; this increase is accenuated by protein insufficiency [17].

Limited *in vitro* data suggest that the decreased breast cancer risk associated with ALA may be related to increased lipid peroxidation products [18]. The addition of ALA to breast cancer cells caused an increase in the formation of lipid peroxidation products in the cell lipids, and their content was correlated with the capacity of arresting cell growth [18]. The addition of the antioxidant vitamin E to the ALA-supplemented cancer cells diminished formation of lipid hydroperoxides and restored cell growth [18]. In addition, vitamin E also suppressed the inhibitory effect of ALA on tumor growth in different models of mammary carcinogenesis in rats [51]. Administration of oxidative compounds to diets high in ALA led to an inhibition of tumor growth in chemically induced mammary carcinogenesis [51].

In an experimental study, a fermented soymilk product induced generation of ROS and caused apoptotic cell death in MCF-7 breast cancer cells. Growth inhibition and ROS generation induced by fermented soymilk product could be inhibited by catalase and deferoxamine, indicating that the ROS production probably was the cause of this apoptotic cell death [80]. The opposite has also been reported, that is, soy having an antioxidant effect [81]. The effect appears to be indirect as the antioxidant potency of isoflavones is weak and the effects appear to be due more to effects on signaling pathways that induce antioxidant enzyme systems or suppress enzymes that produce ROS.

Isothiocyanates

In animal models of breast cancer, tumor growth is inhibited by brassica consumption, or ITC or indole-3-carbinol administration [82-85]. In humans, the relationship between brassica consumption and breast cancer risk is uncertain. Investigations have found null associations [86-89], protective but not statistically significant associations [90-92], and statistically

significant protective associations [93,94]. The failure to consider the lipid peroxidation pathway and the implied modifying effects of oxidative stress-related genes may explain the lack of consistency among human studies. ITCs are potent inducers of lipid peroxidation [20-23]. There is suggestive evidence that ITC-induced apoptosis is mediated by oxidative stress/lipid peroxidation products [22,23]. Depletion of the antioxidant glutathione significantly accelerated ITC-triggered apoptosis [23]. ITCs are also able to induce glutathione *S*-transferases (GSTs) and/or other antioxidant enzymes through the stress-signaling pathway involving oxidative stress [22]. Oxidizing agents enhanced ITC-induced ROS production and ITC-induced GST activities, whereas antioxidants inhibited both [22]. In humans, one study has shown that individuals with genetic polymorphisms encoding lower or no activity in antioxidant enzymes (GSTM1, GSTT1) experience more breast cancer protection from ITCs than those with common alleles [95], putatively because more beneficial peroxidation agents could reach the cancer cells and cause damage (see below). However, this finding has not been confirmed in a second study [94].

Tea

Published epidemiological studies overall suggest that green tea but not black tea consumption is related to decreased risk of breast cancer [96]. A recent meta-analysis [96] included three cohort and one population-based study for green tea, while five cohort and eight case-control studies were analyzed for a link between black tea and breast cancer. Overall summary odds ratio showed an approximately 20% statistically significant reduction in risk of breast cancer associated with high intake of green tea. No such protective effect was found for black tea.

Both green and black tea have demonstrated inhibitory activities against chemically induced mammary tumors in experimental animals [97-99]. Epigallocatechin gallate (EGCG), one of the main constituents of tea that is thought to be responsible for its anticancer properties, not only can function as an antioxidant, but it possesses the chemical property of a pro-oxidant. Previous studies on the antioxidative properties of EGCG have demonstrated that its effects include both trapping of ROS as well as inhibition of lipid peroxidation [25]. However, after neutralizing the peroxyl and/or other radicals, EGCG itself could be converted to a phenoxyl radical [26]. Experimental studies in HT-29 colorectal cancer cells have indicated that oxidative stress is involved in EGCG-induced cell death. The chemical property of EGCG as a potential pro-oxidant is highlighted by the blocking effects of reduced glutathione and *N*-acetyl-L-cysteine against EGCG-induced mitogen-activated protein kinase activation, cytochrome c release and cell death [24]. In a recent study [100], Wu and colleagues noted that the protective effect of tea on breast cancer was confined to those possessing the low-activity genotype of the antioxidant catechol-O-methyl transferase (COMT), putatively because more beneficial peroxidation agents could reach the cancer cells and cause damage.

