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Mini-review

# Dietary agents for prevention and treatment of lung cancer

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ABSTRACT

Lung cancer is a prominent cause of cancer-associated mortality worldwide. The main reason for high mortality due to lung cancer is attributable to the fact that the diagnosis is generally made when it has spread beyond a curable stage and cannot be treated surgically or with radiation therapy. Therefore, new approaches like dietary modifications could be extremely useful in reducing lung cancer incidences. Several fruits and vegetables offer a variety of bioactive compounds to afford protection against several diseases, including lung cancer. A number of research studies involving dietary agents provide strong evidence for their role in the prevention and treatment of lung cancer, and have identified their molecular mechanisms of action and potential targets. In this review article, we summarize data from *in-vitro* and *in-vivo* studies and where available, in clinical trials, on the effects of some of the most promising dietary agents against lung cancer.

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## Introduction

Lung cancer is the leading cause of cancer-related deaths in the United States and is the cause for more mortality than prostate, colon, and breast cancers combined [1]. The American Cancer Society has estimated diagnosis of 224,210 new cases and 159,260 deaths due to lung cancer in the United States in 2014 [2]. It is usually detected at an advanced stage which is not curable as it cannot be treated surgically or with radiation therapy. Tobacco smoking is the main cause for lung cancer and is responsible for approximately 90% of all lung cancer cases [3]. The occurrence of lung cancer drops very slowly after the termination of smoking, suggesting that ex-smokers are also at significant high risk for developing lung cancer [4–7]. Studies have reported that there is an increased risk for lung cancer if there is a family history, which suggests that genetic factors other than tobacco smoke also have a role in the individual's vulnerability to lung cancer [8]. Therefore, in addition to cessation of smoking, dietary modification may be considered as an attractive approach for the prevention and control of the lung cancer. In this review article, we discuss the use of selected dietary agents like green tea polyphenols, isothiocyanates, indole-3-carbinol, genistein, curcumin, pomegranate polyphenols and fisetin as chemopreventive and/or chemotherapeutic agents for the prevention and treatment of lung cancer. The reported *in-vitro* and *in-vivo* effects of these dietary agents against lung cancer are summarized in Tables 1 and 2, respectively and their molecular targets are shown in Fig. 1.

## Green tea polyphenols and lung cancer

### In-vitro *studies*

Tea, derived from the plant *Camellia sinensis*, is the most common beverage consumed globally. Significant data from various studies provide evidence that tea consumption has a preventive effect on carcinogenesis [9–13]. Much of the effects of green tea are related to the various activities of (−)-epigallocatechin gallate (EGCG), a major component of green tea. Several mechanisms including modulation of carcinogen-metabolizing enzymes, induction of apoptosis and cell-cycle arrest, modulation of cell-signaling pathways and suppression of the activation of transcription factors, resulting in the inhibition of cancer development or progression have been suggested for the preventive/therapeutic actions of green tea and its constituents, especially EGCG. Recently, it has been reported that treatment with EGCG (1–40 μM) inhibited anchorage-independent growth of human lung cancer cells by upregulating p53 expression, increase in the phosphorylation of p53 at $Ser^{15}$ and $Ser^{20}$ and enhancement of its transcriptional activity, and inhibition of MDM2-mediated p53 ubiquitination. This study identified p53 as a possible target of EGCG to accomplish its cancer-preventive activity. [14]. Wang et al. reported that EGCG (5–50 μM) regulated cellular activity by targeting HIFα protein and miR-210. It also determined the tumor suppressor activity of miR-210 and suggested an anticancer role of HIF-1α protein. As the authors of the study have pointed out, the effective concentrations of EGCG (25–50 μM) used in this study were higher than those that can be observed in blood and tissues of animals after treatment with EGCG or green tea preparations, probably because of the short exposure time *in-vitro* which does not correspond to the *in-vivo* conditions [15,16].

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**Table 1**
*In-vitro* effects of dietary agents in human lung cancer cells.

| *In-vitro* effects of dietary agents | References |
|---|---|
| Green tea polyphenols | |
| Upregulation of p53 expression, increase in the phosphorylation of p53 and enhancement of its transcriptional activity | [14] |
| Upregulation of the expression of miR-210 | [15] |
| Increased expression of E-cadherin, decreased expression of vimentin, inhibited TGF-β-induced migration and invasion of NSCLC cells and EMT | [17] |
| Reduced telomerase activity, decrease in caspases-3, -9, DNA fragmentation and S-phase cell-cycle arrest | [18] |
| Isothiocyanates | |
| Induction of apoptosis, activation of caspase-3, G2/M phase cell cycle arrest, generation of ROS, decrease in glutathione, Akt, NF-κB, MAPK and AP-1 by BITC | [29] |
| PEITC caused induction of disassembly of actin stress fibers and degradation of tubulin and G2/M phase cell-cycle arrest | [30] |
| BITC, PITC and sulforaphane reduced cell-proliferation, caused induction of apoptosis, mitotic arrest, disrupted microtubule polymerization | [33] |
| Treatment with TRAIL in combination with sulforaphane caused induction of chromatin condensation, DNA fragmentation, annexin V staining, sub-G(1) phase DNA content, caspase-3 activity, PARP cleavage, activation of p38 and downregulation of ERK1/2 and Akt | [34] |
| Indole-3-carbinol | |
| Inhibited production of NO, IL-6 and IL-1β | [41] |
| Decrease in cell-proliferation, induction of cell-cycle arrest at G0/G1 phase, increase in cyclin D1, phosphorylated p53, p21, cleavage of caspase-9, -8, -3 and PARP | [42] |
| Genistein | |
| Inhibition of cell-proliferation, migration, induction of apoptosis, G2/M phase cell cycle arrest, heightened effects of cisplatin, decrease in Cdc25B, cyclin B1, survivin | [87] |
| Combination with erlotinib and gefitinib caused a decrease in EGFR, p-Akt, COX-2, PGE2 and NF-κB | [51] |
| Combination with cisplatin, docetaxel or doxorubicin caused inhibition of cell-growth, induction of apoptosis. Treatment with genistein decreased the NF-κB activity | [53] |
| Curcumin | |
| Inhibition of cell-proliferation and invasion induction of G0/G1 phase cell cycle arrest, inhibition of the Wnt/β-catenin pathway | [59] |
| Increase in the cytotoxicity of erlotinib to erlotinib-resistant NSCLC cells, induction of apoptosis, decreased EGFR, p-EGFR, survivin and inhibited NF-κB. | [60] |
| Induction of autophagy, increase in the phosphorylation of AMPKα and acetylCoA carboxylase. | [61] |
| Neutralized formaldehyde-induced oxidative stress, improvement of DNA–protein crosslinks and inhibited activation of NF-κB and AP-1 | [88] |
| Inhibition of migration, invasion, angiogenesis through suppression of STAT-3, survivin, Bcl-xl, cyclin B1, VEGF, MMP-2, -7 and ICAM-1. | [62] |
| Inhibition of cell migration, invasion and downregulation of Cdc42 gene, and its related target gene expression, induction of the rearrangements of the actin cytoskeleton | [63] |
| Induction of apoptosis by an increase in Bax, decrease in Bcl-2, Bcl-xL, mitochondrial membrane potential, release of cytochrome *c* into the cytosol, activation of caspase-9, -3 and an increase in intracellular ROS | [64] |
| Pomegranate polyphenols | |
| Punicalagin and ellagitannins caused inhibition of the DNA adducts, antagonized the effect of sodium azide, methyl methanesulfonate, B(a)P, and 2-aminoflourine and had antiproliferative effects | [73] |
| Peel extract inhibited luminol-amplified chemiluminescence of resting neutrophils and PMA-stimulated neutrophils and inhibition of myeloperoxidase activity | [74] |
| Fruit extract caused reduction in cell-viability, G0–G1 phase arrest, decrease in cyclins D1, D2, E, cdk-2, -4, -6, increase in cell-cycle regulatory molecules, inhibition of MAPK, PI3K, phosphorylation of Akt, NF-κB, Ki-67 and PCNA | [75] |
| Fisetin | |
| Inhibition of cell-growth, colony formation, decrease in the protein expression of PI3K, inhibition of phosphorylation of Akt, mTOR, p70S6K1, eIF-4E, 4E-BP1, constituents of mTOR signaling complex, phosphorylation of TSC2, phosphorylation of mTOR and its target proteins and increase in the phosphorylation of AMPKα | [78] |
| Inhibition of hypoxia-induced VEGF expression and decreased hypoxia-induced STAT-3 tyrosine phosphorylation | [79] |
| Inhibition of adhesion, migration, and invasion through downregulation of ERK1/2, MMP-2, uPA NF-κB, c-Fos, c-Jun, NF-κB binding and AP-1 | [80] |

