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*Brief Report*

# Towards a dietary prevention of hereditary breast cancer

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**Abstract**

Inheritance of a deleterious mutation in BRCA1 or BRCA2 confers a high lifetime risk of developing breast cancer. Variation in penetrance between individuals suggests that factors other than the gene mutation itself may influence the risk of cancer in susceptible women. Several risk factors have been identified which implicate estrogen-induced growth stimulation as a probable contributor to breast cancer pre-disposition. The protein products of both of these genes appear to help preserve genomic integrity via their participation in the DNA damage response and repair pathways. To date, the evidence for a cancer-protective role of dietary nutrients, for the most part those with antioxidant properties, has been based on women without any known genetic pre-disposition and it is important to identify and evaluate dietary factors which may modify the risk of cancer in BRCA carriers. Here we propose that diet modification may modulate the risk of hereditary breast cancer by decreasing DNA damage (possibly linked to estrogen exposure) or by enhancing DNA repair. The prevention of hereditary breast cancer through diet is an attractive complement to current management strategies and deserves exploration.

## BRCA mutation carriers of today

It has been estimated that between five and ten% of breast cancers are hereditary [1, 2]. Approximately 30–40% of familial cases can be attributed to a germline mutation in one of the two breast cancer susceptibility genes, BRCA1 and BRCA2 [3]. Deleterious BRCA1 or BRCA2 mutations are associated with a very high lifetime risk of breast cancer, currently estimated at 80% by age 70 [3, 4]. Both mutations also confer increased lifetime risks of ovarian cancer and pre-dispose men and women to a range of other malignancies [5–7].

Lifetime risks of breast cancer as low as 38% and as high as 87% have been reported in women carrying a deleterious BRCA1 or BRCA2 mutation [3, 8–12]. The variability in risks between women in different studies has prompted the search for factors other than the gene itself which might influence the risk of cancer in susceptible women. In 1993 we reported in a large American family of BRCA1 mutation carriers that the incidence of breast cancer was five times greater among women born after 1930 than for those before 1930, suggesting an important role for external factors in BRCA-associated carcinogenesis [13]. In the past decade, both genetic and non-genetic factors have been suggested to influence breast cancer risk in BRCA1 and BRCA2 mutation carriers (reviewed in [14–16]). Genetic risk factors include both the type and position of the mutation [17–19], and the presence of specific alleles of modifying genes [20–23]. Non-genetic or environmental factors include hormonal factors, particularly those related to estrogen exposure (reviewed in [14]). Oophorectomy (removal of the ovaries) and breastfeeding are protective [24–27]. A positive relationship between early oral contraceptive use and breast cancer risk has been suggested by one study [28] and confirmatory studies are underway. These

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observations suggest that sex hormones play an important role in BRCA-carcinogenesis.

The cloning of both breast cancer susceptibility genes, along with the introduction of predictive genetic testing, has allowed for the identification of high-risk women. Options currently available for these women include primary prevention and specialized surveillance programs aimed at early detection. Prophylactic bilateral mastectomy appears to be the most effective choice for high-risk women, conferring a decrease of 90% or greater in the incidence of breast cancer [29]; however, the proportion of women who elect to undergo this invasive surgery is low and varies between countries (reviewed in [30]). Currently, non-surgical chemopreventive options available for BRCA carriers are based on interrupting the estrogen-signaling pathway (reviewed in [14, 15]), although the effectiveness of estrogen blockade still remains to be elucidated especially because the majority of BRCA1 tumours are estrogen-receptor negative.

The heterogeneity in penetrance associated with a BRCA mutation suggests that the potential exists to modify risk in carriers. More importantly, the lack of effective chemoprevention suggests a need to pursue novel alternatives, such as dietary or lifestyle strategies. Prospective trials with breast cancer as an endpoint to evaluate chemoprotective agents are generally not feasible in the high risk population. Thus, there is a need to identify biomarkers of cancer susceptibility that can be used as intermediate endpoints. In turn, the identification of molecular or genetic changes which are valid biomarkers of breast cancer risk in carriers will allow for the evaluation of dietary or lifestyle interventions.

