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Review

Prevention of Lung Cancer: Future Perspective with Natural Compounds

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Lung cancer remains the most common cause of cancer death in the United States and worldwide. About 80~90% of cases are smoking-related and smoking cessation programs are of great importance in reducing lung cancer risk. However, the lifetime risk for lung cancer remains elevated even in ex-smokers. Chemoprevention holds the promise to further reduce this risk and thus to decrease lung cancer incidence and mortality. Over the last decades, most chemoprevention trials for lung cancer have yielded negative outcomes. Population-based studies suggest that high intake of certain foods such as soy, red wine or green vegetables may be associated with decreased cancer risk. Because of these observations and their general safety, a plethora of natural compounds is currently being studied for the chemoprevention of cancer. In this review we discuss promising *in vitro* and *in vivo* data of novel natural compounds, their interference with molecular mechanisms responsible for lung cancer development and potential implications for their further preclinical and clinical investigation.

Key Words: Lung Neoplasms; Natural compounds; Chemoprevention

Introduction

1. Clinical background

With an estimated 1.5 million new cases annually and an estimated 1.3 million deaths worldwide in 2007, lung cancer remains the most deadly malignancy[1]. Cigarette smoking is its major risk factor, with an estimated 90% of all lung cancers being directly attributable to smoke carcinogens. Smoking cessation efforts have had a major impact on lung cancer rates and mortality in the United States[2]. The continued increase in tobacco consumption worldwide, however, has led the World Health Organization (WHO) to estimate that all cancer rates could rise by 50% to 15 million annual cases by the year 2020. It is well established that smoking can create a molecular field defect in the airway epithelium of susceptible individuals. Consequently, the individual lung cancer risk of former smokers remains permanently elevated even years after successful cessation[3]. The high death rate of lung cancer combined with the lack of effective screening strategies and the persistently elevated lung cancer risk in former smokers has led to an early interest in chemoprevention strategies.

For the last decades, cancer prevention was mainly directed at the identification and avoidance of carcinogens. The principles of "chemoprevention", defined as the use of synthetic or natural substances to inhibit progression towards cancer or to reverse premalignant molecular changes, were established in the 1970s by Sporn and coworkers[4]. In breast, prostate, and colon cancer, this principle has subsequently been validated[5-7]. The NASBP-P1 trial showed a 43% relative risk reduction for breast cancer in high risk women treated with tamoxifen[5]. Finasteride led to a 24% decrease in relative prostate cancer risk in one study. However these findings have largely not been translated into clinical practice due to the increased risk for high grade prostate cancer

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in finasteride-treated patients[7]. Finally, in subjects with mutations in the APC gene, treatment with high dose celecoxib led to a significant reduction in adenomatous polyps[6]. In lung cancer however, despite intense efforts, most chemoprevention trials have so far yielded either negative or even harmful results.

2. What we have learned from the past

Vitamin A or its derivatives were the first substances to be extensively studied in the prevention of aero-digestive cancers. Strong epidemiologic evidence suggested that there was an inverse relationship between vitamin A levels and lung cancer incidence. Furthermore, *in vitro* and mouse data showed a strong putative anticancer effect. Based on these very promising data, four large randomized studies with more than 100,000 subjects overall were conducted[8-11]. Shockingly, these studies not only failed to show any benefit of chemoprevention with beta-carotene, but demonstrated an increased risk of lung cancer in the beta-carotene cohort, for the most part due to an increase in individuals who continued to smoke actively[8,10]. Naturally occurring retinoids were subsequently replaced with synthetic retinoids which offered the advantage of better tolerability and bioavailablity. Treatment with 13-cis-retinoic acid (isotretinoin) yielded very interesting results in the secondary and tertiary prevention of head and neck cancer[12]. For the prevention of lung cancer however, isotretinoin proved ineffective and, like beta-carotene, harmful in active smokers[13]. The molecular basis for the increased lung cancer risk with retinoids in active smokers has not been fully understood so far.