Liu et al. highlighted epithelial–mesenchymal transition (EMT)-related proteins as a therapeutic target and suggested that treatment of non-small cell lung cancer (NSCLC) cells with EGCG (5–20 μM) inhibited transforming growth factor (TGF)-β-induced EMT through down-regulation of phosphorylated Smad2 and ERK1/2 [17]. The effects of green tea polyphenols on small cell lung carcinoma (SCLC) cells, predominantly on drug-resistant tumor cells, were investigated. It was shown that EGCG had similar cytotoxicity in both drug-sensitive and drug-resistant SCLC cells specifying that it is not part of the drug resistance phenotype that occurs in SCLC. In both cell lines, incubation with EGCG caused 50–60% reduced telomerase activity and decrease in the activities of caspases-3 and -9, but not caspase-8. Treatment of SCLC cells also led to DNA fragmentation in cells and S-phase cell-cycle arrest [18]. Treatment with EGCG (2.5–40 μmol/l) caused inhibition of cell-proliferation in erlotinib-sensitive and resistant cell lines, including those with c-Met overexpression and acquired resistance to erlotinib. This showed that EGCG therapy could be given to patients who have developed resistance to erlotinib. Combination of EGCG and erlotinib treatment also inhibited the growth of H460 xenografts. EGFR tyrosine kinase inhibitors have not shown promise in clinical studies using erlotinib in combination with chemotherapy for patients with NSCLC. [19]. Cotreatment with EGCG (100 μM) and celecoxib induced the expression of both GADD153 mRNA level and protein, while EGCG or celecoxib alone had no effect in human lung cancer PC-9 cells. The synergistic effects of EGCG and celecoxib were also observed in A549 and ChaGo K-1 human lung cancer cells. Cotreatment of EGCG and erlotinib also activated ERK1/2 and p38 mitogen-activated protein kinases (MAPK). The authors also pointed out that GADD153 could be a new molecular target for cancer prevention in combination with EGCG [20].

### In-vivo *studies*

Treatment with green tea and its constituents, especially, EGCG and GTP has shown preventive and therapeutic effects against lung cancer in animals. The growth of tumors in athymic nude mice implanted with H1299 cells was inhibited by dietary supplementation of EGCG (0.1, 0.3 and 0.5% in the diet). Treatment with EGCG caused an increase in the tumor cell apoptosis and oxidative DNA damage assessed by the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and phosphorylated histone 2A variant X. It was demonstrated for the first time that EGCG induced reactive oxygen species (ROS) formation and consequently caused DNA oxidative

