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Lessons Learned from Cancer Prevention Studies with Nutrients and Non-Nutritive Dietary Constituents

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Abstract

Epidemiological studies have observed the association between dietary patterns and the risk of certain types of cancer. Extensive studies have been conducted on the cancer preventive activities of constituents from food and beverages. While laboratory research has shown impressive and promising results, such promising cancer preventive activities have not been demonstrated in many human intervention trials. This article analyzes the major differences between these different types of studies and the limitations of these studies. Animal and cell line studies usually use optimal conditions in order to demonstrate the hypothesized effects, sometimes without considering the human relevance. On the other hand, some clinical trials were designed without a good understanding of the biochemical and pharmacological properties of the agents used. Lessons learned from these studies will be illustrated using vitamin E, β -carotene and selenium as examples for nutrients, and green tea polyphenols as an example for non-nutritive dietary constituents. From the lessons learned, we believe that more interdisciplinary collaboration and integration of laboratory and human studies would effectively advance the field of cancer prevention.

Keywords

Nutrients; tocopherols; tea polyphenols; cancer prevention; Selenium

1. Introduction

The importance of diet and nutrition in human cancer became evident in the early 1980s, especially after the publication of the classical paper “The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today” by Doll and Peto [1]. By then, much information had been accumulated from epidemiological studies, showing correlations between dietary patterns and risk for different types of cancer [1–3]. For example, a dietary pattern rich in fat and meat, but low in cereals, fruit, vegetables and fiber is associated with increased risk for colorectal and mammary cancer. The high incidence of gastrointestinal cancers in many of the developing countries was correlated with deficiency

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or insufficiency of certain nutrients. As diet and cancer became increasingly linked, the concept that there are dietary carcinogens and anticarcinogens was extensively discussed [4, 5]. Dr. Lee Wattenberg proposed the chemoprophylaxis of carcinogenesis by dietary chemicals and the possible mechanisms involved [6, 7]. Dr. Michael Sporn later coined the term “chemoprevention” to advocate cancer prevention by using chemicals, including those of dietary origin [8]. Since then, numerous laboratory studies have been conducted on the cancer prevention activities of constituents from food and beverages [7, 9–12]. The rationale for these studies, in addition to the epidemiological data mentioned above, is the concept that these materials are readily available and that natural products are nontoxic. In spite of the interesting results from many laboratory studies, very few phytochemicals have been shown to be effective cancer preventive agents in human trials. In this article, studies on nutrients such as vitamin E, β -carotene and selenium, and non-nutritive dietary compounds, such as tea polyphenols, are used to illustrate some of the progresses and challenges in this area of research. Most of these compounds are known antioxidants. However, their cancer preventive functions involve many other biological activities and depend on the doses and specific forms of these groups of compounds. The reasons for the discrepancies among the results from laboratory studies, epidemiological observations and human trials are discussed. Ways for improvement in future cancer prevention research are proposed.

2. Early Cancer Prevention Studies on Vitamins E, β -Carotene and Selenium

In early epidemiology studies, an association between micronutrient insufficiencies and risk for many types of cancer has been determined. Esophageal cancer, for example, was found to be prevalent in populations with a monotonous diet, such as those in certain areas of northern China, central Asia, and northern Iran. In these populations, people survived on staples such as corn, wheat or millet and a low intake of vegetables, fruits, and animal products, leading to insufficiencies of vitamins and minerals [13]. Similarly, laboratory studies have demonstrated that a diet with insufficiencies in micronutrients enhances *N*-nitrosomethylbenzylamine-induced esophageal carcinogenesis in rats, which may be attenuated by supplementation with zinc, molybdenum, vitamin A and riboflavin (reviewed in [9]).

The hypothesis that supplementation with micronutrients can prevent esophageal cancer was tested in the 1980s in a large-scale intervention study, the China-U.S. Cooperative Linxian Nutritional Intervention Trial (LNIT). The poor rural population in Linxian (now named Linzhou City) had low intake of micronutrients, and insufficiencies in some nutrients were indicated in blood nutrient analyses [14, 15]. Because many micronutrients had been suggested to be associated with esophageal cancer, there were debates among collaborators at first on the experimental design. If individual vitamins or minerals were used in each group, it would be theoretically possible to determine which individual micronutrient could or could not prevent esophageal cancer, but it would be almost impossible to conduct a trial with so many groups and still have enough subjects to provide the statistical power needed. Alternatively, if all the micronutrients of interest were investigated together in a single experimental group, it would have enough statistical power for the trial, but it would not