The development of selenium as a chemopreventive compound for lung cancer has followed a similar path. Epidemiologic studies suggested an inverse relationship between selenium blood levels and the incidence of several cancers, including lung cancer. It was hypothesized that as a cofactor to glutathione synthase, selenium would be able to reduce oxidative stress. Initial clinical studies were promising. In the Nutritional Prevention of Cancer (NPC) study, there was a statistically significant reduction in the secondary endpoints of lung, prostate and colorectal cancer incidence in 1,312 patients with a history of skin cancer that were randomized to receive 200 μg selenium daily or placebo[14]. With an additional 3 years of follow-up this difference disappeared, however[15]. Based on the promising initial analysis of the NPC study, a large study with 35,533 healthy men was planned with the primary endpoint of prostate cancer prevention and secondary endpoints of the prevention of other cancers, including lung[16]. This was a four-arm study that randomized participants to placebo, vitamin E, selenium or selenium combined with vitamin E. There was no significant difference in any of the primary and secondary endpoints. There was, however, a non-significant trend towards increased cancer risk with vitamin E and towards diabetes mellitus with selenium, highlighting again the fact that despite promising preliminary data, patients may actually incur harm while participating in a chemoprevention trial with seemingly benign compounds. For the tertiary prevention of lung cancer, selenium is currently being tested in a placebo-controlled study by the Eastern Cooperative Oncology Group (ECOG 5597) in patients with completely resected non small cell lung cancer (NSCLC). This trial has recently been closed to accrual due to the Data Monitoring Committee's conclusion that it was unlikely to meet its endpoint.

The experience from these trials that involved considerable time and expense and large numbers of patients has led many investigators to pursue the identification of histologic and molecular surrogate markers that could be used to screen compounds for possible activity much more expeditiously.

3. Surrogate endpoints in lung cancer chemoprevention studies

Surrogate endpoints are frequently used to evaluate the efficacy of a given compound in smaller cohorts of patients than those necessary to prove a reduction in lung cancer-related mortality. Requirements for a clinically useful surrogate endpoint are its involvement in carcinogenesis, its differential expression between normal and premalignant tissue, a high prevalence in pre-

malignancy and malignancy, being targeted by the intervention and having little or no fluctuation without the intervention[17]. Most ongoing chemoprevention trials currently use a combination of histologic endpoints, proliferation markers and molecular endpoints that are specific to the agent tested.

An important consideration is the selection of patients for lung cancer chemoprevention trials, particularly if histologic regression of progenitor lesions is a surrogate endpoint. Squamous metaplasia has a high spontaneous regression rate and the metaplasia index is also frequently reduced in the placebo arms of chemoprevention trials. High grade lesions regress less frequently. Bronchial dysplasia as detected by bronchoscopy, however, is rare in unselected patients at risk for lung cancer. A large bronchoscopy trial for the evaluation of autofluorescence bronchoscopy and several chemoprevention trials have shown a prevalence of dysplasia or worse in about 3～4% of all biopsied lesions[18-20]. In patients in which atypia is detected in expectorated sputum, this prevalence rises to 30% of all biopsied lesions[21,22] and 30～80% of patients undergoing bronchoscopy will harbor at least one dysplastic or higher grade lesion[21-23].

A clear understanding of the pathways targeted by a chemopreventive compound will allow the definition of additional surrogate endpoints based on the pharmacologic and molecular effects of a particular agent. A thorough preclinical evaluation of any compound of interest is therefore mandatory.

Promising Phytochemicals for Lung Cancer Chemoprevention

1. Tea polyphenols

Tea is among the most widely consumed beverages worldwide. Epigallocatechin-3-gallate (EGCG) (Figure 1) is the most prevalent polyphenol in green tea and a powerful antioxidant. Epidemiologic studies link green tea consumption to the decreased risk of breast, prostate, and lung cancer. *In vitro*, EGCG inhibits multiple critical pro-carcinogenic pathways (Table 1). Epige-