**Table 2**
*In-vivo* effects of dietary agents against lung cancer.

| *In-vivo* effects of dietary agents | References |
|---|---|
| Green tea polyphenols | |
| Inhibition of the surrogate markers of proliferation and apoptosis in murine lung cancer xenografts, decrease in the expression of Bcl-xl, Ku70 and an increase in Bax | [89] |
| In athymic nude mice, decrease in the growth of tumors by EGCG in the diet. Increase in the tumor cells apoptosis and oxidative DNA damage | [16] |
| Inhibition of tumor growth, tumor weight and tumor burden in athymic nude mice | [90] |
| Theaflavins and EGCG reduced hyperplasia, dysplasia and carcinoma in situ, reduction in proliferating cells and increase in apoptotic cells against B(a)P-induced lung carcinogenesis | [21] |
| In urethane-induced lung tumorigenesis in A/J mice, reduction in the mean number of tumors, mean lung weights and less smaller lesions in mice | [91] |
| Combination of polyphenon E and atorvastatin inhibited NNK-induced lung tumorigenesis, decrease in tumor multiplicity, tumor burden, induction of apoptosis and decrease in Mcl-1 level in adenomas | [23] |
| In A/J mice, polyphenon E and caffeine decreased the number of visible lung tumors, incidence and multiplicity of lung adenocarcinoma, inhibition of cell-proliferation, induction of apoptosis, decrease in the levels of c-Jun and p-ERK1/2 in tumor tissues | [25] |
| [37] | |
| Green tea solution caused decrease in lung tumor multiplicity, inhibition of angiogenesis and induction of apoptosis in NNK-induced lung adenomas in A/J mice | [26] |
| Isothiocyanates | |
| Decrease in $H_2O_2$, increase in release of cytochrome *c* from mitochondria, decrease in Bcl-2 and increase in Bax and caspases-3 in Swiss albino mice by sulforaphane | [35] |
| Less pulmonary carcinogenicity from cigarette smoke in neonatal mice exposed to cigarette smoke by budesonide, PEITC and NAC | [36] |
| Decrease in the incidence of adenocarcinoma by PEITC in NNK-treated A/J mice. Treatment with sulforaphane–NAC in the diet caused lower lung tumor incidences and sulforaphane and PEITC caused a decrease in malignant lung tumor multiplicity. Reduction in PCNA and induction of apoptosis | [37] |
| In A/J mice, reduction in lung tumor multiplicity induced by cigarette-smoke carcinogens by BITC and more effective than BHA and sulforaphane in the inhibition of lung tumors | [39] |
| Indole-3-carbinol | |
| Reduction in the multiplicity all tumor size classes at different efficacy levels, reduction in hyperplastic foci, adenoma, adenoma with dysplasia, and adenocarcinoma. Increase in the multiplicities of smaller tumors, decrease in the bigger tumors when I3C was given during tumor progression. Increase in the multiplicities of early stage histological lesions, decrease in adenoma with dysplasia and adenocarcinoma. | [43] |
| Inhibition of pulmonary adenocarcinoma when given during the progression phase. | |
| Modulation of PI3K/Akt signaling pathway | |
| Decrease in the multiplicities of tumors on the surface of the lung and adenocarcinoma by I3C and silibinin. Modification of p-Akt, p-ERK, cyclin D1 and PARP cleavage by I3C plus silibinin in NNK-treated mice | [44] |
| In A/J mice, the levels of miR-21, mir-31, miR-130a, miR-146b and miR-377 were decreased in mice treated with I3C in the diet | [45] |
| I3C caused a decrease in tumor multiplicities, cancer incidence, and inhibition NF-κB activation, COX-2, p-Akt, caspase-3 and PARP cleavage. | [46] |
| Myoinositol decreased in the multiplicities of pulmonary surface tumors, adenoma with cellular pleomorphism, lung adenoma and inhibition of p-Akt and FAS expression | |
| I3C reduced the expression of Ki-67, PCNA, p-Akt, p-BAD and induction of apoptosis in the lung tissues | [47] |
| Genistein | |
| Combination of gefitinib and genistein caused a significant decrease in tumor growth in the xenograft model | [55] |
| In irradiated Sprague-Dawley rats, genistein during the phase of pneumonitis caused inhibition of the increase in breathing rate after irradiation and a delay of 50–80-days. There was also a decrease in the levels of TNF-α, IL-1β, TGF-β, collagen content, levels of 8-OHdG, and protection against DNA damage | [56] |
| Inhibition of lung tumor nodule formation, lung collagen hydroxyproline content, serum sialic acid level, and an increase in the life span of C57BL/6 mice on treatment with genistein and diadzein. | [57] |
| Curcumin | |
| Decreased expression of COX-2 in subcutaneous tumors with decrease in weight of intralung tumors and increase in survival rate. Also decrease in tumor growth of orthotopic human NSCLC xenografts and increased survival of athymic nude mice | [66] |
| Liposomal curcumin diminished radiation pneumonitis, reduced fibrosis of lung and sensitized LL/2 cells to irradiation | [68] |
| Decreased tumor growth and invasion, by inhibition of Cdc42 in BALB/c mice | [63] |
| Inhibition of the primary tumor growth of Lewis lung carcinoma in C57BL/6 mice. | [69] |
| Upregulation of α1-antitrypsin and decrease in neutrophil elastase in tumor tissues | |
| Decrease in tumor growth and increase in survival contributed to T cell-mediated adaptive immune response | [70] |
| Reduction in the visible lung tumors in the absence and presence of non-typeable Hemophilus influenzae exposure which induces chronic obstructive pulmonary disease (COPD)-like airway inflammation in an airway conditional K-ras-induced mouse model by dietary curcumin. | [72] |
| Pomegranate polyphenols | |
| In athymic nude mice, significant inhibition of tumor growth and increased survival | [75] |
| In A/J mice, decrease in lung tumor multiplicities tumor incidence in two lung tumor protocols. Inhibition NF-κB, MAPK, PI3K, phosphorylation of Akt, mTOR, c-met, markers of cell proliferation and angiogenesis | [76] |
| Fisetin | |
| Decrease in mitochondrial dysfunction induced by B(a)P | [81] |
| Reduction in histological lesions, lipid peroxidation, PCNA and modulation of the enzymatic and non-enzymatic anti-oxidants in B(a)P-treated Swiss Albino mice | [82] |
| Inhibition of angiogenesis, reduction in tumor growth. | [83] |
| Combination of fisetin and cyclophosphamide caused improvement in antitumor activity, with low systemic toxicity and decrease in microvessel density | |

damage in tumor cells in animals. EGCG is usually known as a strong antioxidant, whereas, in this study, it was shown that EGCG could also serve as a pro-oxidant in some conditions [16]. It has been demonstrated that theaflavin and EGCG could lower the proliferative index at different stages of experimental lung carcinogenesis in a benzo(a)pyrene [B(a)P]-induced lung carcinogenesis mouse model. Treatment with theaflavins (0.02 mg/mouse/day) and EGCG (0.01 mg/mouse/day) reduced hyperplasia, dysplasia and carcinoma in situ which were apparent on 8th, 17th and 26th weeks respectively [21]. In Swiss albino mice, treatment with GTP and black tea polyphenols (BTP) at doses of 0.1 and 0.2% led to low incidence of diethylnitrosoamine-induced alveologenic tumors in lungs of animals and caused inhibition of the expression of Akt, cyclooxygenase (COX)-2 and nuclear factor kappa-B (NF-κB) [22]. Polyphenon E

