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Dietary chemoprevention strategies for induction of phase II xenobiotic-metabolizing enzymes in lung carcinogenesis: A review

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Abstract

Lung cancer is the leading cause of cancer mortality for men and women in the United States and is a growing worldwide problem. Protection against lung cancer is associated with higher dietary intake of fruits and vegetables, according to recent large epidemiologic studies. One strategy for lung cancer chemoprevention focuses on the use of agents to modulate the metabolism and disposition of tobacco, environmental and endogenous carcinogens through upregulation of detoxifying phase II enzymes. We summarize the substantial evidence that suggests that induction of phase II enzymes, particularly the glutathione *S*-transferases, plays a direct role in chemoprotection against lung carcinogenesis. The engagement of the Keap1–Nrf2 complex regulating the antioxidant response element (ARE) signaling pathway has been identified as a key molecular target of chemopreventive phase II inducers in several systems. Monitoring of phase II enzyme induction has led to identification of novel chemopreventive agents such as the isothiocyanate sulforaphane, and the 1,2-dithiole-3-thiones. However, no agents have yet demonstrated clear benefit in human cell systems, or in clinical trials. Alternative strategies include: (a) using intermediate cancer biomarkers for the endpoint in human trials; (b) high-throughput small molecule discovery approaches for induced expression of human phase II genes; and (c) integrative approaches that consider pharmacogenetics, along with pharmacokinetics and pharmacodynamics in target lung tissue. These approaches may lead to a more effective strategy of tailored chemoprevention efforts using compounds with proven human activity.

Keywords

Lung cancer; Phase II enzymes; Chemoprevention; Induction; Phytochemicals

1. Introduction

Lung cancer is by far the leading cause of cancer-related death in both the United States and the world. It is projected that the United States will experience 213,380 new lung and

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bronchus cancer cases and 160,390 deaths for lung and bronchus cancer in the year 2007 [1]. The devastating 5-year relative survival rates for this disease in the United States including all stages is approximately 15.0%, with 5-year survivals at a localized and regional stage approximately 49% [1]. Most of the promise for this disease during the last decade relates to the introduction of novel targeted therapeutic agents, improved staging and surgical techniques, and increased utilization of concomitant chemoradiotherapy for locally advanced lung cancer. However, these interventions have only minimally decreased overall mortality rates.

Several environmental factors associated with an increased risk of developing lung cancer have been identified, including mainstream cigarette smoking; exposure to radon, polycyclic aromatic hydrocarbons (PAHs), nickel, chromate, arsenic, asbestos, chloromethyl ethers and ionizing radiation; and chronic obstructive pulmonary disease with airflow obstruction. The risk of lung carcinoma increases with the number of cigarettes smoked, years of smoking, earlier age of commencing smoking, degree of inhalation, tar and nicotine content, and use of unfiltered cigarettes, and decreases proportionally with the number of years after quitting [2]. However, several studies have demonstrated that former smokers still have a higher lung cancer risk than nonsmokers [3,4], even 10 years after smoking cessation [5]. Therefore, in addition to smoking cessation, new, emerging preventive approaches such as dietary modulation and chemoprevention may be considered for control of the lung cancer epidemic.

Cancer chemoprevention has been defined as the use of dietary and pharmacological intervention with specific natural or synthetic agents designed to prevent, suppress, or reverse the process of carcinogenesis before the development of malignancy [6]. One of the major mechanisms of chemical protection against carcinogenesis, mutagenesis, and other forms of toxicity mediated by electrophiles is the induction of enzymes involved in their deactivation, particularly phase II xenobiotic-metabolizing enzymes such as glutathione *S*-transferases (GSTs), uridine diphosphate-glucuronosyl transferases (UGTs), and NAD(P)H quinine oxidoreductase (NQO1) [6,7]. Indeed, induction of phase II enzymes can be achieved in many target tissues by administering any of a diverse array of naturally occurring and synthetic chemical agents [7–9]. This review focuses on several issues related to lung cancer chemoprevention by modification of phase II metabolism, including lung carcinogenesis, mechanisms of phase II enzyme induction, and identification of small molecules.