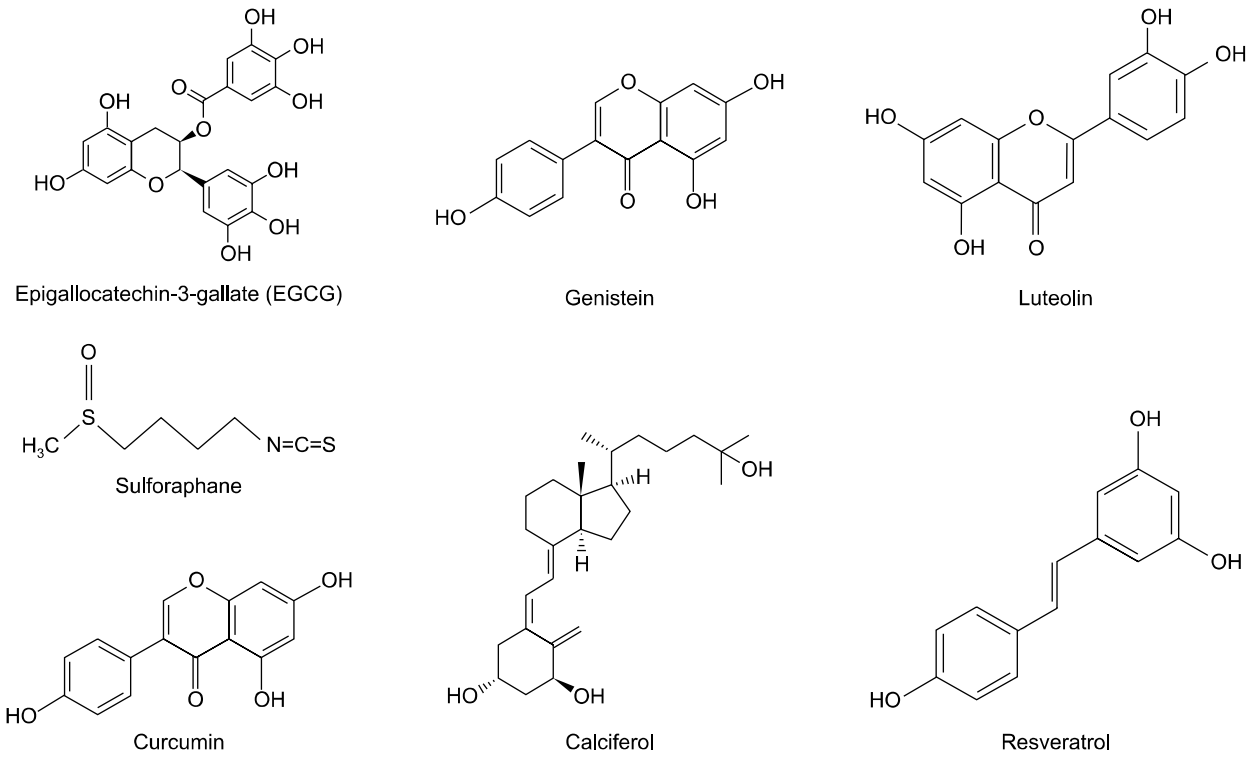

Figure 1. Structures of phytochemical compounds with potential for lung cancer chemoprevention.