reveal which nutrients were effective and which were not. After long discussion, a compromised design was adopted, in which nutrients were divided into 4 groups: (A) retinol, zinc; (B) riboflavin, niacin; (C) ascorbate, molybdenum; and (D) α -tocopherol, β -carotene, selenium, and the nutrients were combined in a factorial design with eight groups: placebo, AB, AC, BC, AD, BD, CD, and ABCD. In the preparation of the trial, it was found that the disease considered to be esophageal cancer in Linxian actually consisted of 60% esophageal squamous cell carcinoma (ESCC) and 40% gastric cardia adenoma (GCA). The latter was therefore classified as gastric cancer in this trial. The study involved 29,584 adults (aged 40 to 69) who were given supplementations as daily pills for 63 months (1985 to 1991). There were 2,127 deaths during the trial period; 32% were due to esophageal and gastric cancer. The study showed that supplementation with a combination of α -tocopherol (50 mg), β -carotene (15 mg) and selenium (50 μ g) to the general population (>40 years old) for 63 months decreased mortality due to gastric cancer (mainly GCA) by 20% and total cancer mortality by 13% [16]. The result is consistent with the findings of the nested case-control studies, which showed that the blood levels of α -tocopherol and selenium were inversely associated with gastroesophageal cancer risk [17, 18]. Other nutrient combinations, however, did not show any effect on the endpoints measured [16]. Furthermore, results from a 10-year follow-up showed that the protective effect of the combination of α -tocopherol/ β -carotene/selenium on GCA was sustained, and that this nutrient combination also protected against ESCC in subjects younger than 55 years old (but not in those older than 55 years) when they were enrolled in the trial [19]. It is possible that the intervention was ineffective in older subjects because they already had precancerous lesions; however it was effective in younger subjects because the younger subjects had lower grade or no precancerous lesions. This is consistent with the result of a parallel trial in Linxian on subjects with esophageal dysplasia, showing a lack of beneficial effect by supplementation with multiple micronutrients [20]. These results are supported by studies in a rat model demonstrating that insufficiencies in vitamin E and selenium enhanced *N*-methylbenzyl nitrosamine-induced esophageal carcinogenesis, and that supplementation of these nutrients at the early stage of the experiment had a stronger protective effect against carcinogenesis than supplementation at a later stage [21]. The concept that chemoprevention is more effective in patients with less severe precancerous lesions was also demonstrated in another study in Linxian. In a randomized, placebo-controlled trial with selenomethionine and celecoxib for 10 months, selenomethionine improved squamous histology in 115 patients with mild esophageal dysplasia, but not in 125 patients with severe dysplasia [22]. Celecoxib had no effect on the squamous histology.

In the 1980s, β -carotene was lauded as a very promising chemopreventive antioxidant [23], a viewpoint based mainly on epidemiological studies showing that decreased cancer risk was associated with vegetable and fruit consumption. The hypothesis that β -carotene can prevent lung cancer was tested in Finnish smokers in the α -Tocopherol and β -Carotene (ATBC) study started in 1985 [24]. This was a randomized, double-blinded, placebo-controlled primary-prevention trial with a total of 29,133 male smokers, 50 to 69 years of age, from southwestern Finland using α -tocopheryl acetate (50 mg per day) and β -carotene (20 mg per day) in a 2 $\times$ 2 factorial design for an average of 4.5 years, with follow-up continued for 5 to 8 years. Among the 876 new cases of lung cancer diagnosed during the trial, no reduction in

incidence was observed among the men who received α -tocopherol. Unexpectedly, increased lung cancer incidence (by 18%) was observed in the β -carotene group. Some consider this study a failure; however, many interesting observations were subsequently gleaned from this cohort, resulting in more than 180 publications. For example, supplementation with α -tocopheryl acetate was found to reduce the incidence of prostate cancer by 45% in a secondary endpoint analysis of the ABTC study [25]. In the β -Carotene and Retinol Efficacy Trial (CARET), a primary-prevention trial involving a total of 18,314 smokers, former smokers, and workers exposed to asbestos in the US, relative risk of lung cancer was increased (~28%) in the group treated daily with a combination of 30 mg of β -carotene and 25,000 IU of retinol (vitamin A) [26]. CARET also indicated the combination of β -carotene and vitamin A may increase the risk of death from lung cancer, cardiovascular disease, and other causes in smokers and workers exposed to asbestos.