Vitamin D and calcium

Increased mammographic breast density is strongly associated with the risk of breast cancer [101]. A recent study showed that an increased intake of vitamin D and calcium was associated with decreases in mammographic breast density [102].

Evidence from both *in vitro* and *in vivo* studies has demonstrated that vitamin D compounds can inhibit the growth of breast cancer cells [103]. The anticancer activity of the hormonal form of vitamin D, 1,25-dihydroxycholecalciferol ($1,25\text{-}(OH)_2D_3$) [104], is associated with inhibition of cell cycle progression, induction of differentiation, and apoptosis. In addition, $1,25(OH)_2D_3$ may exert some of its activity by cooperating with other anticancer agents. $1,25(OH)_2D_3$ and its synthetic analogues increased the susceptibility of cancer cells to the cytotoxic/cytostatic action of tumor necrosis factor [105-107], interleukin 1, interleukin 6 [108], doxorubicin, menadione [109], and radiation [110]. A feature shared by these agents whose potency is increased by $1,25(OH)_2D_3$ is their ability to bring about excessive ROS generation in their target cells [111-113]. This common feature suggests the involvement of ROS in the interaction between $1,25(OH)_2D_3$ and these agents. In addition, the potentiation of the cytotoxic/cytostatic action of the chemotherapy drug doxorubicin or cytokines by $1,25(OH)_2D_3$ is markedly inhibited by the antioxidant N-actylcysteine [108,109]. Importantly, it has recently been shown that $1,25(OH)_2D_3$, acting as a single agent, is also a pro-oxidant in cancer cells [29]. These findings indicate that $1,25(OH)_2D_3$ causes an increase in the overall cellular redox potential that could translate into modulation of redox-sensitive enzymes and transcription factors that regulate cell cycle progression, differentiation, and apoptosis [29].

The evidence that calcium or dairy products are associated with breast cancer risk is still open to debate. A recent pooled analysis of cohort studies failed to find an association [114], but some studies found some limited evidence of a protection [115,116]. As mentioned above, a recent study showed that an increased intake of vitamin D and calcium was associated with decreases in mammographic breast density [102]. Boyapati and colleagues [116] recently reported that dietary calcium intake was negatively associated with the risk of breast cancer in both premenopausal and postmenopausal women. In the Nurses' Health Study both calcium and dairy product intake was associated with a survival benefit for women with breast cancer [117,118].

After ingestion, calcium absorption can occur via an active transport process (transcellular) that requires the action of $1,25\text{-}(OH)_2D_3$, or by passive diffusion (paracellular), which is a vitamin-D-independent process. The vast majority of

calcium absorption (77% to 92%) relies upon the trans-cellular pathway, and thus upon the activity of 1,25-$(OH)_2D_3$ [119]. Therefore, it is difficult to separately establish the potential effects of these two nutrients on health and disease, and it is of clear importance to evaluate the effects of these nutrients simultaneously [120].