2. Lung cancer carcinogenesis and phase II xenobiotic-metabolizing enzymes

Lung carcinogenesis results from the interaction between endogenous factors and inhaled compounds. Many inhaled carcinogens undergo phase I metabolic oxidation that generates highly reactive electrophilic intermediates that electrophilically attack macromolecules including DNA within cells. The metabolic step that detoxifies/reduces these reactive electrophiles can be termed phase II metabolism (Fig. 1), on which this review is focused. Phase II reactions catalyze conjugation by sulfation, glucuronidation, or glutathioylation and neutralize electrophilic chemicals resulting in a reduction of chemical reactivity, and the facilitation of elimination. DNA damage, including the formation of DNA adducts, is recognized as a primary step in chemical carcinogenesis. Accordingly, blocking DNA damage can be considered the first line of defense against lung and other tobacco-related cancers. This defense against chemicals (DNA alkylation and DNA adduct formation) and oxidative stress (oxidative DNA base modification) is generally achieved by increasing the expression and/or activity of phase II detoxifying or antioxidant enzymes.

Subserving this phase II detoxification function members of the GST superfamily of enzymes are prominent. GSTP1 has been identified as the most abundant GST enzyme expressed in the lung [10–12], and is known to be one of the main detoxifiers of the dihydrodiol and epoxide forms of benzo(a)pyrene (BaP), known tobacco mutagens [13]. For example, in an experimental model, Ritchie et al. reported that *GSTP*-null mice were found to have substantially increased numbers of lung adenomas relative to wild-type mice following exposure to BaP, 3-methylcholanthrene, and urethane, suggesting that variability in *GSTP1* expression or function will influence lung tumorigenesis [14].

In epidemiological studies, certain genetic polymorphisms of metabolizing genes have been associated with altering lung cancer risk by modifying the effect of tobacco smoking carcinogens. Among them, polymorphisms in *GSTM1* have received the most attention. A large meta-analysis reported that lung cancer risk increased by 17% in those who were *GSTM1* null (95% CI, 1.07–1.27) [15], while a pooled analysis in Caucasians < 45 years of age also found similar, but not statistically significant, results (OR, 1.1; 95% CI, 0.9–1.3) [16]. The most recent and largest meta-analysis of 130 studies found an 18% increased risk of lung cancer among individuals with the *GSTM1*-null genotype (95% CI, 1.14–1.23), but when analyzing data only from the larger studies there was no association [17]. This group also looked at *GSTP1* and *GSTT1* in these 130 studies. For *GSTP1* coding sequence I105V and A114V polymorphisms, there was no association with lung cancer risk, whereas for the *GSTT1*-null genotype, risk of lung cancer was modestly increased (OR, 1.09; 95% CI, 1.02–1.16). However, when only the larger studies with a lower probability of false-positive findings were evaluated, the *GSTT1* association became non-significant. Both study size and ethnic background were important sources of heterogeneity between studies. There is quantitative variability in *GSTP1* expression in the lung, some of it attributable to promoter polymorphisms [18]. Association studies of *GSTP1* expression levels and lung cancer risk are ongoing.

3. Mechanisms of phase II xenobiotic-metabolizing enzyme induction

Many synthetic and naturally occurring compounds are known to induce the expression of phase II enzymes. Critical DNA sequences are frequently found single or multiply in the promoters of these genes, including antioxidant response element (ARE) and xenobiotic-responsive elements (XREs). Two underlying mechanisms: aryl hydrocarbon receptor (AhR)–XRE and nuclear factor erythroid 2-related factor (Nrf2)–ARE signaling pathway are involved in induction of phase II enzymes (Fig. 2). There may be bidirectional regulation; recent studies suggest that Nrf2 is an AhR target gene, and the Nrf2 expression is directly modulated by AhR activation [19,20]. Therefore, the distinct but overlapping AhR and Nrf2 gene batteries may be coordinately regulated, offering the possibility that phase II enzymes can be synergistically activated by a number of phytochemicals.

3.1. AhR–XRE signaling pathway

AhR, a basic helix-loop-helix (bHLH)-PAS protein functions as a ligand-activated DNA binding protein, and has been identified as a key regulator in tobacco metabolism. In the absence of ligand, the AhR is located in the cytosol in association with heat shock protein 90 (HSP90) [21,22]. Upon entering cells, both synthetic chemicals such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and naturally occurred phytochemicals, sterols, and heme breakdown products bind to and activate AhR in the cytoplasm, and the ligand/receptor complex translocates into the nucleus, where it heterodimerizes with another transcription factor, aryl hydrocarbon receptor nuclear translocator (Arnt) [23]. This AhR/Arnt complex then augments transcription by binding to xenobiotic (enhancer) responsive elements, which are found upstream of both phase I genes including *CYP1A1*, *CYP1A2*, *CYP1B1* and phase II genes, such as *GST*, *NQO1*, *UGT*, *ADH* (Fig. 2) [24,25]. Clearly, the

coordinate induction of phase I and phase II enzymes in lung may yield no net protection; indeed, the induction of phase II enzymes by a mutagen may be more robust than for phase I induction [26].