Selenium is another “antioxidant nutrient” that has been studied extensively as a chemopreventive agent [27–30]. In order to determine whether a nutritional supplement of selenium would decrease the incidence of skin cancer, a multicenter, double-blind, randomized, placebo-controlled cancer prevention trial known as the Nutritional Prevention of Cancer Study (NPC) was conducted in the eastern United States [31]. A total of 1312 patients with a history of basal cell or squamous cell carcinomas of the skin were randomized from 1983 through 1991. Patients took 200 μ g of selenium as selenium-enriched yeast or non-enriched yeast as placebo orally per day for a mean of 4.5 years, and had a total follow-up of 6.4 years. After a total follow-up of 8271 person-years, it was found that selenium treatment did not significantly affect the incidence of basal cell or squamous cell skin cancer. However, analysis of secondary endpoints revealed that, compared to controls, patients treated with selenium had significant reductions in total cancer mortality, total cancer incidence and incidences of lung, colorectal and prostate cancers. The blinded phase of the trial was stopped early, primarily because of the apparent reductions in total cancer mortality and total cancer incidence in the selenium group. In contrast, the Vitamins And Lifestyle (VITAL) prospective cohort study, which contained detailed questionnaires about vitamin E and selenium supplement intake from 35,242 men recruited between 2000 and 2002 in western Washington State, showed that a 10-year average intake of selenium at >50 μ g/day was not associated with a reduced prostate cancer risk overall. Nevertheless, risk of advanced prostate cancer (regionally invasive or distant metastatic) was significantly decreased with greater intake of supplemental vitamin E [32].

3. Recent Studies on Cancer Prevention by Tocopherols

Tocopherols, the major forms of vitamin E, contain a chromanol ring system and a phytyl chain of 16 carbons (Figure 1). Depending on the number and position of methyl groups on the ring, they exist as α -, β - γ - or δ -tocopherol (α -, β -, γ - and δ -T) [33]. The possible cancer prevention activities by tocopherols have been studied extensively (reviewed in [34–36]). Of particular interest in prostate cancer prevention is the result of a nested case-control study (CLUE II), showing that the prostate cancer risk was inversely associated with serum levels of γ -T [37]. A recent report by Weinstein *et al.* on the Prostate, Lung, Colorectal and Ovarian Cancer (PLCO) Screening Trial and some previous reports indicated that higher

serum levels of α -T were associated with decreased risk of prostate cancer, particularly among smokers ([38] and references cited).

3.1 Disappointing results from recent intervention studies with α -tocopherol

Because α -T is the most abundant form of tocopherols in blood and tissues and has the highest activity in the classical fertility-restoration assay, α -T is generally considered to be “the vitamin E” [33]. Therefore, many studies on vitamin E have been conducted with α -tocopheryl acetate. The results from several large-scale intervention studies with α -T, however, have been disappointing [39–42]. For example, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) was designed with great optimism based on the encouraging results from the ATBC and the NPC Nutritional trial. However, daily supplementation with 400 mg of α -tocopheryl acetate or 200 μ g selenium (from L-selenomethionine) in a 2 $\times$ 2 design, for an average of 5.5 years, did not prevent prostate or other cancers [41]. In the subsequently published results on the 7–12 year follow-up of this study, subjects receiving α -tocopheryl acetate had a hazard ratio of 1.17 for developing prostate cancer [42]. It was noted that, in the SELECT, the α -T supplementation caused a 50% decrease in median plasma γ -T levels [41]. A possible interpretation for the lack of cancer prevention effect of α -T is that the supplementation of a nutrient to a population that is already adequate in this nutrient may not produce any beneficial effect. The mean baseline median plasma level of α -T in subjects of the SELECT was at an adequate level of 12.5 μ g/ml [41]. Since γ -T has been suggested to have strong anti-inflammatory and cancer preventive activities [34, 43–45], the decrease in blood and tissue levels of γ -T, caused by high doses of α -T, may have partially contributed to the increased prostate cancer risk in the SELECT [41, 42]. Another possibility is that some of these subjects already had preneoplastic lesions when entering the trial, and the supplementation with high doses of α -T promoted prostate cancer development. The exact reasons for these negative results from the SELECT and other trials are still not known. Nevertheless, the disappointing outcome of these large-scale trials reflects our insufficient understanding of the biological activities of tocopherols and points to the need for systematic studies of the disease preventive activities of the different forms of tocopherols.