Table 1. Phytochemicals with potential lung cancer chemopreventive effects

Agent	Natural source	Lung cancer chemoprevention trial	Mechanism of action	Molecular target	Synergy
Green tea polyphenols	Camellia sinensis (green tea)	British Columbia Cancer Agency; University of Arizona	Antioxidant, antiinflammatory, antiangiogenesis, apoptosis	DNMT1, EGFR, AKT, p53, p73, NF-kB, mVEGF, COX-2	Curcumin, erlotinib, luteolin, genistein
Isothiocyanates	Cruciferous vegetables (broccoli, cabbage, kale)	Masonic Cancer Center, University of Minnesota; Johns Hopkins University	Inhibition of phase I enzymes, induction of phase II enzymes, cell cycle arrest, antiangiogenesis, apoptosis	Nrf-2 upregulation, phase I and phase II enzymes, VEGF, caspase2, p53, SIRT1	EGCG
Luteolin	Artichoke, broccoli, celery, spinach, cauliflower	Emory University (in preparation)	Antioxidant, antiproliferative, antiinflammatory	p53, p21, BAX, EGFR, IGF-1R, AKT, NF-kB, CDK	EGCG
Calciferol	Fish, fortified milk	Roswell Park Cancer Institute	Antiinflammatory, antiproliferative	Vitamin D receptor, E-cadherin, cdks	Genistein
Resveratrol	Red wine, red grapes	None currently	Antiinflammatory, antioxidant, antiproliferative	Glutathione, AKT, NF-κB, p53, p21, BAX	EGCG, quercitin, luteolin, genistein
Curcumin	Curcuma longa (turmeric)	None currently	Antiinflammatory, antiproliferative, antiangiogenic	EGFR, IGFR, AKT, NF-κB, p53, p21, Bax, VEGF	EGCG, genistein, retinoic acid
Genistein	Soybeans	None currently	Antiinflammatory, antiproliferative, antiangiogenic	DNMT1, HDAC, AKT, survivin, p53, p21, Bax, ER, IGF-1R	EGCG, resveratrol, vitamin D

EGFR: epidermal growth factor receptor; EGCG: epigallocatechin-3-gallate.

netic effects[24] and inhibition of the Ras-GTPase-activating protein SH3 domain-binding protein 1 (G3BP1) constitute the underlying functional mechanism[25]. In xenograft models, green tea extract inhibits colon cancer growth and breast cancer metastasis. EGCG has completed a phase I clinical trial for the treatment of chronic lymphocytic leukemia (CLL)[26] and a phase II trial for the short-term, pre-operative treatment of prostate cancer[27] in the phase I study, doses were escalated to as high as 2,000 mg bid and no dose limiting toxicity was observed. In the phase II study, green tea polyphenon E (PPE) with an EGCG equivalent of 800 mg was given once daily without any discernable toxicity[26,27]. Median initial reports suggested a possibility of hepatotoxicity at higher doses of EGCG, although this was not observed in either one of these studies. In both studies, responses either in terms of lymphocyte reduction or PSA reduction were observed[26,27]. After single-dose administration of an 800 mg dose of EGCG, plasma levels peaked at around 400 ng/mL[28] and trough concentrations at 3 ng/mL[26]. In lung cancer cell lines, EGCG can induce apoptosis[29] and synergize with celecoxib[30] and erlotinib[31]. Green tea extract is currently being investigated in two clinical studies including a trial for the secondary prevention of lung cancer in subjects with bronchial dysplasia and as adjunct to therapy with erlotinib in the second-line setting for the treatment of advanced NSCLC (clinicaltrials.gov).

2. Broccoli extract

Sulforaphane is one of the major derivatives of cruciferous vegetables and broccoli extract in particular. The enzyme myrosinase transforms the broccoli glycosinolate constituent glucoraphanin into the isothiocyanate-form sulforaphane (Figure 1). Other isothiocyanates are similarly derived from the hydrolysis of glycosinolates in other cruciferous vegetables. Epidemiologic studies have linked high intake of these vegetables to

decreased risk of lung[32], colon, breast and prostate cancer. Sulforaphane and other isothiocyanates target multiple cellular processes. Traditionally, it had been assumed that the major mechanism of action was mediated through induction of the Nrf-2 transcription factor and the induction of phase II detoxifying enzymes. However, additional mechanisms are related to inhibition of phase I enzymes in the cytochrome p450 system which mediates the activation of many tobacco carcinogens. Sulforaphane and other isothiocyanates can induce apoptosis in various cancer cell lines, activate p53 signaling, cause cell cycle arrest, inhibit angiogenesis and cause microtubular disruption (Table 1). Broccoli extracts were evaluated in a phase I study of healthy subjects given two different doses of the glycosinolate glucoraphanin and the isothiocyanate sulforaphane. Thyroid, liver, hematologic and renal parameters were monitored closely and no significant toxicities were observed[33]. Broccoli extract and other isothiocyanates are currently being evaluated for the prevention of lung cancer in active smokers (clinicaltrials.gov).