There are several proposed mechanisms of action for calcium, including inhibition of cellular proliferation [121,122], and induction of apoptotic cell death [123,124] through oxidative stress [30,31,125,126]. It is, however, unclear how calcium, especially through intake, might have these hypothesized effects when blood levels are so tightly regulated. It seems that the elevation in the intracellular free calcium, which correlates with the induction of apoptosis in breast cancer cells, is brought about by vitamin D compounds [124,127-129]. The latter studies further support the need for investigations that consider the role of vitamin D and calcium simultaneously. The effects of these two nutrients may be strongly related to one another, and separate studies may not capture their true effects on breast neoplasia. Generally, apoptotic cell death is triggered by intracellular signaling pathways, including a rise in intracellular free calcium [30], the generation of ROS and membrane lipid peroxidation products [125,126]. Calcium strongly stimulates the release of ROS [130] (including superoxide anion [131-133], hydrogen peroxide and hydroxyl radicals [134]), which induce membrane lipid peroxidation [130]. This calcium-dependent increase in membrane lipid peroxidation triggers apoptotic cell death [30,31,125,126]. Calcium, lipid peroxidation and apoptosis are possibly interlinked through signals, as is evident from the increased activity of nuclear factor kappa-B, a critical molecule in oxidative-stress-induced apoptosis, and generation of nitric oxide [135].

Oxidative stress metabolism: modifying effect of oxidative stress genes on the relationships between dietary factors and breast cancer

If lipid peroxidation/ROS plays a role in breast cancer protection, it is likely that differences in the ability to protect cells from these beneficial products will determine, to some degree, the effect of these protective factors on breast cancer. Critical to the effects of products of lipid peroxidation are the enzymatic and non-enzymatic defenses that protect the cells from oxidative stress through reduction of reactive molecules, including mitochondrial manganese superoxide dismutase (MnSOD), glutathione peroxidase, and catalase. These enzymes form the first line of defense against superoxide and hydrogen peroxide [136]. The resultant secondary oxidation products may still damage DNA, proteins and lipid, and require further detoxification. This second line of defense against ROS/lipid peroxidation is provided by enzymes such as the GSTs. Several examples support a modifying effect of oxidative stress genes on the relationships between dietary factors and breast cancer.

We will summarize next the potential modifying roles of oxidative stress genes on the relationships between n-3 fatty acids, ITCs, and tea and breast cancer.

Glutathione S-transferases GSTM1, GSTT1, and GSTP1

Glutathione-associated metabolism is a major mechanism for cellular protection against agents that generate oxidative stress, protecting cells against cytotoxic products of lipid peroxidation [58,59]. GSTs are induced under conditions of oxidative stress, and alpha-, pi-, mu-, and theta-class GSTs are active in detoxification of numerous products, including reactive oxidant damage to DNA and lipids, such as organic epoxides, lipid hydroperoxides, and unsaturated aldehydes [58,59]. Individuals lacking these enzymes may have reduced removal of lipid peroxidation products and, thus, may experience higher cancer protection, as supported by results from our study [19]. We found that women with genetic polymorphisms causing lower or no activity in detoxifying genes (*GSTM1*, *GSTT1*, *GSTP1*) had more protection from marine n-3 fatty acids than those with common alleles, putatively because more cytotoxic peroxidation agents could reach the cancerous and pre-cancerous cells and cause damage. Recent results on cyclin D1 (CCND1), an intracellular cell-cycle regulatory protein with checkpoint function, and breast cancer risk further support our hypothesis of an oxidative stress-induced apoptosis mechanism underlying the diet-induced breast cancer protection. CCND1 has been shown to modulate growth arrest and apoptosis following exposure to ionizing radiation oxidative DNA damage [137,138]. In a recent study, the protective effect of the heterozygous *CCND1 GA* genotype on breast cancer risk was restricted to situations of elevated oxidative stress, characterized by absence of antioxidant GSTM1 and GSTT1 enzymes [139]. In addition, *CCND1* also interacted with marine n-3 and n-6 fatty acids to influence breast cancer risk, with the protection being restricted to women with a high intake level of n-6 fatty acids, or a low intake level of marine n-3 fatty acids [139].