3.2 New insights from recent laboratory studies of specific forms of tocopherols

Previous cancer prevention studies in different animal models, mainly with α -T, have obtained inconsistent results [46]. However, recent studies from our research team at Rutgers University have demonstrated the inhibition of cancer formation and growth in the lung, colon, mammary gland and prostate by γ -T, δ -T and a tocopherol mixture that is rich in γ -T (named γ -TmT) [35, 47]. γ -TmT, a product derived from vegetable oil and usually contains (per g) 130 mg α -T, 15 mg β -T, 568 mg γ -T, and 243 mg δ -T, was shown to significantly inhibit the formation of colon adenoma in a mouse carcinogenesis model induced by azoxymethane and dextran sodium sulfate. We also demonstrated that δ -T was more active than γ -T in inhibiting cancer cell growth in culture, human lung cancer H1299 cell tumorigenesis in a xenograft model, colon carcinogenesis induced by azoxymethane in rats, and PhIP-induced prostate carcinogenesis in CYP1A-humanized mice; whereas α -T was ineffective in these models [48–50]. Takahashi *et al.* also demonstrated that γ -T (0.005% or 0.01% in the diet), but not α -T, decreased the number of adenocarcinomas in the ventral lobe

in the “Transgenic rat for adenocarcinoma of prostate” model [51]. In these studies, the commonly observed inhibitory actions of δ -T and γ -T were the quenching of reactive oxygen and nitrogen species, the lowering of prostaglandin E2 and leukotriene B4 levels, and the enhancing of cancer cell apoptosis [35, 52]. Recent studies in prostate cancer cell lines also showed that δ -T was more effective than γ -T and α -T in inhibiting cell proliferation and the P13K/AKT [53]. The inhibition of PHLPP by γ -T to dephosphorylate AKT (S473) in prostate cancer cells [54] and activation of PPAR γ by tocopherols in breast cancer cells [51] have also been proposed. Some of these activities are shown in Figure 2.

Based on the above discussed epidemiological and laboratory studies, we propose that at the nutritional levels, all forms of vitamin E are cancer preventive. At the supra-nutritional levels, γ -T, δ -T and γ -TmT prevent cancer, whereas α -T is not effective. The preventive activities of δ -T, γ -T and γ -TmT warrant further investigation in preclinical and clinical studies.

4. Lessons Learned from Studies on Nutrients

As discussed above, epidemiological studies have shown an association between insufficiencies in certain micronutrients and increased cancer risk. Some nutrients have been shown to reduce tumor formation in laboratory animals, especially when they were fed a diet insufficient in the respective nutrients. However, most intervention trials failed to demonstrate a beneficial health effect of the nutrients [55]. While the reasons for this contradiction may be complex, some of the possibilities are as follows:

4.1 Nutritional status of the population

In most situations, supplementation of a nutrient is only effective in preventing cancer in subjects with insufficiency in that nutrient. This concept is consistent with the result from the LNIT in studying a population with general micronutrient insufficiency. The NPC trial also showed that a beneficial effect of selenium supplementation was observed only in individuals with low baseline serum levels of selenium [31, 56]. Thus, trials would probably not work in subjects with sufficient levels of the intervening nutrients. This may be the case with the SELECT [41]. The mean baseline median plasma level of α -T was 12.5 μ g/mL and of serum selenium was 135 ng/mL, indicating the sufficiency of these nutrients. There are no obvious examples to show that nutrients can be used as pharmaceutical agents for cancer prevention. Much effort has been made to develop metabolites or derivatives of nutrients as chemopreventive agents. For example, retinoic acid derivatives [57] have shown promise in the prevention of head and neck cancers in early studies [58], but later studies demonstrated the lack of chemopreventive effects [59, 60].

4.2 Doses of the nutrients used

Without an accurate method to estimate the dosage, the doses of intervening agents used may be too high or too low [12]. To avoid using dosages that could be too low, many studies tended to use high doses. In the case of the SELECT, the supplementation of α -tocopheryl acetate at a daily dose of 400 mg might have been too high, as it actually decreased plasma

γ -T levels by 50% [41]. If γ -T plays a role in prostate cancer prevention [37, 51], its decrease could result in the increase of prostate cancer risk that was observed [42].

4.3 Time of the intervention

Some nutrients may exert their protective effects early in life or many years before the onset of pre-malignant lesions. Clinical intervention studies are not able to study the population at such an early stage or for a long period of time. Some intervention studies might have started too late in individuals who already had precancerous lesions. This may be the situation in the LNTIT for individuals enrolled at ages >55 years [19] and in the Linxian multiple-nutrient trial in subjects with esophageal dysplasia [20]. It has been reported that in rats, supplementation with folic acid at an early stage of carcinogenesis decreased colon carcinogenesis, whereas supplementation at the late stage enhanced colon carcinogenesis [61]. The latter phenomenon is also consistent with the results of a human trial on colon cancer [62]. In the SELECT [41, 42], many of the subjects may have already had precancerous or cancerous lesions at the beginning of the intervention.