3. Luteolin

Luteolin is a flavonoid (Figure 1) that is abundant in green vegetables such as artichoke, celery, spinach, green pepper and cauliflower. Both anti-inflammatory properties and anti-cancer properties have been described. Luteolin causes cell cycle arrest and apoptosis in a variety of cancer cell types. In mouse xenograft models, luteolin inhibited prostate cancer metastasis[34]. In gastric cancer cell lines synergy between luteolin and cisplatin was observed[35]. Luteolin proved effective in colon[36] and breast cancer[37] prevention in rodent models. Preliminary data from our institution show synergy between luteolin and EGCG in lung and head and neck cancer cell lines. Based on these data, we are currently preparing a phase I study to evaluate the safety and pharmacodynamics of luteolin and EGCG for the secondary prevention of lung cancer.

4. Calciferol

Vitamin D deficiency is a common phenomenon in the developed world, with studies suggesting that as many as 75% of American adults and adolescents are vitamin D deficient[38]. Numerous epidemiologic studies have found links between vitamin D deficiency and cancer, most notably breast, colon, and lung cancer, with a relative risk reduction in vitamin D-exposed versus non-exposed subjects ranging between 25～50%[39]. A recent update of the Women's Health Study showed a lower risk for the development of breast cancer (Hazard Ratio 0.65) in premenopausal women with the highest vs. the lowest amount of vitamin D consumption[40]. Cholecalciferol, the active form of vitamin D (Figure 1), is a steroid hormone. Forming a complex with its receptor, it acts as a transcription factor that regulates cell cycle control by regulating p21 and cdk expression. It furthermore leads to transcription of E-cadherin, the loss of which is a hallmark of epithelial-mesemchymal transition associated with proliferation and invasion of the malignant cell. In biopsies of human bronchial epithelium and lung cancer progenitor lesions, a progressive loss of cytoplasmic vitamin D receptor staining was observed with increasing histologic grade suggesting the involvement of the vitamin D signaling pathways in lung carcinogenesis[41]. In a randomized study of vitamin D and calcium vs. placebo in postmenopausal women at risk for osteoporosis, a statistically significant reduction in the risk of developing any cancer was observed for women who took vitamin D and calcium[42]. Sample size and cancer incidence rates, however, were low with very large confidence intervals so that these findings should only be considered to be hypothesis generating.

Vitamin D deficiency has also been associated with chronic obstructive lung disease (COPD), a major risk factor for the development of lung cancer. In the Third National Health and Nutrition Examination Survey (NHANES III), the pulmonary function parameters FEV_1 and FVC were significantly lower in subjects with the lowest quintile of vitamin D levels when compared with the highest quintile[43]. Certain polymorphisms in the vitamin D binding protein (VDBP) seem to be protective against COPD[44]. Studies examining vitamin D supple-

mentation for the prevention of lung cancer are currently ongoing (clinicaltrials.gov).

5. Resveratrol

Resveratrol, a major component of red wine and grapes, has received significant recent attention, primarily due to its cardioprotective effects and has been identified as the most important explanation of the "French Paradox" of significantly lower coronary heart disease rates in France compared with most other Western countries. Resveratrol is a phytoestrogen (Figure 1) and can act as an agonist of the estrogen receptor. The possible chemopreventive effects of resveratrol have been studied quite extensively *in vitro*[45] and in multiple different cell lines and various animal models. Like most other phytochemicals, resveratrol affects multiple pathways important for cancer development (Table 1). In animal models, resveratrol has been shown to be an effective chemopreventive compound against esophageal and breast cancer development[46-48]. In lung cancer chemoprevention models, the data are conflicting. In Balb/C mice, resveratrol inhibited the induction of benz(a)pyrene-induced diol epoxide-DNA adducts consistent with its ability to inhibit phase I and phase II enzymes[49]. However, in A/J mice exposed to benz(a)pyrene, resveratrol failed to inhibit lung cancer development[50,51]. A possible explanation for the strong *in vitro* activity but limited *in vivo* effectiveness is the low bioavailability of oral resveratrol[52]. Resveratrol was evaluated in a phase I study of healthy volunteers and found to be safe even at high doses[53]. Currently, resveratrol is under clinical investigation in studies with various vascular endpoints and in colon cancer. No specific study for the evaluation of lung cancer has been registered with clinicaltrials.gov.