## The BRCA gene products, DNA repair capacity, and biomarkers of breast cancer susceptibility

The BRCA1 gene has multiple functions, and is involved in DNA transcription, cell cycle checkpoint control, DNA damage repair, protein ubiquitination, regulation of apoptosis and chromatin remodeling [31–33]. In addition to double-strand DNA break repair by homologous recombination, other repair functions attributed to BRCA1 include transcription-coupled repair and global genomic repair, both of which are sub-pathways of nucleotide-excision repair [32, 34–36]. Both BRCA1 and BRCA2 have been shown to interact with Rad51, a protein believed to participate in homologous recombination [37–40]. Although not as diverse, the molecular functions of BRCA2 are better understood than that of BRCA1. In addition to regulating Rad51 [41], BRCA2 has recently been implicated in stabilizing DNA structures at stalled replication forks [42]. Collectively, the data suggests that BRCA1 and BRC2 participate in a common DNA damage repair pathway associated with homologous recombination-mediated and double-strand DNA repair [43, 44].

Various chromosomal instability disorders have been identified (including xeroderma pigmentosum, ataxia telangiectasia, Bloom syndrome, Fanconi anemia and Nijmegen breakage syndrome) which pre-dispose an individual to cancer due to the inheritance of a defective gene critical for the repair of DNA lesions (reviewed in [45–47]). Individuals with mutations in genes involved in DNA repair demonstrate hypersensitivity to DNA-damaging agents, an increased tendency to accumulate mutations, chromosomal instability, and a high risk of developing cancer [46, 48]. These individuals are identified through cytogenetic tests that quantify chromosome breaks [49] or that assess the ability to repair radiation-induced DNA damage [50, 51].

The functional roles of BRCA1 and BRCA2 proteins suggest that the inheritance of a mutated gene may be associated with faulty DNA repair, chromosomal instability and consequently pre-disposition to breast and ovarian cancer. An impaired cellular response to DNA damage appears to be a plausible mechanism by which BRCA carriers are at an increased risk of breast cancer [52]. Hence, the evaluation of an individual's capacity to repair DNA may serve as a biomarker of breast cancer risk in carriers. Although there are numerous techniques to assess DNA repair capacity, there is presently no gold standard for humans [53, 54]. Various cytogenetic endpoints, including counting chromosomal aberrations, micronuclei and sister chromatic exchanges, have been utilized as biomarkers of cancer susceptibility [55]. The epidemiological evidence is strongest for the association between an elevated frequency of chromosomal aberrations and an increased risk of cancer [56]. However, each of these endpoints has limitations including the need to use proliferating cells and the large volume of blood required. The techniques are often laborious and time-consuming [57]. Other markers of DNA repair capacity include an assessment of DNA damage in the form of DNA adducts or strand breaks [53, 58].

When there is an imbalance between the rate of oxidant production and the antioxidant defense mechanisms, oxidative stress occurs. The deleterious effects of oxidative stress have been implicated in aging and other chronic conditions, including cardiovascular disease, immune-system decline and cancer [59–63]. Of particular importance for carcinogenesis is oxidative damage to DNA. Carcinogenesis is a multi-step process and damage to DNA is believed to be a critical step in this process. Given the role of DNA mutation in

carcinogenesis, it is not surprising that oxidative damage to DNA has been implicated in the etiology of various cancers, including the breast [64, 65]. Quantification of 8-oxo-2′-deoxyguanosine (8-oxodG) in blood or urine is the most commonly utilized biomarker of *in vivo* oxidative DNA damage [36, 58, 66, 67]. Elevated levels of 8-oxodG are believed to reflect deficient DNA repair capacity and an increased risk of cancer [68–70]. However, no studies have evaluated the role of oxidative stress or oxidative DNA damage in mutation carriers.