4.4 Selection of the preventive agents

The intervening agents may or may not possess cancer preventive activity in the population studied. In some cases, the existing epidemiological association of cancer prevention was due to factors other than the hypothesized nutrient. This mistake was actually made in the 1980s, when the possible cancer preventive effect of vegetables and fruits against lung and other cancers was attributed to β -carotene. As discussed earlier, the hypothesis that β -carotene prevents lung cancer was tested in smokers in the ATBC trial, which obtained the unexpected result of increased lung cancer incidence [24].

A recent report on the large-scale Physicians Health Study Randomized Controlled Trial II (PHSII) showed that men taking multivitamins had a significant reduction in the incidence of total cancer [40]. The effect is considered to be modest, but is significant in demonstrating the preventive effect in a well-nourished population and even in individuals with a history of cancer. These results are not consistent with Points 4.1 and 4.3 as discussed above. Research on nutrition and cancer prevention is a rather complicated field. It is possible that many of the inconsistent observations in human studies were due to the lack of power to detect a protective effect in some intervention trials or due to chances for false negative or positive results when multiple endpoints were analyzed.

5. Studies on Cancer Prevention by Non-Nutritive Dietary Constituents

Dietary phytochemicals have been studied for their cancer preventive activities. These include a variety of polyphenols, such as resveratrol, curcumin, genistein, chlorogenic acid, epigenin, delphinidin, luteolin and silibinin; of terpenoids such as carnosol and limonene; and of organosulfur compounds such as sulforaphane, phenethyl isothiocyanate, indole-3-carbinol, and diallyl sulfide. Many interesting results from laboratory studies have been accumulated [10–12]. However, convincing cancer preventive activities from human studies are lacking. We will use studies on green tea polyphenols as examples to illustrate the problems of extrapolation results from *in vitro* studies to animals and from studies in animal

models to human situations. Tea, made from the leaves of the plant *Camellia sinensis*, has been used historically as a medicinal herb and is now a popular beverage worldwide. Because of the abundance of both laboratory and human studies on green tea polyphenols, it provides an unique opportunity for us to analyze the similarities and differences between these two types of studies. The major tea polyphenols, also known as catechins, are (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epicatechin (EC) and their structures are shown in Figure 3. EGCG is the most abundant and most active tea polyphenol in green tea and has been studied extensively. The levels of these characteristic tea polyphenols in blood and urine can be used as exposure biomarkers in epidemiological studies.

5.1 Studies on tea in animal models

The cancer preventive activities of green tea extracts, tea polyphenols and EGCG have been demonstrated in many animal models (reviewed in [63, 64]). These studies have used chemically induced and genetic models for lung, oral, esophageal, stomach, small intestinal, colorectal and prostate cancers, and have shown cancer preventive activities when tea extracts or tea polyphenols are administered through the drinking water or diet. The inhibitory activity against lung carcinogenesis has been observed when tea preparations are administered to mice during the initiation, promotion or progression stages [63]. These results demonstrate the broad cancer preventive activities of tea catechins.

5.2 Studies on tea *in vitro* and mechanistic considerations

The biological activities of tea catechins, especially EGCG, have been studied extensively *in vitro* and *in vivo* (reviewed in [63, 64]). EGCG is known for its anti-oxidative action, but it can also produce ROS, especially *in vitro*. The phenolic groups are donors for H-bonding, and EGCG binds strongly to many different proteins via multiple H-bonds (reviewed in [65]). Some of these proteins have been proposed as targets of EGCG for inhibitory actions against cancer cells [63]. For example, EGCG has been proposed to bind to different molecular targets, inhibit the activities of many key enzymes, and inhibit several receptor-dependent signaling pathways [63]. A study with NMR spectroscopy showed the direct binding of tea polyphenols to the BH3 pocket of anti-apoptotic Bcl-2 proteins, with an inhibition constant (K_i) of 0.33–0.49 μM [66]. However, higher EGCG concentrations were needed to induce apoptosis [66]. The difference in effective concentrations is probably due to the nonspecific binding of EGCG to other proteins, which compete with the target protein. Binding of EGCG to the 67 kDa laminin receptor with a dissociation constant (K_d) value of 0.04 μM was observed using a surface plasmon resonance assay, and this was proposed to be a mechanism for the anti-cancer actions of EGCG [67, 68]. Dong *et al.* identified vimentin, IGF-1R, GRP78, 2AP70 and G3BP1 as high affinity EGCG binding or EGCG target proteins [63, 69–75]. An X-ray crystallography study showed that EGCG binds to both the WW and PPIase domains of Pin1 (peptidyl prolyl cis/trans isomerase) [75]. Pin1 is an important enzyme known to regulate the activities of AP-1, NF κ B, β -catenin and many other signaling molecules and was proposed to be responsible for the cancer preventive activities of EGCG [75]. Biochemical studies, however, showed a rather high dissociation constant of 21.6 μM between EGCG and Pin1 [75]. Some of the proposed targets for EGCG in cancer prevention are shown in Figure 4.