Similarly, ITCs' protective effect on breast cancer found in some studies [95], although not in others [94], was mainly confined to those possessing the low activity genotype of GSTs. There is suggestive evidence that ITC-induced apoptosis is mediated by oxidative stress/lipid peroxidation products [22,23]. Seow and colleagues [140] also found that individuals with genetic polymorphisms causing lower or no activity in antioxidant genes (*GSTM1*, *GSTT1*, *GSTP1*) had more colorectal cancer protection from ITCs than those with common alleles. These findings were attributed to the direct effects of GSTs on ITC excretion [140]. However, we proposed that the oxidative products of ITCs may be responsible, at least in part, for their anti-cancer effect, and this would explain why the protection appears more pronounced among subjects with lowest antioxidant GST activity [11], in parallel with what we had described for the marine n-3 fatty acids/GST/breast cancer relationship [19]. A

subsequent colon cancer study, in which the effect of the *CCND1* A870G polymorphism on colorectal cancer risk was found to be modified by *GSTM1*, *GSTT1*, *and GSTP1* genotypes and ITC intake [141], further supports our proposed oxidative stress-based hypothesis. In that study, the presence of at least one *CCND1* A-allele was associated with increased risk among low dietary ITC consumers with a high activity GST profile. In contrast, the presence of at least one A-allele was associated with a decreased risk among all remaining subjects, which led the investigators to hypothesize that subjects with low intake levels of ITCs and functional GST enzymes are left with low levels of pro-oxidative, anti-cancer acting ITCs at a cellular level [141].

The genetic polymorphisms of *GSTM1* and *GSTT1* have also been found to influence the risk-enhancing effect of alcohol in breast cancer. Zheng and colleagues [142] found that breast cancer risk was about 7-fold increased for postmenopausal women with the *GSTT1*-null genotype who consumed more than 250 kg of spirit equivalents. In our prior study [19], the *GSTT1*-null genotype was associated with a 30% reduced risk of breast cancer. This finding is consistent with another study that reported a decreased risk among premenopausal women lacking the *GSTT1* gene [60]. Most studies have found no increased risk for breast cancer with null genotypes for *GSTM1* and/or *GSTT1* (reviewed in [61]), although some positive associations have been reported [143-147].

Catechol-O-methyl transferase

COMT is an antioxidant enzyme that catalyses the methylation of hydroxylated sites on the aromatic ring of catechol compounds, which prevents their conversion to semiquinones and quinines and, therefore, blocks the generation of ROS [148-150]. In a recent study, the *COMT-L* low activity allele-containing genotypes (*HL* or *LL)* tended to be at decreased risk of developing breast cancer, especially the advanced stage of disease in premenopausal women and local carcinoma in postmenopausal women [151,152]. A tendency of decreasing risk can also be seen for both pre- and postmenopausal women in the study of Millikan and colleagues [153]. Similarly, in the case-control study of Lavigne and colleagues [154], a tendency of decreasing risk was seen among premenopausal women, although the results were based on a rather small number of subjects. Thompson and colleagues [155] observed increased risk for premenopausal women carrying the *COMT-L* allele-containing genotypes and decreased risk for postmenopausal women with these genotypes. Among Asian-American women in Los Angeles, the protective effect of tea in breast cancer was mainly confined to those possessing the low activity genotype of COMT [100]. A recent study was conducted among breast cancer families participating in the Metropolitan New York Registry, one of the six centers of the Breast Cancer Family Registry [156]. The study found that *COMT* genotypes were not statistically significantly associated with breast cancer risk, although the study population was of modest size (160 sib-ships).