Other assays quantify DNA damage in the peripheral blood lymphocytes of study subjects compared to controls following exposure to ionizing radiation or a challenge with a mutagen such as bleomycin or hydrogen peroxide (reviewed in [53]). Studies using lymphoblastoid cell lines from female heterozygous BRCA1 or BRCA2 carriers demonstrate greater sensitivity to the chromosome damaging effects of gamma radiation, and of $H_2O_2$, compared to cells from healthy controls (as assessed by the micronuclei test or the radiation-induced chromatid break assay) [71–73]. The use of induced micronucleus frequencies or other indices of mutagen sensitivity as biomarkers of faulty DNA repair mechanisms have the potential for evaluating cancer predisposition in BRCA mutation carriers. Employing a surrogate end-point that will allow for the differentiation between carriers and non-carriers, and which will serve as a predictor of breast cancer risk, will not only aid in the identification of genetically susceptible subgroups but will also allow future studies to evaluate the capacity of intervention strategies to modify risk.

## The maintenance of genomic stability in BRCA mutation carriers by dietary intervention

The epidemiological evidence is consistent for a cancer protective effect of a diet high in fruits and vegetables [61, 74–77]. Although the scientific literature regarding the role of specific dietary constituents including vitamins, minerals, flavonoids, carotenoids and various other phytonutrients in the etiology of cancer has expanded in recent years, definite conclusions have not been reached. Nonetheless, there is a high level of public interest in various micronutrients and it is not surprising that women with BRCA mutations often inquire about what they should or should not eat to help prevent cancer. If dietary modifiers of risk exist, what are they?

One frequent question is why does cancer in BRCA-mutation carriers primarily occur in the breast? It is possible that estrogen signaling is an important cofactor (reviewed in [78, 79]). This hypothesis forms the basis for the chemopreventive options presently available [80, 81]. Candidate genetic and non-genetic modifiers of risk include genes involved in DNA repair and estrogen metabolism (reviewed in [14]). These findings implicate estrogen-induced DNA damage and faulty DNA repair mechanisms as probable contributors to hereditary breast cancer. Thus, the prevention of DNA damage, possibly linked to estrogen exposure, and enhanced DNA repair represent promising therapeutic targets for the prevention of hereditary breast cancer. Although a wide variety of dietary constituents have been suggested to play a role in breast cancer etiology to date, these studies have involved women without a genetic predisposition and the evidence is lacking to support a role of nutrition in the etiology of hereditary breast cancer.

## Candidate dietary modifiers of breast cancer risk

When identifying possible dietary compounds that may help in the prevention of hereditary breast cancer, it is important to consider that the mechanism underlying hereditary pre-disposition is likely to be different from that in the general population. DNA repair is clearly an intrinsic problem in BRCA mutation carriers, but has not been implicated in the etiology of breast cancer etiology in the general population. Because the risk factors are not identical, the dietary prevention agents may also be different. It is important to note that cells of BRCA mutation carriers have one functional and one mutant (null) allele. Thus candidate nutrients that may help prevent BRCA-related cancers include those that may limit DNA damage, alter estrogen metabolism, or upregulate expression of the normal BRCA allele, and ultimately enhance DNA repair. Below is an overview of several candidate nutrients that we believe merit investigation as potential dietary modifiers of breast cancer risk.

### *Selenium*

Selenium is an essential trace element and is an important cofactor of various antioxidant enzymes including glutathione peroxidase, selenoprotein-P, gastrointestinal glutathione peroxidase, phospholipid hydroperoxidase glutathione peroxidase and thioredoxin reductase [82, 83]. Other functions of selenium include a role in testosterone and thyroid metabolism [84, 85]. Common sources of dietary selenium include meats, seafood, poultry, whole grains and dairy products. Nuts, in particular Brazil nuts, are believed to be the best sources of selenium because they contain large, concentrated quantities of selenium [86, 87].

Two-thirds of more than 100 experimental studies of tumorigenesis have demonstrated that selenium is effective at reducing tumor incidence [88, 89]. Anticarcinogenic activities of selenium include antioxidant effects [90, 91], suppression of DNA synthesis and cell proliferation [92], enhanced immune response [93], altered carcinogen metabolism [94] and induction of apoptosis [89, 95–97]. Although there is extensive literature from animal studies suggesting cancer preventive properties of selenium compounds, the exact mechanism of cancer inhibition has not been determined. Recent studies suggest that selenium acts early in the cancer process to prevent the clonal expansion of premalignant lesions by inhibiting cell proliferation and promoting apoptosis [96–101].