Based on studies in cell lines, many mechanisms of action have been proposed for tea constituents; however, it is uncertain whether many of these proposed mechanisms are relevant to cancer prevention (reviewed in [63]). Since most of the *in vitro* studies were conducted in cancer cell lines, some of the results may be more relevant to cancer therapy, if the effective concentrations of EGCG are low enough [68]. Another point that was neglected by researchers is that EGCG can be autoxidized under the conditions of cell culture studies. For example, at concentrations of 20 and 50 μM , most EGCG is autoxidized within two hours (mediated by superoxide in a chain reaction) to dimers, which are unstable and converted to unidentified products [76]. The dimers of EGCG may have even higher affinities for protein binding. It is unclear whether many of the *in vitro* binding studies are affected by the formation of dimers or other oxidized products of EGCG and whether some of the reported cell-killing effects of EGCG are mediated by its autoxidation products, superoxide and hydrogen peroxide.

Apparently, mechanisms derived from cancer prevention studies in animal models are likely to be more relevant. These include the induction of apoptosis in different animal models, inhibition of phosphorylation of c-JUN and ERK1/2 in lung tumorigenesis models, suppression of nuclear β -catenin and phospho-AKT levels in colon cancer models, inhibition of the IGF/IGF-1R axis in colon and prostate cancer models, and suppression of VEGF-dependent angiogenesis in lung and prostate cancer models [63, 77–81]. It is still unclear whether these molecules are direct targets for EGCG or downstream events of the primary action. In theory, the high affinity binding proteins mentioned above could serve as initial targets, but this point remains to be substantiated *in vivo*. From the limited human studies that are available, the action of tea constituents in reducing oxidative stress and enhancing the elimination of carcinogens could also be important mechanisms for the cancer preventive activity of tea [82–84].

5.3 Possible cancer prevention by tea in humans

Although there is strong evidence for the cancer preventive activity of tea constituents in animal models, results on such activities in humans from epidemiological studies have not been consistent (reviewed in [63, 85]). The differing results between human and animal studies are likely to be due to the lower quantities of human tea consumption as compared to the doses used in animal studies. In animal studies, the experimental conditions, including the doses of tea preparations, are selected to maximize the opportunity to detect a cancer prevention effect. In epidemiological studies, lifestyle factors, genetic polymorphisms and other interfering factors reduce the power to detect a cancer preventive effect. For example, the protective effect of green tea consumption against upper-gastrointestinal cancer became clear after adjusting for confounding factors [85]. Smoking appears to be a strong interfering factor. In a case-control study on the effect of green tea consumption on esophageal cancer in Shanghai by Gao *et al.* [86], a protective effect was only observed in non-smokers, who were mostly women. A recent systematic review of cohort studies in Japan on green tea consumption and gastric cancer indicated no overall preventive effect of green tea. However, a small consistent risk reduction was found in women, and the result became statistically significant after pooling data of six cohort studies [87]. A possible explanation is that women were mostly non-smokers, whereas most men were smokers in this Japanese

population. The relationship between tea consumption and cancer risk may become clearer if we better quantify tea consumption, correct for confounding factors, and consider genetic polymorphisms of the populations. Nevertheless, the above results suggest the cancer preventive effect of tea is only mild in humans.

The results of human intervention studies with green tea polyphenols, mostly small randomized clinical trial (RCT), have been inconsistent. An earlier RCT on oral cancer prevention in China, with a mixed tea product (3 g/day administered orally or topically) in patients with oral mucosa leukoplakia for 6 months, showed significant decrease in the number and total volume of proliferation index and silver-stained nucleoli organizer regions [88]. A later phase II RCT in the U.S. with green tea extract (500, 750 or 1000 mg/m² 2 times daily) for 12 weeks, to patients with oral pre-malignant lesions (n = 28), showed possible beneficial effects in lessening oral pre-malignant lesions, in part through reducing angiogenic stimulus (stroma VEGF), but it was not statistically significant [89]. In an RCT in Japan, on patients with adenomas removed by polypectomy, supplementation with green tea extracts (1.5 g/day) for one year significantly decreased the development of colorectal adenomas [90]. In this study, the patients were regular tea drinkers (average of 6 cups/day); the dose response relationship of this study is difficult to understand, and further studies are needed.