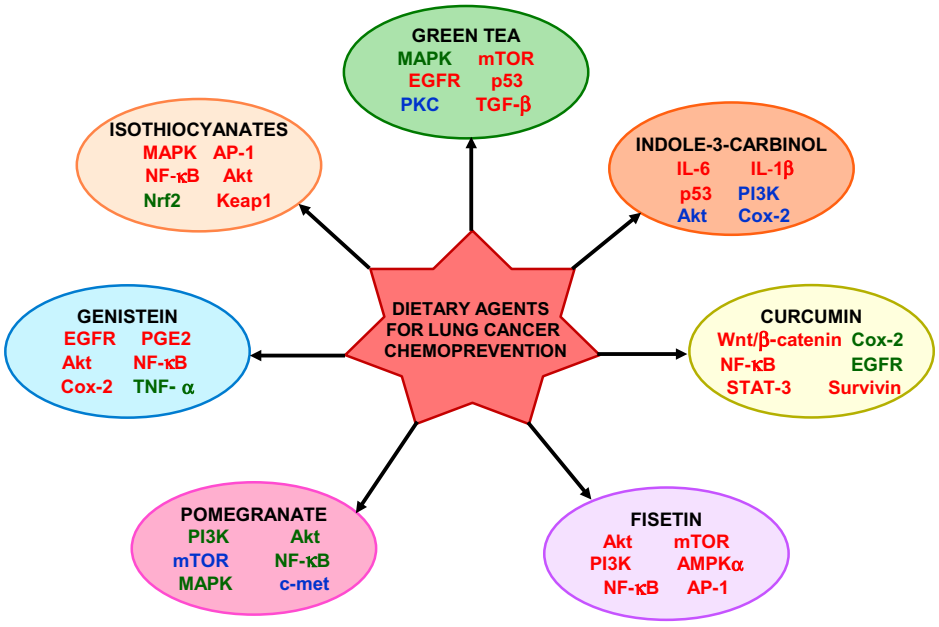


**Fig. 1.** Molecular targets of selected dietary agents against lung cancer chemoprevention. Red color denotes the *in-vitro* molecular targets of dietary agents against lung cancer; blue color denotes the *in-vivo* molecular targets of dietary agents against lung cancer and green color denotes both the *in-vitro* and *in-vivo* molecular targets of dietary agents against lung cancer. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(0.25% or 0.5% in drinking fluid) and atorvastatin combination inhibited 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis in mice. The low-dose combination of polyphenon E and atorvastatin significantly reduced both lung tumor multiplicity and tumor burden with enhancement of apoptosis and suppression of myeloid cell leukemia 1 (Mcl-1) level in adenomas. This study showed that the combination of polyphenon E and atorvastatin effectively inhibited lung tumorigenesis and the action between these two agents was synergistic in both *in-vitro* and *in-vivo* [23]. The effects of Polyphenon E (1% wt/wt in diet) and aerosolized difluoromethylornithine (DFMO) administration were investigated in A/J mice injected with B(a)P. Treatment with polyphenon E treatment did not inhibit average tumor multiplicity but reduced per animal tumor load with a significant decrease in largest carcinomas [24]. Supplementation of Polyphenon E (0.5% in drinking fluid) and caffeine significantly decreased the number of visible lung tumors, incidence and multiplicity of lung adenocarcinoma induced by NNK in A/J mice. This was accompanied by inhibition of cell-proliferation, induction of apoptosis and decrease in the levels of c-Jun and p-extracellular signal-regulated kinase (ERK) 1/2 in tumor tissues, which might play a role in the inhibition of the progression of lung adenoma to adenocarcinoma [25]. Liao et al. determined the effects of treatment with 0.1, 0.2, 0.4, and 0.6% green tea solution on NNK-induced lung tumorigenesis in female A/J mice. Treatment with only 0.6% green tea solution reduced lung tumor multiplicity in mice and caused inhibition of angiogenesis and induction of apoptosis in lung adenomas [26]. Limited doses of GTP treatment by oral intubation (5 mg in 0.2 ml water) 30 min before the administration of carcinogen afforded protection against forestomach and lung tumorigenesis [27].

## Isothiocyanates and lung cancer

### In-vitro *studies*

Isothiocyanates (ITC) are present in cruciferous vegetables as glucosinolates and are converted to ITC by the enzyme myrosinase. They are released by cutting, chewing or by intestinal microflora existing in living persons [28]. Benzyl isothiocyanate (BITC), phenethyl isothiocyanate (PEITC) and sulforaphane are widely studied for their chemopreventive role against cancer. It has been reported recently that BITC inhibited gefitinib-resistant human NSCLC cell growth by induction of apoptosis, activation of caspase-3, cell cycle arrest at G2/M phase, generation of reactive oxygen species (ROS), depletion of glutathione, suppression of Akt activity and NF-κB transcriptional activation and activation of MAPK and activator protein (AP)-1. This showed that BITC diminished gefitinib resistance in lung cancer cells [29]. The anticancer activity of ITC is reported due to reduction in the activation of carcinogens and increase in their detoxification. PEITC decreases phase I enzymes involved in the activation of several carcinogens. They also activate phase II enzymes responsible for the metabolism of many reactive carcinogens and the diminution of oxidative stress. Isothiocyanates also demonstrate anticarcinogenic actions by induction of apoptosis and inhibition of the phases of cell-cycle [30]. It was found that PEITC (3, 6 and 9 μM) exhibited lower cytotoxicity to A549 cells containing wild-type p53 and caused induction of disassembly of actin stress fibers and degradation of tubulin. Treatment of cells with PEITC also caused G2/M phase cell-cycle arrest but the fraction of G2/M cells decreased in a dose and time-dependent manner in favor of cells with sub-G1 DNA content. It was suggested that the decrease in cell-viability of A549 cells was due to the apoptosis-inducing activity of PEITC, but in the case of large cell lung carcinoma H1299 cell line at doses of 1.5, 3 and 4.5 μM, there was a greater proportion of cells displaying morphological features of mitotic catastrophe [30]. Treatment with BITC and PEITC at doses of 10–40 μM induced oxidative stress and inhibited the metastasis of lung cancer cells by modulation of metastasis-related gene expression and inhibition of the Akt/NF-κB pathway [31]. BITC (7.5 and 10 μM) and PEITC (12.5 and 20 μM) caused induction of apoptosis, activation of caspases-3, and G2/M phase cell-cycle arrest in L9981 NSCLC cells. There was also activation of c-Jun N-terminal kinase, ERK1/2 and p38, and downregulation of AP-1 transcriptional activation and cyclin D1 expression [32]. Treatment of A549 cells with BITC (10 μM), PITC (10 μM) and sulforaphane (10 and 30 μM) reduced cell-proliferation and caused induction of

apoptosis and mitotic arrest. Treatment with ITC (5–40 μM) also disrupted microtubule polymerization *in-vitro* and *in-vivo*. It was also shown that cysteine in tubulin was covalently modified by isothiocyanates supporting the fact that tubulin is a target of isothiocyanates. These data establish that tubulin is a target of ITC and the interaction of ITC and tubulin causes downstream growth inhibition. This is an important study as it describes the chemical basis of ITC-induced cell growth inhibition and identifies structural information which may lead to the development of more specific ITC-related compounds for chemoprevention/chemotherapy of cancer [33]. Treatment with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in combination with sulforaphane (5 and 10 μM) sensitized TRAIL-resistant A549 cells to TRAIL-mediated apoptosis. Caspase-3 through downregulation of ERK1/2 and Akt emerged as a key regulator of apoptosis in response to combined sulforaphane and TRAIL in human lung adenocarcinoma cells. The authors advocated the use of TRAIL in combination with subtoxic doses of sulforaphane for the treatment of resistant NSCLC [34].