The role of selenium in cancer prevention was suggested by Shamberger and Frost [102, 103] who noted an inverse relationship between selenium levels of forage crops and mortality due to various cancers, including that of the breast. Since this discovery, several other epidemiological investigations have evaluated the anticarcinogenic role of selenium, generally reporting an inverse association between nutritional selenium status and cancer risk (reviewed in [88, 89, 104]). Of particular importance was a finding from a double-blind, randomized, placebo-control trial investigating the effect of selenium on the risk of skin cancer [105]. Clark *et al.* [105] found that supplementation with selenized yeast reduced mortality from lung, colon and prostate cancer by almost 50%, although it had no effect on the incidence of skin cancer. The findings from this study intensified interest in the cancer protective role of selenium, and has since prompted investigations, such as the SELECT trial to assess the effect of supplementation with selenium and vitamin E on the development of various cancers, particularly of the prostate [106].

There is limited data in the literature regarding the role of dietary selenium intake and the risk of breast cancer [107–109]. Since the selenium content of food is directly affected by the selenium content of the soil in which it grows, the geographical variability in soil selenium makes it difficult to assess the selenium content of individual foods. Therefore, selenium status based on dietary intake is not regarded as accurate indicator of selenium status. Instead, serum or plasma concentrations (which are indicative of short-term changes in dietary intake), and whole blood or red blood cell selenium (which represent long-term selenium intake) have been used to assess individual selenium status [110], though measurements of toenail selenium reflects long-term dietary selenium intake and is regarded as a more suitable biomarker [111]. The majority of epidemiological studies have reported no association between serum selenium levels and the risk of breast cancer [107, 112–117], although a few earlier studies did observe lower serum selenium levels in cases compared with controls [118–120]. Studies using toenail selenium concentrations have indicated no significant association with the risk of breast cancer [107, 121–127].

Despite the uncertainties from epidemiological studies of non-hereditary breast cancer, recent studies implicating an alternate mechanism of cancer prevention by selenium suggests that there may be a role for selenium in preventing hereditary cancer syndromes. Seo *et al.* [128] recently demonstrated that selenomethionine (SeMet), the major dietary source of selenium, can activate a DNA-repair subpathway of p53 that is dependent on Ref-1 [100]. SeMet protected p53 wild-type cells from UV-induced DNA damage by activating p53-dependent DNA repair pathways but p53 null cell were unprotected. This group has also shown that SeMet enhances DNA repair in normal human fibroblasts *in vitro* and protects against DNA damage in response to various DNA damaging agents. Traditionally, the p53 gene has been regarded as the ''guardian of the genome'' that functions to maintain genomic stability by regulating growth arrest and apoptosis; however, the p53 gene product may also maintain genomic stability via involvement in the activation of DNA-repair [129–131].

This observed enhancement of DNA repair by SeMet is potentially important in hereditary cancer prevention, especially for BRCA mutation carriers because their susceptibility to DNA breakage is linked to DNA-repair. One study has examined the role of chromosome breakage and selenium supplementation in BRCA1 carriers [132]. Lubinski *et al.* reported that heterozygous BRCA1 carriers demonstrated an elevated frequency of bleomycin-induced chromosome breaks in cultured blood lymphocytes, in comparison to non-carrier relatives. Furthermore, supplementation with oral selenium for up to three months reduced the mean number of chromosome breaks in BRCA1 carriers to levels similar to those observed in controls [132]. Whether selenium supplementation confers a preventive role by decreasing DNA damage or a corrective role through the functional rescue of DNA repair, the potential for selenium to decrease breast cancer incidence in BRCA carriers deserves exploration.

Selenium exists in both organic and inorganic forms. Selenomethionine is an organic form and the major component of dietary selenium. It has been used in large-scale cancer prevention trials and is the predominant form used in supplements. Other multivitamin preparations exist which use inorganic forms such as sodium selenite or sodium selenate [133]; however in

most studies the organic forms are preferred as they are readily absorbed and have a better safety profile [134–137].