The most impressive study was conducted in Italy, in which 30 men with high-grade prostate intraepithelial neoplasia (PIN) were given 600 mg of green tea catechins daily for 12 months [91]. Only 1 patient developed prostate cancer, whereas 9 of the 30 patients with high-grade PIN in the placebo group developed prostate cancer. The difference is statistically significant. However, a recent trial in Florida with a similar design using Polyphenon E (a proprietary mixture of green tea catechins) containing 400 mg of EGCG in 97 men with high-grade PIN and/or atypical small acinar proliferation (ASAP), supplementation for 6 to 12 months showed no differences in the number of observed prostate cancer cases between the treatment group (n = 49) and the placebo group (n = 48) [92]. However, there was a decrease in the cumulative rate of prostate cancer plus ASAP in the treatment group, in subjects without ASAP at baseline. A decrease in serum prostate specific antigen was also observed in the Polyphenon E supplemented group. Some recent intervention studies on breast cancer and esophageal adenocarcinoma were limited to bioavailability and some biomarker studies. At present, the earlier optimistic expectation of cancer preventive activity by tea polyphenols has not materialized in RCTs.

6. Lessons Learned from Cancer Prevention Studies with Non-Nutritive Phytochemicals

A great number of laboratory studies suggest the potential cancer preventive activities of many non-nutritive phytochemicals, but evidence from human studies is lacking. Tea is one of the few agents that has been extensively investigated for cancer preventive activities in laboratory, epidemiological and intervention studies. For many other dietary chemicals, a large number of laboratory studies but only a few human trials have been conducted. Epidemiological data of most of these dietary chemicals are also lacking because they are

not uniquely present in specific food items and there are no specific exposure markers. The effects of the agents of interest are usually interfered by other constituents in the diet. What we have learned in this area of research is summarized below:

6.1 Problems in interpreting results from cell line studies

At high enough concentrations, EGCG and other phytochemicals are likely to inhibit growth or induce death of cancer cells in culture, and a large number of associated molecular changes can be observed [63]. The effective doses of EGCG *in vitro* and how they can be related to the concentration of EGCG in animal tissue are important issues for consideration. Numerous mechanisms for the action of EGCG and other agents have been proposed [63]. A critical question that remains unanswered is how a molecule like EGCG can function through so many different mechanisms and how another molecule with a different chemical structure can act via many of the same mechanisms. It is possible that most of the proposed mechanisms in cell lines may be just the effects of EGCG observed under the experimental conditions. It remains unclear how most of these results are related to cancer prevention.

6.2 Bioavailabilities of chemopreventive agents and dose-response relationship

A major issue in correlating cell line and animal studies with phytochemicals, such as EGCG and curcumin, is the bioavailability. An agent is only effective if sufficient concentrations can reach the target organs. Without considering the poor bioavailability of certain compounds (such as theaflavins from black tea), cell line studies could be misleading. For most agents, rather high doses of agents are required to demonstrate a cancer preventive effect, and these doses are usually higher than the levels of human consumption. However, a recent study of *Apc*^{Min} mice on a high-fat diet showed a nonlinear dose-response of resveratrol: the lowest dose of resveratrol (0.00007% in the diet) suppressed intestinal adenoma development better than a higher dose (0.0143% in diet) did [93]. Human colorectal tissues exposed to low concentrations of resveratrol *ex vivo* also produced biomarkers (activation of AMPK and enhanced autophagy) similar to the mouse model. The dose of 0.00007% in the diet (corresponding to 0.35 mg for a person taking 500 g of food in dry weight per day) is extremely low. The results are intriguing, and more studies are needed.

6.3 High dose toxicity

When increasing the doses to gain a cancer preventive effect in humans, the toxicity issue needs to be considered. For example, liver toxicity due to intake of green tea extract-based supplement, especially in large bolus doses for weight reduction, is well documented [94, 95]. In clinical trials with Polyphenon E (2,000 mg EGCG, twice daily for up to 6 months) to patients with chronic lymphocytic leukemia, beneficial clinical effects were observed; however, there was also liver toxicity and other adverse effects [96]. Such adverse effects may be tolerable in short-term therapies for cancer patients, but are not acceptable for long-term use in cancer prevention. The concept that natural products are nontoxic is based on observations from the intake of moderate doses or from agents with low bioavailabilities. When the bioavailability is significantly increased, for example, by the use of nanoparticles, toxicity should be an important concern.