In-vivo *studies*

Several mechanisms have been postulated to determine the *in-vivo* mechanisms of ITC against lung cancer. Importantly, Mi et al. identified tubulin as one of these *in vivo* targets for ITC binding and demonstrated covalent binding of BITC, PEITC, and sulforaphane to tubulin. This binding was associated with their ability to cause induction of apoptosis and mitotic arrest [33]. The role of oral administration of sulforaphane (9 μmol/mouse/day) in alleviating the oxidative damage caused by B(a)P (100 mg/kg body weight, i.p.) in Swiss albino mice was determined. There was a decrease in the production of hydrogen peroxide, increase in the release of cytochrome *c* from mitochondria, decrease in Bcl2 and increase in Bax expression and caspases-3 [35]. Neonatal mice were exposed to cigarette smoke for 120 consecutive days, starting at birth. Budesonide (2.4 mg/kg in diet), PEITC (1000 mg/kg in diet) and NAC (1000 mg/kg body weight in drinking water) were administered orally until 210 days. There was a high incidence of multiplicity of benign lung tumors and an increase in malignant lung tumors on exposure to cigarette smoke while budesonide, PEITC and NAC treated mice had less pulmonary carcinogenicity from cigarette smoke. Budesonide, PEITC and NAC treatment decreased the yield of both benign and malignant mainstream cigarette smoke-induced lung tumors, in an experimental situation mirroring an intervention in current smokers [36]. Conaway et al. investigated the effect of PEITC, sulforaphane and their *NAC* conjugates during progression of lung adenomas to adenocarcinomas in A/J mice. There was a decrease in the incidence of adenocarcinoma in the PEITC (3 and 1.5 mmol/kg diet) treated group and PEITC-NAC (8 and 4 mmol/kg diet) group as compared with the NNK-treated control group. Treatment with sulforaphane–NAC (8 and 4 mmol/kg diet) in the diet also caused lower lung tumor incidences. This study reported that PEITC, sulforaphane and their NAC conjugates inhibited the progression of lung adenomas to adenocarcinomas by a decrease in cell proliferation and induction of apoptosis in the tobacco carcinogen-treated A/J mice [37]. The effects of BITC (1 μmol/g diet), PEITC (3 μmol/g diet) and a mixture of BITC + PEITC (1 and 3 μmol/g diet) on DNA and hemoglobin (Hb) adducts of B(a)P and NNK, and on two urinary metabolites of NNK, were investigated. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide NNAL-Gluc were measured in urine. At 8 and 16 weeks, PEITC or BITC + PEITC caused a significant reduction in the levels of 4-hydroxy-1-(3-pyridyl)-1-butanone releasing DNA adducts of NNK in the lung, but BITC had no effect. Treatment with PEITC or BITC + PEITC also inhibited the formation of Hb adducts of NNK from 2 to 12 weeks whereas there were no effects on Hb adducts of B(a)P. There was also a significant increase in the levels of NNAL and NNAL-Gluc in the urine of the rats after treatment with PEITC or BITC + PEITC. These studies showed that PEITC or BITC + PEITC inhibited the formation of HPB releasing DNA adducts in the lungs of rats treated with B(*a*)P + NNK. However, there was no effect of BITC on adducts derived from B(*a*)P or NNK [38]. Treatment with BITC (6.7 and 13.4 μmol), prior to each of three carcinogenic polycyclic aromatic hydrocarbons (PAH) found in cigarette smoke (B(a)P, 5-methylchrysene (5-MeC) and dibenz[a,h]anthracene [DBahA]), inhibited lung tumor multiplicity, more effectively than BHA and sulforaphane. This is the first study to demonstrate the chemopreventive efficacy of BITC against cigarette smoke PAH other than B(a)P [39].

## Indole-3-carbinol and lung cancer

In-vitro *studies*

Indol-3-carbinol (I3C) is an autolysis product of glucosinolate present in *Brassica* plants like cabbage, cauliflower kale, broccoli, Brussels sprouts and has reported anticancer effects [40]. However, the anticancer effects of I3C on human lung cancer cells have not been well reported. Treatment with I3C (25–100 μM) diminished the production of pro-inflammatory intermediaries like nitric oxide (NO), interleukin (IL)-6, and IL-1β in lipopolysaccharide (LPS)-induced Raw264.7 cells and THP-1 cells through suppression of the TRIF-dependent signaling pathway. Thus, it was shown that I3C had a protective role in the treatment of inflammatory diseases including acute lung injury caused by microbial infection [41]. Treatment of lung cancer cells with I3C (400 μM) caused a reduction in proliferation of cells, increased formations of fragmented DNA and apoptotic bodies, and induction of cell-cycle arrest at the G0/G1 phase. It also increased the protein levels of cyclin D1, phosphorylated p53, p21 and expression of Fas mRNA, cleavage of caspase-9, -8, -3 and PARP. Thus, treatment of lung cancer cells with I3C caused induction of both cell-cycle arrest at the G0/G1 phase and apoptosis via activation of Fas and caspase-8 pathways [42].

In-vivo *studies*

The efficacy of I3C to inhibit tobacco carcinogen-induced lung adenocarcinoma in A/J mice was assessed when given following post-initiation or progression protocol. There was a reduction in the tumor multiplicity, hyperplastic foci, adenoma, adenoma with dysplasia, and adenocarcinoma after treatment with I3C during the post-initiation period. An increase in the multiplicities of smaller tumors and decrease in the bigger tumors were observed when I3C was given during tumor progression. I3C was found to efficiently inhibit the development of pulmonary adenocarcinoma when given during the progression phase. It was also shown that cancer preventive effects of I3C were mediated via modulation of the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway [43]. In NNK-treated mice, treatment with I3C (10 μmol/g/diet) and silibinin (7 μmol/g/diet) caused reduction in the multiplicities of tumors on the surface of the lung and decrease in adenocarcinoma. The expressions of p-Akt, p-ERK, cyclin D1 and poly (ADP-ribose) polymerase (PARP) cleavage were strongly modified by I3C plus silibinin as compared with I3C or silibinin alone. Thus, findings from this study proved that the combinatorial treatment of I3C and silibinin afforded more protection against the development of lung cancer in A/J mice and could be utilized for the prevention of cancer in current and former smokers [44]. The effect of I3C (100 or 150 μM) on vinyl carbamate (VC)-induced deregulation of microRNA (miRNA) levels in lung tissues of female A/J mice was investigated. The levels of miR-21, mir-31, miR-130a, miR-146b and miR-377 were decreased in mice treated with VC and I3C in the diet as compared to mice treated with VC only. There is a link between abnormal

miRNA expression and the development of lung cancer. These results determined distinctive changes in the expression of several *miRNAs* in lung tumors compared to normal lungs and it was shown that I3C had effects on most of the *miRNAs* levels [45]. The effects of I3C (30 or 70 μmol/g/diet) and myoinositol (MI; 56 μmol/g/diet) against VC-induced lung cancer were investigated. There was a decrease in the multiplicities of tumors on the surface of the lung, incidence of cancer, multiplicity, size and adenoma with cellular pleomorphism on treatment of mice with higher dose of I3C, whereas the lower dose showed less effects. Treatment with higher dose of I3C also caused inhibition of IκBα degradation, NF-κB activation, COX-2, p-Akt and activation of caspase-3 and PARP cleavage [46].