*Indole-3-carbonyl and diindolylmethane*

Cruciferous vegetables such as broccoli, cabbage, cauliflower, and brussel sprouts, are rich dietary sources of indolyl gluccosinolates, which upon hydrolysis, yield indoles such as diindolylmethane (DIM) and its precursor indole-3-carbinol (I3C). The use of these two phytonutrients is increasing due to reports of their ability to promote beneficial and protective estrogen metabolism and possibly to reduce the risk of breast cancer [138]. Metabolism of estradiol (E2), the principal estrogen produced and secreted by the ovaries of premenopausal women, occurs via two hydroxylation pathways yielding products with contrasting estrogenic properties. The first pathway yields 2-hydroxyestrone (OHE), the less potent estrogen. This E2 metabolite has been associated with antiproliferative and apoptotic activities [139, 140]. The alternate route favors the production of 16α-OHE, the more potent estrogen metabolite, due to its ability to induce unscheduled DNA synthesis and promote anchorage independent growth [141–143].

Several prospective cohort and case–control studies have reported an increased risk of either pre- or postmenopausal breast cancer in women with a low ratio of 2-OHE to 16α-OHE [144–149]. A recent prospective study of 10,876 Italian women showed that among premenopausal women, a 2-OHE to 16α-OHE ratio in the highest *versus* the lowest quintile was associated with a reduced risk of breast cancer, but the association was not significant (OR = 0.58; 95% CI = 0.25–1.34) [147]. Data from one case–control study of post-menopausal women reported a significantly lower 2-OHE to 16α-OHE ratio in the cases compared with the matched controls and a strong inverse association between the ratio and risk of breast cancer (OR = 9.73; 95% CI = 1.27–74.84 and OR = 32.74; 95% CI = 3.36–319.09, for the intermediate and lowest tertiles relative to the highest, respectively) [146].

Results from additional studies have shown increased levels of 16α-hydroxylation in breast cancer cases *versus* the controls and in women with a family history of breast cancer [150, 151]; whereas another group reported stable levels of 16α-OHE production but a significant decrease in 2-OHE production [152]. Collectively, the evidence suggests an inverse relationship between a low 2-OHE/16α-OHE ratio and the risk of breast cancer, indicating the potential significance of this ratio as a predictive biomarker of breast cancer risk. These results along with the carcinogenic properties associated with elevated 16α-OHE levels has prompted the search for dietary compounds which may stimulate the enzymes responsible for altering the ratio of estrogen metabolites. To date, studies attempting to decrease 16α-hydroxylation by dietary modulation have been unsuccessful [153]. Alternatively, increasing 2-hydroxylation has been more successful especially since cigarette smoke and certain dietary components have been shown to induce the cytochrome p450 enzymes responsible for catalyzing 2-hydroxylation of E2.

The prevention of estrogen-dependent carcinogenesis by the cruciferous indoles DIM and I3C has been linked to their ability to induce CYP1A1 and CYP1A2 activity, subsequently enhancing the production of 2-OHE and limiting the production of 16α-OHE, and possibly 4-OHE, another suspected carcinogen [154–156]. This adjusted ratio of 2-OHE to 16α-OHE appears to be the primary mechanism by which the indoles exert a protective effect against breast cancer [143]. Yuan *et al.* [157] have shown that I3C can inhibit expression of CYP1B1, the enzyme responsible for 4-hydroxylation of estradiol, thus inhibiting synthesis of the carcinogen 4-OHE.

The anticarcinogenic mechanisms of I3C and DIM may be mediated not only by altering estrogen metabolism, but also at the level of transcription regulation. Meng *et al.* [158] have demonstrated anti-invasion and anti-migration properties of I3C in both ER-positive and ER-negative breast cancer cell lines along with increased expression of E-cadherin/catenin complexes and BRCA1. Furthermore, I3C has also been shown to repress ER- signaling in estrogen-responsive cells in a dose-dependent manner [159]. The latter findings along with a previous study implicating BRCA1 as a potent inhibitor of ER-transcriptional signaling, suggest a role for I3C in the modulation of BRCA1 expression [160]. Additional studies have shown that both I3C and its dimer DIM upregulated BRCA1 expression in human breast cancer cell lines [159, 161–163]. Whether phytochemicals from cruciferous vegetables are able to upregulate BRCA1 expression in women with a germline mutation is important because one functional allele is present in a cell. I3C and DIM have been shown to regulate the transcription of genes involved in G1 cell cycle arrest and apoptosis, and antiproliferative mechanisms which may suppress cell growth and prevent oxidant-induced DNA strand breaks [164–167].