3.2. Nrf2–ARE signaling pathway

The ARE is present in many phase II genes, and has been identified to be a *cis*-acting element that is responsible for phase II gene expression [27], but not phase I induction. Identification of the transcription factors that bind to ARE sequences is not complete and varies between cell types, however, Nrf1 and Nrf2 are involved. Both Nrf1 and Nrf2 can bind AREs as heterodimers with Maf proteins to positively regulate ARE-driven gene expression. Whereas Nrf1 gave primarily cytoplasmic staining that was coincident with that of an endoplasmic reticulum (ER) marker, Nrf2 gave primarily nuclear staining [28]. It is known that in the uninduced cells, Nrf2 is sequestered in the cytoplasm by a cysteine-rich cytoskeleton binding protein named Kelch-like ECH-associated protein 1 (Keap1). Keap1, a 69-kDa protein, is normally anchored to actin. Upon interaction with inducers, dissociation of Nrf2 from Keap1 allows it to translocate to the nucleus. Neither Nrf1 transactivation activity nor its cytoplasmic localization is completely controlled by Keap1. It has been reported that Nrf1 is cleaved and translocated from the endoplasmic reticulum to the nucleus in response to ER stress [29], through the functional contribution of Nrf1 to the ER stress response has not been well described. Finally, Nrf1/Nrf2 bind with other transcription factors including several members of the small Maf family to active the phase II gene transcription through the ARE (Fig. 2) [30].

The mechanistic signal for Nrf2 release by Keap1 is under investigation. On the one hand, Keap1 plays a crucial role in the regulation of Nrf2. Zipper and Mulcahy [31] showed that mutation of Ser-104 in the BTB/POZ domain of Keap1 disrupted Keap1 dimerization and eliminated the ability of Keap1 to sequester Nrf2 in the cytoplasm, which is associated with the release of Nrf2 *in vivo*. Notably, Keap1 contains many cysteine residues. Phase II enzyme inducers can cause oxidation or covalent modification of these cysteine residues, which could induce a conformational change of Keap1, leading to release of Nrf2 from its complex [32]. Recent studies suggest that Keap1 facilitates the ubiquitination of Nrf2 by the Keap1-Cul3 E3 ubiquitin ligase complex in which Keap1 functions as a substrate adaptor, which might be the primary effect on Nrf2 half-life and distribution in the cell [33–35]. On the other hand, the activation of Nrf2 involves phosphorylation by multiple cellular kinase pathways. First, the mitogen-activated protein kinases (MAPKs), namely, c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 kinase, are important cellular signal networks that have been examined for Nrf2 activation [36,37]. Secondly, the protein kinase C (PKC) directed phosphorylation of Nrf2 is a key step before its nuclear accumulation [38–40]. Huang et al. have reported that PKC can directly phosphorylate Nrf2 at Ser-40 [39]. Subsequently, Yoshida and co-workers also reported that Nrf2 phosphorylation is mediated by TPA-insensitive atypical PKC [40]. Thirdly, phosphatidylinositol 3-kinase (PI3K) has been identified as a kinase that is essential for the nuclear translocation of Nrf2 and Nrf2 DNA binding [41]. PI3K also phosphorylates the CCAAT/enhancer binding protein- β (C/EBP- β), inducing its translocation to the nucleus and binding to the CCAAT sequence of C/EBP- β response element with XRE, in conjunction with Nrf2 binding to ARE [42].

There is a substantial literature that supports the view that ARE-regulated phase II enzyme induction is a highly effective strategy for reducing susceptibility to carcinogens. This conclusion has received strong support from experiments on mice in which Nrf2 was deleted. In 2002, Cho et al. [43] reported that hyperoxia-induced mRNA and activity levels of *NQO1*, *GSTY α* , *GSTY γ* , and *UGT* were significantly lower in Nrf2^{-/-} mice compared with Nrf2^{+/+} mice. Although the susceptibility to lung cancer in Nrf2-deficient mice has not

been investigated, the study has suggested that Nrf2-deficient mice are highly susceptible to cigarette smoke-induced emphysema [44]. Accelerated DNA adduct formation in the lung of Nrf2-deficient mice when exposed to diesel exhaust has been reported [45]. Recent studies suggest that Nrf2^{-/-} mice were more susceptible to genotoxic carcinogens because the spontaneous mutation frequency of the guanine phosphoribosyltransferase gene in the lung was approximately three times higher in Nrf2-null (Nrf2^{-/-}) mice than Nrf2 heterozygous (Nrf2^{+/-}) and wild-type (Nrf2^{+/+}) mice, and a single intratracheal instillation of BaP increased the lung mutation frequency 3.1- and 6.1-fold in Nrf2^{+/-} and Nrf2^{-/-} mice, respectively, compared with BaP-untreated Nrf2^{+/-} mice [46]. Overall, Nrf2 is considered an important molecular target for cancer prevention.