Dietary administration of I3C (1, 10, 30, 71 and 112 μmol/g/diet) to NNK plus B(a)P-treated mice beginning at 50% in the carcinogen treatment phase caused reduction in lung tumors. Treatment with I3C (112 μmol/g/diet) beginning 1 week after the last dose of the carcinogen led to reduction in the NNK plus B(a)P-induced lung tumor multiplicity. It was concluded that administration of I3C during the postcarcinogen treatment phase led to a significant reduction in lung tumor multiplicity and modified the expression of proteins that play a role in cell-proliferation and apoptosis [47]. Pretreatment with I3C (25 or 125 μmol/mouse/day by gavage for 4 days) inhibited lung tumor multiplicity and pulmonary $O^6$-methylguanine formation, but enhanced hepatic DNA methylation after NNK administration in A/J mice. I3C-pretreated mice also showed increased formation of α-hydroxylation products in hepatic microsomes, whereas pulmonary microsomes had no significant effect. This study confirmed the ability of I3C to inhibit NNK-induced lung neoplasia by a decrease in $O^6$-methylguanine formation in mice lung caused by treatment with I3C, which was probably due to the decreased bioavailability of NNK and NNAL in the lungs of I3C-treated mice, because of increased hepatic metabolism [48].

## Genistein and lung cancer

### In-vitro *studies*

Genistein (4,5,7-trihydroxyisoflavone), the most abundant isoflavone in soybean, has been widely reported for its chemopreventive and chemotherapeutic effects. Genistein (10 μM) has been reported to enhance trichostatin A (TSA)-induced apoptosis and exerts its effect, at least partly, by increasing caspase-3 activity in human lung carcinoma A549 cells but not in normal human lung fibroblasts [49]. A follow-up study proved that genistein (25 μM) enhanced the anticancer activity of TSA through tumor necrosis factor receptor (TNFR)-1 signaling and suggested that the combination of genistein and TSA could be explored for the therapy of lung cancer [50]. Treatment with genistein (25 μM) augmented the antitumor effects of epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib in H3255, H1650, and H1781 (wild-type EGFR) NSCLC cell lines. The combination treatment caused a reduction in the expression of EGFR, p-Akt, COX-2, prostaglandin (PG) E2 and downregulation of NF-κB. These results showed that genistein improved the antitumor effects of EGFR-TKIs in NSCLC cell lines with different EGFR gene mutation status and numerous sensitivities to EGFR-TKIs, in part due to down-regulation of the DNA-binding activity of NF-κB [51]. Treatment of human lung adenocarcinoma cell line SPC-A-1 with genistein (20–40 μM) caused cell-cycle arrest, inhibition of proliferation and induction of apoptosis in lung cancer cells, through regulation by many apoptotic-related genes [52].

Combination of genistein (15–30 μmol/l) with cisplatin, docetaxel or doxorubicin caused greater inhibition of cell-growth and induction of apoptosis as compared with either of them alone in lung, prostate, breast and pancreatic cancer cells. This showed that pretreatment with genistein inactivated NF-κB and, in turn, sensitized cancer cells to inhibition of growth and apoptosis induced by chemotherapeutic agents [53].

### In-vivo *studies*

It has been recently reported that a derivative of genistein, 7-difluoromethyl-5,4′-dimethoxygenistein, dose-dependently suppressed lung tumor growth *in-vivo* and apparently had no toxicity [54]. In a xenograft model, treatment of mice with a combination of gefitinib and genistein caused a significant decrease in tumor growth [55]. In rats receiving genistein (750 mg/kg body weight) during the phase of pneumonitis, there was inhibition of the increase in breathing rate after irradiation with 18 Gy at approximately 0.5 Gy/min and a delay of 50–80-days in Sprague-Dawley rats. At 28 weeks after irradiation, treatment with genistein also decreased the levels of TNF-α, IL-1β, TGF-β, decreased collagen content, levels of 8-OHdG, and protection against DNA damage measured in surviving rats. Treatment with genistein after irradiation also reduced DNA damage in the form of micronucleus formation and provided indication that DNA damage is caused by the production of ROS [56].

The effects of dietary soybean isoflavones, genistein and daidzein, were investigated for lung metastasis induced by B16F-10 melanoma cells in C57BL/6 mice. Treatment with genistein (200 μmol/kg body weight) caused a significant inhibition of lung tumor nodule formation, lung collagen hydroxyproline content and the serum sialic acid level, as compared to untreated tumor-bearing animals. Importantly, treatment with genistein increased the life span of the tumor-bearing animals [57].

## Curcumin and lung cancer

### In-vitro *studies*

Curcumin (diferuloylmethane), derived from the plant *Curcuma longa*, has been widely studied for its antioxidant, antiangiogenic, analgesic, anti-inflammatory, and antiseptic properties [58]. Treatment of NSCLC cells with curcumin inhibited the proliferation and invasion of cells, and induced G0/G1 phase cell-cycle arrest through inhibition of metastasis-associated protein 1 (MTA1)-mediated inactivation of Wnt/β-catenin pathway [59]. Treatment with curcumin caused a significant increase in the cytotoxicity of erlotinib to erlotinib-resistant NSCLC cells, enhanced erlotinib-induced apoptosis, decreased the expressions of EGFR, p-EGFR, survivin and inhibited NF-κB activation in erlotinib-resistant NSCLC cells. In erlotinib-resistant NSCLC cells, treatment with combination of curcumin and erlotinib displayed the same effects on apoptosis as the combination of curcumin and cisplatin [60]. Treatment with curcumin (50–100 μM) induced autophagy in human lung adenocarcinoma cells and increased the phosphorylation of AMP-activated protein kinase (AMPKα) and acetylCoA carboxylase. It was also shown that blocking of AMPK signaling with the pharmacological inhibitor compound C or genetic knockdown of AMPK*a*1 eliminated the increase of LC3-II/I and reduction of SQSTM1, the two protein markers of autophagy. Hence, this data suggest that curcumin induced autophagy through activation of the AMPK signaling pathway [61]. Yang et al. determined the role of IL-6/JAK/signal transducer and activation of transcription (STAT)-3 signaling in the progression of SCLC and provided evidence that this pathway was targeted by curcumin (15 μM) for treatment of SCLC [62]. Knocking down of Cdc42 expression by shRNA against Cdc42 suppressed lung cancer cell migration, invasion and induced rearrangements of the actin cytoskeleton. Treatment of a highly metastatic lung cancer cell line, 801D, with curcumin (5–20 μM) inhibited cell migration, invasion and downregulated the Cdc42 gene, which is