More recently, methyl-substituted DIM compounds were shown to bind Ah receptors and inhibit estrogen-induced growth of human breast cancer cells and carcinogen-induced rat tumors, suggesting a possible role of methyl-substituted DIM compounds as selective

AhR modulators (SAhRMs) and a new prospect for cancer treatment [168]. Of particular interest is a study by Bjeldanes *et al*. [155] who found not only antiproliferative effects of DIM in human prostate cancer cells but more importantly strong anti-androgenic properties of the compound similar to those exerted by Casodex, a synthetic anti-androgen [169]. These results implicate the use of DIM as a pure androgen antagonist in the prevention and treatment of hormone-responsive prostate cancer. This group has also shown growth-inhibitory effects of I3C in both estrogen-dependent and estrogen-independent MCF-1 human breast cancer cell lines [165]. Furthermore, combined treatment of I3C and tamoxifen was more effective at suppressing the growth of estrogen-responsive human breast cancer cells, and inhibiting cyclin-dependent 2 kinase-specific activity and subsequent Rb phosphorylation than administration of either compound alone, suggesting a possible combinatorial therapy for estrogen-dependent tumors [170].

Although both I3C and DIM are associated with anti-carcinogenic potential, the latter appears to be a more potent and specific inducer of estradiol 2-hydroxylation [154, 171, 172]. Supplementation with DIM is also favored since I3C is highly reactive and poorly absorbed as it does not appear to leave the stomach or enter the blood stream after oral administration to humans [173]. I3C is a pro-drug that requires bioactivation by gastric acid for conversion to its active product DIM [174].

### *Lycopene*

Lycopene, a red pigment naturally synthesized by plants and microorganisms, has gained increasing importance in the prevention of chronic diseases. It is the most potent singlet oxygen quencher among all the carotenoids [175, 176]. In an extensive review of 72 epidemiological studies, an inverse association between tomato or lycopene intake or blood lycopene levels and the risk of cancer at various anatomic sites was observed in 57 studies, 35 of which were statistically significant [177]. With regards to the risk of breast cancer, those studies evaluating the role of dietary lycopene intake showed no association; however, three of the four studies using serum or breast adipose tissue levels of lycopene as biomarkers of lycopene status did support an inverse association between lycopene and the risk of breast cancer (reviewed in [177]). This inconsistency in results, along with two studies indicating that serum and tissue levels do not necessarily correlate with dietary carotenoid intake, suggest that the method of assessing lycopene status and the limitations associated with the use of dietary questionnaires may have influenced the results [178, 179]. Both *in vitro* and animal models have provided evidence for a protective role of lycopene in breast cancer [180–182].

Lycopene is the predominant carotenoid found in human plasma and has been shown to accumulate specifically in certain tissues, such as the testes, liver, adrenal and prostate glands, and may be responsible for the stronger anti-carcinogenic effects observed in certain sites [183–186]. Although the strongest protective effects observed by Giovannucci were for the prostate, lung and stomach, based on the tissue specific distribution of lycopene, mean lycopene levels in human prostate gland and breast are comparable [177]. Common sources of dietary lycopene include red fruits and vegetables such as apricots, watermelons, and pink grapefruits, although tomatoes and tomato-based products account for approximately 85% of dietary lycopene intake in North America [187, 188]. Studies have shown that processed tomato products such as ketchup, spaghetti sauce and salsa appear to be better sources of lycopene than fresh tomatoes possibly due to the processing of fresh tomatoes which releases the carotenoid in the more bioavailable *cis*-conformation [187, 189, 190]. Daily consumption of one to two servings of tomato products has been shown to significantly reduce oxidative damage to lipids, low density lipoproteins, proteins and DNA [191–193]. In addition, a human intervention trial with 330 ml of tomato juice daily supplemented with 40 mg of lycopene reduced the frequency of DNA strand breaks in healthy individuals [194]. The data suggest that diets high in lycopene may decrease the risk of chronic disease by reducing oxidative DNA damage.