4. Phase II enzyme inducers and lung cancer prevention

Substantial scientific evidence indicates that increased consumption of fruit and vegetables is associated with reduced risk to developing lung cancer [47,48]. Since previous trials of single agent active in other selected pathways showed null or adverse effects on lung cancer for (beta-carotene, alpha-tocopherol, retinol, retinyl palmitate, *N*-acetylcysteine, or other agents) [49,50], attention has shifted to other pathways, including phase II enzyme inducers inherent in edible plants. Three of the most highly examined phase II enzyme inducers are the cruciferous vegetable-derived isothiocyanates (ITCs) and dithiolethiones, and tea-derived catechins such as epigallocatechin gallate (EGCG) (see Table 1). While there are other target pathways for prevention, such as DNA damage, cell cycle arrest, apoptosis and phase I enzyme inhibition that may contribute to their chemopreventive effects [51,52], we will focus on discussing their effects on phase II induction, as well as touch on their *in vivo* and *in vitro* effects on lung cancer chemoprevention overall. The few clinical trails on lung cancer using these three agents will also be discussed.

4.1. Isothiocyanates

Isothiocyanates are sulfur-containing phytochemicals with the general formula R-NCS naturally occurring as glucosinolate conjugates in cruciferous vegetables such as broccoli, cauliflower, kale, turnips, collards, Brussels sprouts, cabbage, radish, turnip and watercress. These include the methylsulfinylalkyl isothiocyanates, such as sulforaphane (SFN) and aromatic ITCs, including phenethyl isothiocyanate (PEITC) and benzyl isothiocyanate (BITC).

ITCs are believed to exert their chemoprotective effect partly by inducing phase II enzyme [53–61], thereby enhancing the elimination of activated carcinogens. SFN is the principal and perhaps most potent phase II enzyme inducer found in cruciferous plants, with particularly high levels detected in broccoli and broccoli sprouts. *In vitro*, SFN has been shown to increase NQO1, UGT1A1 GSTA1, and GSTP1 expression, at mRNA, protein and activity levels [54–56]. SFN has also been found to be effective at inducing the phase II enzyme response *in vivo*. Mice treated by gavage with 15 μ mol SFN/mouse per day for 5 days had an increase in QR and glutathione *S*-transferase in the lung [57]. A dose-escalation safety study in healthy human subjects showed that NQO1 activity in skin tissues increased in a dose-dependent manner, with maximum increases of 1.5- and 4.5-fold after application of 150 nmol SFN, once or three times (at 24-h intervals), respectively [58].

The existing reported evidence for direct PEITC and BITC influence on phase II enzyme induction in lung tissue or cells is sparse. However, PEITC has proven to increase GSTs, and NQO1 and UGT activities in the rat liver [59] and in murine Hep1c1c7 cells [60]. In a study *in vivo*, a single gavage treatment of 1 mmol/kg PEITC induced NQO1 5-fold and GST activity 1.5-fold in the liver, but activity of these enzymes were not significantly affected in the lung or nasal mucosa [59], which indicates PEITC may be a tissue specific

inducer of phase II enzyme. Additionally, BITC, as the most potent component of papaya juice, induced GST activity in small intestine and liver of female ICR/Ha mice in an early chemoprevention study by Wattenberg and co-workers [61]. In addition, BITC was also found to significantly induce *GSTP1* in cultured rat liver epithelial cells [62].