involved in survival, invasion and metastasis of human cancer cells and Cdc42-related target gene expression and induced rearrangements of the actin cytoskeleton. Hence, curcumin could be utilized for investigating the beneficial effects of Cdc42 targeted treatment for lung cancer [63]. Curcumin treatment caused apoptosis by upregulation of Bax expression, downregulation of Bcl-2 and Bcl-xl, decrease in mitochondrial membrane potential, release of cytochrome *c* into the cytosol, activation of caspase-9, -3 and an increase in intracellular ROS level in SCLC NCI-H446 cells. This suggests that the death receptor-mediated pathway was not associated with curcumin-induced apoptosis and the ROS-mediated mitochondrial pathway was involved in the process of curcumin-induced apoptosis of human SCLC cells [64]. The effect of curcumin on cell anoikis (an apoptosis triggered by loss of cell anchorage) as a possible mechanism of its anti-tumorigenic action and the role of Bcl-2 and Cav-1 was investigated. It was shown that there was an induction of anoikis resistance of lung carcinoma H460 cells by ectopic expression of either Bcl-2 or Cav-1. Treatment with curcumin decreased Bcl-2 protein during anoikis and sensitized the cells to detachment-induced apoptosis, while there was no effect on Cav-1 protein expression. [65].

In-vivo *studies*

Treatment with curcumin (0.6%) decreased the expression of COX-2 in subcutaneous tumors *in-vivo* and led to a decrease in weight of intralung tumors accompanied by an increase in survival rate. It also caused a significant decrease in the tumor growth of orthotopic human NSCLC xenografts and increased survival of athymic nude mice [66]. Treatment with combination of curcumin and erlotinib significantly inhibited tumor growth of erlotinib-resistant NSCLC cells *in-vivo*, suggesting that curcumin might be a prospective adjuvant for NSCLC patients during treatment with erlotinib [60]. Co-administration of phospho-sulindac (200 mg/kg/day) and curcumin (500 mg/kg/body/day) synergistically inhibited the growth of human lung cancer xenografts in nude mice, which was credited to the inhibition of efflux transporters by curcumin, leading to improved bioavailability of phospho-sulindac [67]. Shi et al. developed a water-soluble liposomal curcumin system to investigate its prevention and sensitizing effects in mice models. Liposomal curcumin inhibited the NF-κB pathway, TNF-α, IL-6, IL-8, and TGF-β induced by thoracic irradiation. The combination treatment of liposomal curcumin and radiotherapy caused an increase in intratumoral apoptosis and microvessel responses to irradiation *in-vivo* and greater inhibition of tumor growth was observed in a murine lung carcinoma (LL/2) model. It was concluded from these experiments that liposomal curcumin efficiently diminished radiation pneumonitis, reduced fibrosis of lung and sensitized LL/2 cells to irradiation [68]. In BALB/c mice, treatment with curcumin suppressed tumor growth and invasion, by inhibition of Cdc42 [63]. Treatment with curcumin (100 mg/kg/day and 300 mg/kg/day) inhibited the primary tumor growth of Lewis lung carcinoma (LLC) in C57BL/6 mice. In tumor tissues of mice treated with curcumin, there was upregulation of α1-antitrypsin and decrease in the protein level of neutrophil elastase which play important roles in modulating lung tumor proliferation in inflammatory microenvironment. This study highlighted a new inflammation-related mechanism of curcumin against tumor proliferation and advocates the possible role of curcumin in the treatment of other inflammation-related diseases in lungs such as emphysema [69]. Decrease in tumor growth and increase in survival contributed to T cell-mediated adaptive immune response which was found in tumor-bearing mice treated with low-dose curcumin (50 mg/kg body weight) for ten days. High-dose curcumin (100 mg/kg body weight) decreased T cells but a low-dose increased T cells derived from 3LL tumor-bearing mice, particularly CD8 + T cells which exhibited the enhancement of IFN-γ secretion, proliferation and cytotoxicity against 3LL tumor cells. These results point out that curcumin may result in the induction of effective T cell-mediated antitumor immune response and support the immune system in lung tumor-bearing models, thus advocating the possible use of curcumin as an immunologically safe drug for the treatment of cancer [70]. Treatment with curcumin (30 and 45 mg/kg body weight) caused a reduction in lung tumor incidence, size and weight compared with the control group in athymic nude mice injected with human lung large cell carcinoma NCI-H460 cells [71]. It was shown that 1% curcumin in the diet suppressed non-typeable Hemophilus influenzae NTHi-induced chronic obstructive pulmonary disease (COPD)-like airway inflammation and K-ras-initiated lung cancer in mice. These data provide further evidence about the effectiveness of curcumin, alone or in combination as a therapeutic agent, against lung cancer [72].

## Pomegranate polyphenols and lung cancer

In-vitro *studies*

Pomegranate (*Punica granatum*, Punicaceae) is an edible fruit widely cultivated in Afghanistan, India, China, Japan, Russia and the United States which is about 80% juice and 20% seed. Punicalagin (50–500 μM) and ellagitannins (50–500 μM), found in the fruit peel of *P. granatum*, showed a significant inhibition of the resultant DNA adducts on incubation of B(a)P with rat liver microsomes, appropriate cofactors, and DNA [73]. Treatment with pomegranate peel aqueous extract (200 mg/kg body weight) dose-dependently inhibited luminol-amplified chemiluminescence of resting neutrophils and phorbol myristate acetate-stimulated neutrophils and caused direct inhibition of myeloperoxidase activity *in-vitro*. [74]. We have earlier reported that treatment with pomegranate fruit extract (PFE) at doses of 50–150 μg/ml caused reduction in cell-viability of human lung cancer A549 cells but had insignificant effects on normal human bronchial epithelial cells. Treatment of human lung cancer cells with PFE also lead to arrest of cells in the G0–G1 phase of the cell cycle, decrease in the protein expressions of cyclins, cyclin-dependent kinases and increase in cell-cycle regulatory molecules. There was also inhibition of MAPK, PI3K, phosphorylation of Akt, NF-κB and markers of cell-proliferation on treatment of A549 cells with PFE. Based on the results of this study, it was concluded that PFE and its associated antioxidants have strong potential for development as a chemopreventive/chemotherapeutic agent against lung cancer [75].