Apart from an antioxidant role, alternate mechanisms by which lycopene may exert a protective role in the prevention of disease have also been proposed. Lycopene has been suggested to improve gap-junction communication, modulate hormonal and immune response, function as a hypocholesterolemic agent by inhibiting HMGCoA reductase, and regulate gene function and metabolism (reviewed in [187]). Lycopene has also been shown to inhibit cell cycle progression from the G1-to S-phase of breast and endometrial cells via the down-regulation of cyclin D [195]. This antiproliferative nature of lycopene is similar to that exerted by anti-estrogens and tamoxifen, both of which reduce cyclin D1 levels and block entry of cells into the S-phase [196, 197].

### *Green tea*

Green tea contains various polyphenols that possess strong antioxidant qualities. Epigallocatechin-3-gallate (EGCG), epicatechin, and epigallocatechin are referred

to as the green tea polyphenols which possess the strongest antioxidant properties [198]. EGCG accounts for approximately 40% of the polyphenols in green tea and is believed to have strong anti-carinogenic effects [198, 199]. The possible role of green tea in cancer prevention has been suggested by the lower incidence of breast cancer in areas such as Japan and China where tea consumption is high [200]; however, no consensus has been reached for Western countries.

Several studies using animals have demonstrated anti-carcinogenic effects of polyphenols [201–203]. While there is significant evidence of a chemopreventive effect observed from *in vitro* and *in vivo* models, epidemiological evidence is limited. Epidemiological studies conducted in Western populations have mainly focused on the association of breast cancer risk with black tea consumption [204–208]. Two studies using a cohort of Japanese women who survived the atomic bomb reported no association between green tea intake and risk of breast cancer [209, 210]; whereas two other Japanese case–control studies reported a lower risk of breast cancer recurrence with green tea consumption in women with stage I or II breast cancer [211, 212]. A recent case–control study of Asian-American women demonstrated a significant reduction in breast cancer risk associated with green tea consumption, especially in women with low soy intake (OR = 0.45; 95% CI = 0.26–0.78) [213]. Potential mechanisms by which green tea may protect against cancer development includes the scavenging of ROS, inhibition of carcinogen formation, enhanced carcinogen detoxification, enhanced DNA repair and modulation of estrogen metabolism [198, 214]. Green tea has also been linked with cell cycle arrest and apoptosis [201, 215]. Even at low concentrations, green tea catechins are effective at reducing oxidative-induced DNA single-strand breaks and DNA base damage [216]. EGCG has also been shown to inhibit the activation of the epidermal growth factor (EGF) and Her-2/neu receptors, possibly via preventing their phosphorylation, thereby reducing downstream activation of the downstream NF-$\kappa$B signaling pathway [217–219]. Overexpression of both EGF and Her-2/neu receptors is associated with enhanced tumor proliferation, resistance to chemotherapeutic agents and shorter survival time [220–223].

## Future role of dietary modification in BRCA mutation carriers

The well-established role of nutrition in the etiology of cancer, along with the emerging knowledge of the molecular basis of disease, has directed scientists into an era presently referred to as nutrigenomics [224–226]. This innovative stream of science has encouraged the exploration of gene-nutrient interactions, more specifically, the effect of dietary constituents on gene expression. The ability to identify and understand how certain nutrients may correct the molecular processes involved in the etiology of cancer has opened a new avenue to exploit in the prevention and treatment of this complex disease. However, routine recommendations cannot be made to BRCA mutation carriers until the protective effect of these nutrients is assessed.

However, in evaluating the protective role of modifying factors, the use of incident breast cancer is not a practical approach, as cancer may take several years to develop and requires large, lengthy and expensive epidemiological studies [227]. Molecular epidemiological studies that employ surrogate end-points of disease are more feasible. To carry out such studies, there is a need to ascertain a valid biomarker of cancer susceptibility that will allow one to distinguish between carriers and non-carriers and will predict cancer risk. The identification of molecular or genetic changes as potential predictors of breast cancer risk in carriers will allow for the effective evaluation of dietary or lifestyle interventions and their ability to alter the biomarker and subsequently, the risk of breast cancer.