6.4 Investigation of new chemopreventive agents

Many plant materials, such as traditional medicinal herbs, have been considered as rich sources of chemopreventive agents. Many of the anti-inflammatory agents from plants could be investigated for use in cancer prevention because of the importance of inflammation in carcinogenesis. However, caution should be applied before launching studies with new agents in this area. Studies in cell lines may be used to identify new potent phytochemicals for *in vivo* studies in animal models. It is important that only agents that showed very high potency, with low or null toxicity, in animals warrant further investigations.

7. Concluding Remarks

Important advances have been made in the field of cancer chemoprevention in the past 30 years however, there are also many inconsistent and negative results [97, 98]. There is even the statement that “Chemoprevention of cancer is an almost universal failure” [99]. We do not agree with this statement, and instead agree with the viewpoint of Adhami *et al.* [100], “Cancer chemoprevention is not a failure.” A recent analysis of this field titled “Cancer Prevention: Obstacles, Challenges and Road Ahead” has been published [101]. After we understand why some of the previous human trials failed, we will learn to design better studies in the future. Among the lessons that we have learned, we would like to emphasize the importance of integration of laboratory and human studies. For laboratory researchers, it is important to have human relevance in mind when pursuing studies. With a trend of emphasizing translational research, caution should be applied to avoid over-interpreting data and rushing one’s favorite agents prematurely into human trials. It is also important for epidemiologists and human trial investigators to appreciate the value of laboratory studies and recognize the limitation in the power of their studies in testing (accepting or rejecting) a hypothesis. With institutional pressure for more publications and publicity to gain recognition and funding, we have to remind ourselves to maintain a high level of scientific integrity. Cancer prevention is an important field of research aimed to reduce human suffering. More interactions between laboratory researchers and scientists in human studies, in the spirit of mutual respect, will lead to fruitful collaborations to advance the field of cancer prevention.

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Abbreviations

ASAP	atypical small acinar proliferation
ATBC	α -Tocopherol and β -Carotene
COX2	cyclooxygenase 2
EGCG	(-)-epigallocatechin-3-gallate
ESCC	esophageal squamous cell carcinoma

G3BP	GTPase activating protein (SH3 domain) binding protein
GCA	gastric cardia adenoma
GRP78	78 kD glucose-regulated protein
IGF1R	insulin like growth factor 1 receptor
LNIT	Linxian Nutritional Intervention Trial
NPC	Nutritional Prevention of Cancer
PHLPP	PH domain and leucine rich repeat protein phosphatase
Pin1	peptidylprolyl cis/trans isomerase
PLCO	Prostate, Lung, Colorectal and Ovarian
PPARγ	peroxisome proliferator-activated receptor gamma
RCT	randomized clinical trial
RNS	reactive nitrogen species
ROS	reactive oxygen species
RYK	receptor tyrosine kinase
SELECT	Selenium and Vitamin E Cancer Prevention Trial
ZAP70	zeta chain of T cell receptor associated protein kinase 70kD

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Figure 1.
Structures of tocopherols

Figure 2.

Some proposed mechanisms of cancer prevention by tocopherols.

Abbreviations: **AKT**, v-akt murine thymoma viral oncogene homolog; **COX2**,

cyclooxygenase 2; **PHLPP**, PH domain and leucine rich repeat protein phosphatase;

PPAR γ , peroxisome proliferator-activated receptor gamma; **RNS**, reactive nitrogen species;

ROS, reactive oxygen species; **RYK** receptor tyrosine kinase.

Figure 3.
Structures of major tea polyphenols

Figure 4.

Some proposed targets for EGCG in cancer prevention.

Abbreviations: **67LR**, 67b kD laminin receptor; **Bcl2**, B-cell CLL/lymphoma 2; **CDKs**, cyclin-dependent kinases; **DHFR**, dihydrofolate reductase; **DNMT**, DNA methyltransferase; **EGCG**, (–)-epigallocatechin-3-gallate; **FYN**, fyn proto-oncogene, Src family tyrosine kinase; **G3BP**, GTPase activating protein (SH3 domain) binding protein; **GRP78**, 78 kD glucose-regulated protein; **HIF-1α**, hypoxia inducible factor 1, alpha subunit; **IGF1R**, insulin like growth factor 1 receptor; **MAP kinases**, mitogen-activated protein kinases; **MMPS**, matrix metalloproteinases; **Pin1**, peptidylprolyl cis/trans isomerase; **ROS**, reactive oxygen species; **STAT1**, signal transducer and activator of transcription 1; **ZAP70**, zeta chain of T cell receptor associated protein kinase 70kD.