Two main mechanisms have been proposed to explain the induction of phase II enzymes by ITCs, namely disruption of Nrf2–Keap1 interactions and MAPK activation. The disruption of Nrf2–Keap1 interactions, the translocation of Nrf2 to the nucleus, and the induction of ARE-containing genes has been extensively reviewed for SFN [63,64]. The importance of Nrf2 was illustrated through the use of Nrf2 knockout mice, in which the response to SFN in induction of the aforementioned classes of phase II genes was abrogated. Analysis of gene expression profiles by an oligonucleotide microarray revealed that SFN upregulated expression of *NQO1*, *GST* and glutamylcysteine synthetase in the small intestine of wild-type mice, whereas the Nrf2-null mice displayed much lower levels of these enzymes [65]. Furthermore, a recent study using microarray demonstrated that PEITC could induce *NQO1* and *GST* subunits alpha2, mu1, mu3 and theta3 gene expression in wild-type, but not Nrf2-deficient mice [66].

An additional mechanism through which ITCs might activate ARE-driven genes is via the MAPK pathway. SFN was shown to increase ERK2 activity, which is downstream from Raf-1 and MEK [67]. Use of a MAPK inhibitor attenuated activation of ARE reporter activity, and SFN directly activated Raf-1 [68]. Subsequently, MAPK activation was observed in rat livers 2 h after rats were treated by gavage with 50 μmol SFN [67]. This, along with other data reviewed in [69], suggests that MAPK activation may play a role in the activation of Nrf2, and that SFN may regulate ARE-mediated transcription through its effects on Keap1–Nrf2 destabilization, as well as MAPK activation. BITC and PEITC also differentially regulated the activation of MAPKs and Nrf2, ARE-mediated luciferase reporter-gene activity, and phase II gene induction [67,69].

Experimental studies in animals have demonstrated the efficacy of ITCs in inhibiting lung carcinogenesis by known carcinogens, such as PAH, BaP and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) [70–74]. It was recently reported that SFN, PEITC and their *N*-acetylcysteine conjugates inhibit the growth of lung carcinomas from benign tumors in the A/J mice treated with NNK plus BaP, two important carcinogens in cigarette smoke [70]. In addition, both PEITC and BITC, separately and in combination, were effective inhibitors of PAH, BaP and NNK induced lung adenoma in rats and A/J mouse [71–74].

Epidemiological studies have been designed to specifically evaluate dietary ITCs consumption as protective in human lung cancer. London et al. provided the first direct evidence that links dietary ITCs to reduced incidence of lung cancer in humans [75]. They evaluated the direct relationship between total ITCs in urine, *GST* genotype, and subsequent risk for lung cancer among 232 incident cases of lung cancer and 710 matched controls from a cohort of 18,244 men in Shanghai, China. Individuals with ITCs in the urine showed a decreased risk for lung cancer (smoking adjusted OR, 0.65; 95% CI, 0.43–0.97). Interestingly, the protective effect of dietary ITCs was more pronounced in person with the homozygous *GSTM1*-null genotype (OR, 0.36; 95% CI, 0.20–0.63) and was particularly strong in subjects with deletion of both *GSTM1* and *GSTT1* (OR, 0.28; 95% CI, 0.13–0.57). Subsequent case–control studies by Spitz et al. [76] and Zhao et al. [77] also showed that lung cancer risk is reduced by dietary sources of ITCs, and that persons with the *GSTM1*-null and *GSTT1*-null genotypes clearly benefit more extensively from diets rich in ITCs. A recent study by Brennan et al. [78] investigated the role of cruciferous vegetables in lung cancer after stratifying by *GSTM1* and *GSTT1* status in 2141 cases and 2168 controls. They found that weekly consumption of cruciferous vegetables protected against lung cancer in

those who were *GSTM1* null (OR, 0.67; 95% CI, 0.49–0.91), *GSTT1* null (0.63, 0.37–1.07) or both (0.28, 0.11–0.67), but not in people who were both *GSTM1* and *GSTT1* positive (0.88, 0.65–1.21).

There remains no evidence for induction of phase II gene transcription by ITCs in dietary intervention studies in humans. Gasper et al. [79] reported that no evidence was found for induction of phase II gene transcription in the gastric mucosa after a short-term broccoli intervention. Recently, Traka et al. [80] adopted an empirical approach in humans to elucidate the mechanisms that underlie the beneficial effects of a broccoli-rich diet, and explored the interaction with *GSTM1*, by analysis of global gene expression profiles within a target tissue before and after a 12-month dietary intervention. They found significant differences between *GSTM1* genotypes on the broccoli-rich diet, associated with transforming growth factor beta 1 (TGF β 1), epidermal growth factor (EGF) and insulin signaling pathways, but not phase II metabolism pathway. To the best of our knowledge, SFN, PEITC and BITC in pure form have, however, not yet been investigated for intermediate marker modulation or impact on outcomes in clinical trials of subjects at risk of lung cancer.