An important question is whether repair capacity in biological samples such as blood or urine accurately reflects DNA repair capacity in the tissue from which the cancer arises. Despite this limitation, the goal of future studies should involve the elucidation of a valid and reliable biomarker of breast cancer risk in BRCA1/2 mutation carriers. Because of the proposed roles of BRCA1 and BRCA2 in DNA repair, the prospect that interventions including lifestyle modification and dietary changes may modulate the risk of hereditary breast cancer by decreasing oxidative damage to DNA or enhancing DNA damage repair pathways, is an attractive alternative.

Currently, the recommended dietary allowance (RDA) for selenium has been set at 55 $\mu$g per day for both men and women and is based on the amount of selenium needed to maximize glutathione peroxidase (GPX) synthesis [228]. Based on the current evidence, the cancer protective effects of selenium may not only be associated with its role as an essential constituent of the antioxidant enzyme GPX, but instead as a source of metabolites [229]. Thus, recommendations as high as 300 $\mu$g per day have been suggested to maximize levels of selenium metabolites for cancer protective effects [230]. Although an upper limit of 400 $\mu$g per day has been set by the National Academy of Sciences, studies have shown that adverse effects identified as selenosis (symptoms include

gastrointestinal upsets, hair and nail loss, white blotchy nails, and mild nerve damage) do not occur until intakes approach more than 900 $\mu$g per day [231]. Based on the dosages used in various cancer prevention randomized-control trials, a daily dose of 200 $\mu$g of 1-selenomethionine (which is four times the recommended dietary intake) is believed to keep serum selenium levels at the highest acceptable physiological level without causing toxicity [105, 106, 232].

In order to achieve the recommended daily dose of 30–40 mg of DIM per day, one would need to consume two pounds of broccoli a day. However, supplementation with 100–200 mg of DIM in an absorbable formulation per day has been demonstrated to result in changes in the ratios of estrogen metabolites [233]. No side effects were reported when the normal daily dose of 150 mg was tripled to 450 mg/day in human subjects [234]. Although no recommended daily allowance of lycopene has been established, the suggested value is 5–10 mg per day for adults as part of a healthy regimen [193, 235]. Based on previous studies, we recommend a dose of 30–40 mg of lycopene for BRCA mutation carriers (which is equivalent to drinking two glasses of tomato juice per day) since this dose has been shown to help in the prevention of oxidative DNA damage [193, 194, 236]. In a recent review, Fujiki *et al.* [237] recommended a daily consumption of about ten Japanese-cups of green tea, preferably in the decaffeinated form to prevent side-effects. This dose was based on the cancer-protective effects observed in a prospective study of 8552 individuals aged over 40 in Japan [238, 239] and is equivalent to 0.5–1.3 g of green tea extract containing 340–540 mg of EGCG. A combination of green tea and supplements was also suggested.

In summary, we believe female BRCA mutation carriers should consider a daily supplement regimen containing 300 $\mu$g of 1-selenomethionine, 30–40 mg of lycopene, 100–200 mg of absorbable, formulated DIM (containing 25–50 mg of actual DIM) and 340–540 mg of EGCG. Along with increased fruit and vegetable intake, additional recommendations to carriers should include important lifestyle habits such as regular physical activity and maintenance of a healthy body mass index, and diets which restrict alcohol, salt and fat intake [240]. Under the assumption that impaired DNA repair capacity is able to predict future health risk, future studies can be designed to evaluate the ability of dietary interventions or lifestyle changes to increase the fidelity of DNA-double strand break repair as reflected in biomarker analyses and subsequently to modify the risk of hereditary breast cancer. The tailoring of unique diets to prevent hereditary breast cancer may therefore be possible by protecting genomic stability in the presence of an inherited BRCA mutation. This is indeed an attractive alternative to the currently-available strategies however the benefits of these recommendations are not yet known and warrant further studies.

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