In-vivo *studies*

In athymic nude mice implanted with human lung cancer A549 cells, we observed that oral administration of PFE caused significant inhibition of tumor growth and the latency period for the appearance of small solid tumors was prolonged in animals [75]. We have also investigated the effect of oral consumption of a human achievable dose of PFE (0.2%, w/v) on growth, progression, angiogenesis, and signaling pathways in two models of lung cancer induced by B(a)P and N-nitroso-tris-chloroethylurea (NTCU) in A/J mice [76]. A significant decrease in lung tumor multiplicities and decrease in tumor incidence were found in mice treated with PFE and B(a)P or NTCU. In lungs of B(a)P- and NTCU-treated mice, oral administration of PFE caused inhibition NF-κB, MAPK, PI3K, and phosphorylation of Akt, mTOR, c-met, markers of cell proliferation and angiogenesis. These results provide understanding of the protective effects of PFE against lung cancer by targeting multiple signaling pathways and associated events, which are critical for the development and progression of lung cancer [76].

## Fisetin and lung cancer

### In-vitro *studies*

Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a naturally occurring flavonoid and is found in strawberry, persimmon, grape, apple, cucumber and onion. It has antiproliferative, apoptotic and antiagiogenic properties in cancer cells [77]. We have recently shown that treatment of NSCLC cells with fisetin (5–20 μM) inhibited cell growth and colony formation with the simultaneous inhibition of PI3K/Akt and mTOR signaling [78]. There was a decrease in the protein expression of PI3K, and inhibition of phosphorylation of Akt and mTOR. This study provided information for the first time that fisetin at physiologically achievable concentrations exerts dual inhibition of PI3K/Akt and mTOR signaling in human NSCLC cells without affecting normal human bronchial epithelial cells [78]. Anso et al. studied the effect of a group of 20 flavonoids, including flavones, flavonols and isoflavones, on the production of VEGF induced by hypoxia in lung cancer squamous cell carcinoma NCI-H157 cell line. They reported that apigenin, luteolin, fisetin and quercetin caused inhibition of hypoxia-induced VEGF expression. Treatment of cells with fisetin, luteolin, galangin or quercetin induced HIF-1alpha expression and reduced expression of VEGF. Treatment with flavonoids also decreased hypoxia-induced STAT-3 tyrosine phosphorylation which correlated with their effectiveness as inhibitors of VEGF [79]. In human lung cancer A549 cells, treatment with fisetin (1, 5 and 10 μM) inhibited adhesion, migration, and invasion through downregulation of ERK1/2, MMP-2, urokinase-type plasminogen activator (uPA) at both the protein and mRNA levels. Treatment with fisetin also decreased the nuclear levels of NF-κB, c-Fos, c-Jun and inhibition of the NF-κB binding and AP-1. This study indicates the effectiveness of fisetin for the control of tumor metastasis due to its inhibitory effect on migration and invasion of lung cancer cells, and therefore, it could be used as an antimetastastic agent in the treatment of cancer [80].

### In-vivo *studies*

In a recent study, it has been reported that treatment with fisetin (25 mg/kg body weight) alleviated the mitochondrial dysfunction in a model of lung carcinogenesis induced by B(a)P [81]. In a previously published study, it has been shown that treatment with fisetin (25 mg/kg body weight) decreased histological lesions and levels of lipid peroxidation and modulated the enzymatic and non-enzymatic anti-oxidants in B(a)P-treated Swiss Albino mice. [82]. Using the matrigel plug assay, it was shown that treatment with fisetin (223 mg/kg body weight) inhibited angiogenesis in LLC-bearing mice. Treatment with fisetin also caused reduction in tumor growth inhibition, similar to low-dose cyclophosphamide (30 mg/kg body weight). The combination treatment of fisetin and cyclophosphamide resulted in striking improvement in antitumor activity accompanied by low systemic toxicity and decrease in microvessel density. This study provides the first evidence that fisetin exhibits antiangiogenic and anticancer activities in mice bearing LLC [83].

## Conclusion

The majority of lung cancer patients have disease diagnosed at later stages which is not curable by current treatment options. Therefore, effective chemopreventive agents against lung cancer are greatly desired. Indications from these research studies on lung cancer suggest that the dietary agents modulate several signaling pathways and inhibit inflammatory processes. In Western countries, dietary factors account for about 30% of cancers, suggesting that diet plays an important role in the prevention of cancer [84]. Therefore, the concept of chemoprevention would be very beneficial for persons who are in the high risk category for developing lung cancer, such as heavy smokers, ex-smokers and patients with resected primary lung cancer.

The compounds described in this article have shown promising results against lung cancer *in-vitro* and *in-vivo*. Certain epidemiological and case–control studies have reported the effects of green tea consumption against lung cancer. Drinking of green tea (3 cups of green tea/week for 1 year or more) was related with a decreased risk of lung cancer among non-smoking women and the risk decreased with increasing consumption in a population-based case–control study in Shanghai, China [85]. In another randomized controlled tea (4 cups/day) intervention phase II trial, the levels of 8-hydroxydeoxyguanosine (-OHdG), which is a product of oxidative DNA damage and considered as a relevant marker for cellular oxidative stress, were investigated. There was a significant decline in urinary 8-OHdG after drinking decaffeinated green tea among smokers [86].

Several dietary agents discussed in this article inhibited lung carcinogenesis in animal models and altered various signaling pathways, but there are many limitations for their use in clinical trials, the main reasons being lack of intermediate biomarkers and gaps in our understanding of the pathogenesis of lung cancer along with lack of good models for risk prediction. The clinical trials for small cell lung cancer are especially difficult to design because the sequence of events in the development of cancer is less well-defined and the development of validated markers of early pulmonary carcinogenesis is crucial for effective clinical trials of chemopreventive agents.

The results of clinical trials with dietary agents are not very encouraging for lung cancer. However, clinical trials investigating the natural or dietary agents for the chemoprevention/chemotherapy of lung cancer are critically required. The studies establishing the mechanistic basis of these dietary agents and identification of biomarkers are vital for the successful planning of the clinical trials. Therefore, future clinical studies should be designed keeping in mind the current trials and to identify and incorporate clinical markers of lung cancer risk with molecular biomarkers which might help in the early detection of lung cancer. The dietary agents discussed in this review article inhibit lung carcinogenesis in cell-culture and animal models and act through varied mechanisms. Therefore, a combination of these agents would be more desirable to pursue in future clinical trials. For successful chemoprevention, it is more reasonable to use a combination of chemopreventive agents as it would reduce the dose of each compound resulting in less toxicity and maximum efficacy by targeting multiple signaling pathways.

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## Conflict of interest

None.

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