4.2. Dithiolethiones

The dithiolethiones are another class of chemoprotective compounds isolated from cruciferous vegetables such as Brussels sprouts and cabbage. 3*H*-1,2-dithiole-3-thione (D3T) is a representative compound from these groups and has been studied extensively as cancer chemoprotective agent. In addition, synthetic substituted dithiolethiones, including oltipraz (5-[2-pyrazinyl]-4-methyl-1,2-dithiol-3-thione), and anethole dithiolethione (ADT; 5-[4-methoxyphenyl]-1,2-dithiole-3-thione), have been developed for pharmaceutical applications because of their antioxidant, chemotherapeutic, radioprotective, and chemopreventive properties [81].

The dithiolethiones appear to act via some alternate mechanisms, including the inhibition of cell replication, as well as the predominant mechanism of increasing the expression or activity of phase II enzymes, such as GSTs. D3T and oltipraz were found to increase glutathione and phase II enzyme levels in several organs of the rodent host [82–85]. ADT increases activities of phase II enzymes in mouse tissues to a similar degree to oltipraz [86]. Up to now, phase II genes recognized to be induced by dithiolethiones *in vivo* include alpha, mu and pi isoforms of GSTs, UGTs and NQO1.

Nrf2–Keap1 signaling system is a main signaling pathway in the induction of phase II enzyme by chemopreventive dithiolethiones. This issue has been directly examined by exploring the effects of disruption of the *Nrf2* gene *in vivo* on induction of phase II enzymes [87]. Dithiolethiones elevated transcript levels, protein levels and activities of multiple phase II genes in wild-type mice, but not in the homozygous *Nrf2*-mutant mice [88].

In animal models of carcinogenesis, dithiolethiones exert chemoprotective properties against the development of lung and other cancers [89]. For example, to directly test the cancer chemoprotective efficacy of oltipraz, Wattenberg and Bueding [86] examined the capacity of oltipraz to inhibit carcinogen-induced neoplasia in mice. Oltipraz administered could reduce by nearly 70% the number of both pulmonary adenomas and tumors of the forestomach induced by BaP. Based on efficacy in preclinical models, oltipraz is one of the broadest acting chemoprevention agents in development. Phase I and II clinical trials investigating the chemopreventive potential of oltipraz have been completed at several centers. However, it has been generally regarded as too toxic to consider for routine chemopreventive purpose [90].

Another synthetic substituted dithiolethione, ADT, was predicted to have chemopreventive activity on the basis of its activity in inducing the expression of phase II genes [82]. It has been shown to be a promising chemopreventive agent for lung cancer in a randomized phase IIb study by Lam et al. [91]. They determined the protective effects of ADT in smokers with bronchial dysplasia, identified by an autofluorescence bronchoscopy-directed biopsy. One hundred and twelve current or former smokers with at least one site of bronchial dysplasia were randomly assigned to receive placebo or ADT at 25 mg orally thrice daily for 6 months. The primary endpoint was response defined by improvement in histology. No response difference was seen between the two groups. However, rates of progression of pre-existing dysplastic lesions by two or more grades or presence of new lesions were statistically significantly lower in the ADT group (8%) than in the placebo group [17%; $P < 0.001$, difference in progression rate, 9% (95% CI, 4–15%)], and the rate of disease progression was significantly lower in patients receiving ADT (32%) than in those receiving placebo (59%). Also of note, although nearly half the participants on the active arm of the trial underwent dose reduction because of abdominal bloating or flatulence, no severe toxicities were noted. This trial suggests that ADT or analogues merit further evaluation as a potential chemoprevention agent for lung cancer.

4.3. Epigallocatechin gallate

Tea is second only to water as the most consumed beverage in the world. Green tea, which accounts for 80% of the world's tea, is primarily consumed in Asian nations. The major anti-cancer components of teas are catechins (also known as polyphenols). By dry weight, a cup of green tea is typically 30–40% catechins. These catechins include epicatechin, epigallocatechin, epicatechin-3-gallate and epigallocatechin gallate. EGCG, as the most abundant catechin in green tea, has strong anti-proliferative and anti-tumor effects *in vitro* and in animal models [92].