Manganese superoxide dismutase

MnSOD is an antioxidant enzyme that is induced by free radical challenge, such as marine n-3 fatty acid-induced lipid peroxidation [136,157,158], and inhibits polyunsaturated fatty acid-induced lipid peroxidation and the subsequent killing of human breast cancer cells [159]. In the mitochondrion, MnSOD catalyzes the dismutation of two superoxide radicals, producing hydrogen peroxide and oxygen. A substitution variant in the mitochondrial targeting sequence was found that changes the amino acid codon at the -9 position in the signal peptide from valine to alanine [160-162]. Hiroi and colleagues [163] reported that processing efficiency of the valine-type SOD leader peptide in the presence of mitochondria was significantly lower than that of the alanine-type, which may reduce protection against superoxide radicals. This decrease in the efficiency of transport into mitochondria for the valine isoform of the protein may result in increased ROS [160,163]. An association between the valine allele with lung cancer risk has been reported in a recent study [164]. Contrarily, an association between the alanine allele and risk of breast cancer has been found in three studies, two conducted in the US [162,165] and one in Finland [166]. In the study by Ambrosone and coworkers [162], premenopausal women who were homozygous for the alanine allele had a four-fold increase in breast cancer risk in comparison to those with one or two valine alleles [162]. Risk was most pronounced among women who consumed below the median amount of fruit and vegetables and of ascorbic acid and α-tocopherol. In addition, in the Finnish study, MnSOD genotypes containing the alanine allele were found to be associated with a 1.5-fold (95% CI = 1.1, 2.0) increased risk of breast cancer compared with those homozygous for the valine/valine genotype [166]. The association between this polymorphism and breast cancer risk was weaker in the other US study (odds ratio = 1.27; 95% CI = 0.91, 1.77) [165], and no association was found in a case-control study within the Breast Cancer Family Registry [167]. However, recent results suggest that this polymorphism is also associated with breast cancer risk among Chinese in Shanghai [168].

Conclusion

Accumulating evidence suggests that oxidative stress-induced apoptosis may play an important role in the anti-carcinogenic effect of several chemopreventive agents, including retinoids, nonsteroidal anti-inflammatory drugs, polyphenols, tamoxifen, vanilloids, and rotenoids [169]. In this review, we describe how several breast cancer chemo-preventive factors may exert their anti-cancer effect through lipid peroxidation-induced apoptosis, including marine n-3 fatty acids, soy, ITCs, vitamin D and calcium. We also describe the modifying effect of oxidative stress-related genes such as *GSTM1*, *GSTT1*, *GSTP1*, *CNDN1*, and *COMT* in the relationships between marine n-3 fatty acids, ITCs, and tea and breast cancer. We believe that the lack of consideration of the lipid peroxidation pathway and the

implied modifying effects of related gene polymorphisms may account for some of the lack of consistency in previous epidemiological studies of diet and breast cancer. In addition, because several of the dietary factors discussed in this manuscript have been shown to have multiple cellular effects, not only lipid peroxidation-related effects, we believe that it will be important to study the cross-talk between the lipid peroxidation pathway and other pathways, such as estrogen, insulin resistance, inflammation, and possibly other pathways. The lipid peroxidation hypothesis might be further investigated by measuring the levels of lipid peroxidation markers such as F2 isoprostanes, which comprise the 'gold standard' marker of oxidative stress *in vivo*, in fluid nipple aspirate or breast tissue of women with breast cancer, and compare the values with those from control women.

Our hypothesis has practical implications for breast cancer prevention. Lipid peroxidation could be proven to be a pre-diagnostic marker for breast cancer. Lipid peroxidation levels in breast ductal cells may represent a promising cancer biomarker to detect, through non-invasive methods such as nipple fluid aspirate sampling, for example, women at high risk for breast cancer. In addition, a better understanding of the relationship between breast cancer risk factors and oxidative stress/lipid peroxidation-related biomarkers and genes may prove useful in identifying the dietary or non-dietary exposure-genotype combinations that put women at the lowest risk. In addition, lipid peroxidation markers could also be used as indicators of prognosis. Decreased plasma malondialdehyde (MDA), another lipid peroxidation marker, has been found to be significantly associated with severity of prognosis factors for breast cancer. MDA concentrations were significantly lower in the plasma of patients with large tumors or in whom nodes and/or metastasis were present [170-172].

Competing interests

The authors declare that they have no competing interests.

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