Although green tea or green tea polyphenols have many mechanisms of action that may contribute to *in vivo* efficacy, such as induction of apoptosis, they have been shown to modulate various phase II enzymes, including GSTs, NQO1 and UGTs, in animal studies and *in vitro* systems [93]. Phase II enzyme induction by green tea polyphenols is generally believed to involve the activation of MAPK pathway via the electrophilic-mediated stress response, resulting in the activation of transcription factors that bind to ARE located on many phase II genes [94]. It has been shown that exposure of HepG2 cells to the green tea extract induces expression of phase II detoxifying enzymes through ARE, and the upregulation is accompanied by activation of ERK2 and JNK1, as well as immediate-early genes c-JUN and c-FOS [94]. Subsequent studies have also shown that EGCG transcriptionally activates the phase II enzyme gene expression in HepG2 cells, as determined by the ARE reporter-gene assay [95]. In this experiment, EGCG strongly activates all three MAPKs (ERK, JNK and p38) and induces caspase-3-mediated cell death.

Green tea/EGCG are prepared with minimal oxidation of polyphenols, and have been shown in animal studies and human epidemiological studies to prevent cancer, including lung cancer [96]. In animal models, it has been shown that green tea and one of its components, EGCG inhibits mouse lung tumorigenesis induced by NNK, *N*-nitrosodiethylamine, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine and cisplatin [97]. However, Yan et al. recently reported that polyphenon E, containing 65% EGCG, 25% other catechins, and ~0.5% caffeine decreased lung tumor load by approximately 59%, but EGCG, both at the same dose and at a higher dose, failed to inhibit lung carcinogenesis in A/J mice treated with BaP [98]. They explained that metabolites of EGCG or other catechins, which would not be available to the lung following aerosol administration, may be involved. Alternatively, it is possible that EGCG could still be the active compound, but for its anti-tumor activity, it may require

another component that is present in PolyE formulation and, as such, none of the other components in the PolyE may be biologically active without EGCG or vice versa.

In a randomized control trial [99], 133 smokers who smoked at least 10 cigarettes per day during the past year were randomized into three groups consuming at least four cups daily of either (i) decaffeinated green tea, (ii) decaffeinated black tea, or (iii) water. After 4 months of intervention, smokers who drank decaffeinated green tea had a significant decrease in their urinary 8-hydroxydeoxyguanosine (8-OHdG) level, but no significant changes were observed in both water and black tea groups. Only one clinical trial used green tea for treatment of lung cancer [100]. In this phase I study, 17 advanced lung cancer patients were given green tea extract at a single dose of 3 g/m² per day for 4 weeks. The dosage equates to 20 cups of green tea, which has been found to be the maximum tolerated dose. Unfortunately, no effect of the treatment in advanced lung cancer was observed. Therefore, green tea is unlikely to be an effective therapeutic cytotoxic agent against existing tumors.

5. Conclusions and perspectives

Currently, there is a general paucity of clinical trials testing plant-derived or synthetic chemoprevention agents for lung cancer. The published prospective, randomized, controlled trials in human lung cancer chemoprevention have so far produced either neutral or harmful primary endpoint results, whether in the primary, secondary, or tertiary prevention settings. These clinical trials lag, in number, the availability of candidate agents, and complex mixtures, which have shown activity in animals, but have been sparsely piloted in humans.

Discovery of new candidate chemopreventive agents remains a bottleneck to eventual clinical use, as well. Monitoring the induced expression of one or more phase II genes may represent a rational method for screening libraries of naturally occurring and synthetic chemical agents to identify new chemopreventive molecules. With the development of rapid molecular assays, efficient approaches such as gene expression-based high-throughput small molecule screening, in which a gene expression signature is used as a surrogate for cellular carcinogenesis and redox states, could be employed for identification of compounds that induce the phase II enzymes [101,102]. The commercial availability of large, chemically defined libraries of naturally occurring products as well as panels of chemicals generated through combinatorial chemistry now provide a significant resource for finding more potent and less toxic chemopreventive agents.

Additionally, there is evidence that suggests that inherited genetic characteristics might determine chemopreventive success [18]. To realize the full chemopreventive potential of phase II inducers for lung cancer, it will be important to identify individuals who are likely to benefit from the agents, as well as those who are more likely to experience toxicity. The promise of pharmacogenomics lies in maximizing the efficacy of a dietary intervention while minimizing the toxicity. The pharmacological-pathway approach entailing the use of knowledge regarding agent absorption, excretion, activation and other metabolic functions are specifically of importance for the regulation of chemopreventive activity. Understanding the interactions of inherited genetic characteristics within a biological or pharmacological pathway will allow for an important ability to predict chemoprevention response. Therefore, an approach that combines information from host pharmacogenetics/pharmacogenomics, and agent pharmacokinetics and pharmacodynamics in the lung, may represent a more effective strategy tailored to the needs of the individual at risk for lung cancer.

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Abbreviations

PAHs	polycyclic aromatic hydrocarbons
GSTs	glutathione <i>S</i> -transferases
UGTs	uridine diphosphate-glucuronosyl transferases
NQO1	NAD(P)H:quinine reductase
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
BaP	benzo(a)pyrene
AhR	aryl hydrocarbon receptor
AHH	arylhydrocarbon hydroxylase
CYP	cytochrome P450
ITC	isothiocyanate
SFN	sulforaphane
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
HSP90	heat shock protein 90
Arnt	aryl hydrocarbon receptor nuclear translocator
XREs	xenobiotic-responsive elements
ARE	antioxidant response element
Nrf2	nuclear factor erythroid 2-related factor 2
ER	endoplasmic reticulum
Keap1	Kelch-like ECH-associated protein 1
MAPKs	mitogen-activated protein kinases
JNK	c-Jun N-terminal kinase
ERK	extracellular signal-regulated kinase
PKC	protein kinase C
PI3K	phosphatidylinositol 3-kinase
EBP-β	enhancer binding protein-β
PEITC	phenethyl isothiocyanate
BITC	benzyl isothiocyanate
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
QR	quinone reductase
TGFβ1	transforming growth factor beta 1
EGF	epidermal growth factor
D3T	3 <i>H</i> -1,2-dithiolethione-3-thione
ADT	anethole dithiolethione
EGCG	epigallocatechin gallate
8-OHdG	8-hydroxydeoxyguanosine

OR	odds ratio
CI	confidence interval

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Fig. 1.
Mechanism of chemical carcinogenesis and carcinogen detoxification in lung carcinogenesis.

Fig. 2.

The two mechanisms of phase II xenobiotic-metabolizing enzyme induction by phase II inducers. In the AhR–XRE signaling pathway, chemopreventive inducers bind to the AhR and facilitate the dissociation of HSP90 from this complex and the translocation of the receptor and ligand into the nucleus. Once translocated, AhR complexes with the transcription factor Arnt, binds to XRE and enhances transcription of both phase I and phase II genes at the same time it induces Nrf2 expression. In the Nrf2–ARE signaling pathway, chemopreventive inducers are thought to modify the cysteine-rich sensor protein Keap1, in such a way that it releases the transcription factor Nrf2, which is then free to translocate into the nucleus. Additionally, Nrf1 is translocated from the endoplasmic reticulum (ER) to the nucleus in response to ER stress, through the functional contribution of Nrf1 to the ER stress response has not been well described. Finally, Nrf1 and/or Nrf2 heterodimerize with Maf and other transcription factors and bind as a complex to the ARE, leading to enhanced expression of phase II genes.

Table 1

Common phase II enzyme inducers, their major sources and effects on lung cancer in animal model.

Phase II inducers	Major source(s)	Molecular target (phase II genes)	Effects on lung cancer (in animal model)	Reference
Sulforaphane	Broccoli	$\uparrow GSTA1/2/3$, $\uparrow GSTM1/2$, $\uparrow GSTP1$, $\uparrow NQO1$, $\uparrow UGT1A1$	Inhibit lung tumor induced by NNK and BaP	[52–56,68]
Phenethyl isothiocyanate	Chinese cabbage, watercress	$\uparrow GSTA1$, $\uparrow GSTM1/3$, $\uparrow GSTT1$, $\uparrow NQO1$	Inhibit lung tumor induced by NNK and BaP	[57,58,68–72]
Benzyl isothiocyanate	Garden cress, radish	$\uparrow GSTP1$	Inhibit lung tumor induced by PAH and BaP	[59,60,70–72]
3H-1,2-dithiolethione-3-thione	Brussels sprouts and cabbage	$\uparrow GSTs$, $\uparrow NQO1$, $\uparrow UGTs$	No investigation	[78,80,81]
Oltipraz	Synthesised	$\uparrow GSTA$, $\uparrow GSTM$, $\uparrow GSTP$ $\uparrow UGTs$, $\uparrow NQO1$	Inhibit lung tumor induced by BaP	[78,79,82]
Anethole dithiolethione	Synthesised	$\uparrow GSTs$, $\uparrow UGTs$, $\uparrow NQO1$	No investigation	[82]
Epigallocatechin gallate	Green tea	$\uparrow GSTs$, $\uparrow NQO1$, $\uparrow UGTs$	Inhibit lung tumor induced by NNK and cisplatin, but not BaP	[89–94]