6. Curcumin

Curcumin is the major ingredient in the culinary spice turmeric. It has been well recognized for its chemopreventive properties in many solid tumors and lymphoma. Like other phytochemicals (Figure 1), it exerts its effects through the targeting of multiple different pathways (Table 1). *In vitro*, curcumin inhibits lung cancer cell growth, induces cell cycle arrest and apoptosis[54-56]. Curcumin has been tested in early clinical trials including those for the prevention of cancer and has been shown to be well tolerated[57,58]. *In vitro*, there is synergy with EGCG[59,60], genistein and the chemotherapeutics vinorelbine, 5FU and gemcitabine. The available preclinical data form a strong rationale for the potential role of curcumin in lung cancer chemoprevention, although no active studies are currently listed at clinicaltrials.gov.

7. Genistein

Genistein is a soy isoflavone (Figure 1) and has been tested extensively in the prevention of prostate and breast cancer. Like resveratrol, genistein is a phytoestrogen. Its function is mainly regulated through the estrogen receptor beta. The molecular targets of genistein are listed in Table 1. Genistein has been shown to enhance the effects of docetaxel[61] and radiation[62] in prostate cancer cells. In humans, a pilot study in subjects with prostate cancer and rising PSA treated with 100 mg of genistein per day demonstrated efficacy[63]. Given the important role of epigenetic changes in the development of lung cancer and given its presumed properties as an inhibitor of both the DNA methyltransferases (DNMTs) and histone deacetylases (HDACs), genistein is an attractive chemopreventive compound against lung cancer. In lung cancer cell lines, genistein has been shown to induce apoptosis[64] and to synergize with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs)[65]. In a large population study from Japan, genistein intake was reported to be correlated with reduced lung cancer incidence in never-smoking men, with a trend towards reduced lung cancer in never-smoking women, but not in active or former smokers[66].

Molecular Pathways Commonly Involved in Lung Cancer

Molecularly, lung cancer is a very heterogeneous disease. Recent research identifying activating EGFR

mutations[67,68] and the EML4-Alk translocation[69] as well as the sequencing of the cancer genome of adenocarcinomas has only limited impact on the chemoprevention field since most of these specific abnormalities occur in non-smokers while smokers are the primary candidates for chemoprevention studies. A distinct advantage of chemoprevention with natural compounds is the fact that these generally target multiple carcinogenic pathways in parallel. The likelihood of achieving successful lung cancer prevention in an individual patient might therefore increase.

1. Introduction to signaling pathways

Mammalian cells require growth factor stimulation to take up nutrients from the environment. In contrast, cancer cells overcome this growth factor dependency by acquiring genetic mutations, the accumulation of which functionally changes receptor-initiated signaling pathways. Mutation or gene amplification of cell surface receptors (such as EGFR) activates multiple downstream signaling cascades (Figure 2) responsible for carcinogenesis. PI3K-AKT-mTOR and Ras-MAPK are two major signaling pathways constitutively activated in cancer cells. Loss/mutation of PTEN, expression of PI3KCA (mutation of PI3K which constitutively activates PI3K-AKT signaling) or mutation of K-ras/H-ras also contribute to the activation of PI3K-AKT-mTOR signaling. K-Ras/H-Ras mutations also constitutively activate the MAPK pathway. Moreover, activation of the Wnt pathway was observed in many cancers contributing to the activation of mTOR signaling. Loss of LKB1, an important signaling molecule in the LKB1-AMPK metabolic pathway is also found in many cancers and is linked to mTOR signaling. Interactions between these signaling pathways and their modulations by various natural compounds are shown in Figure 2.

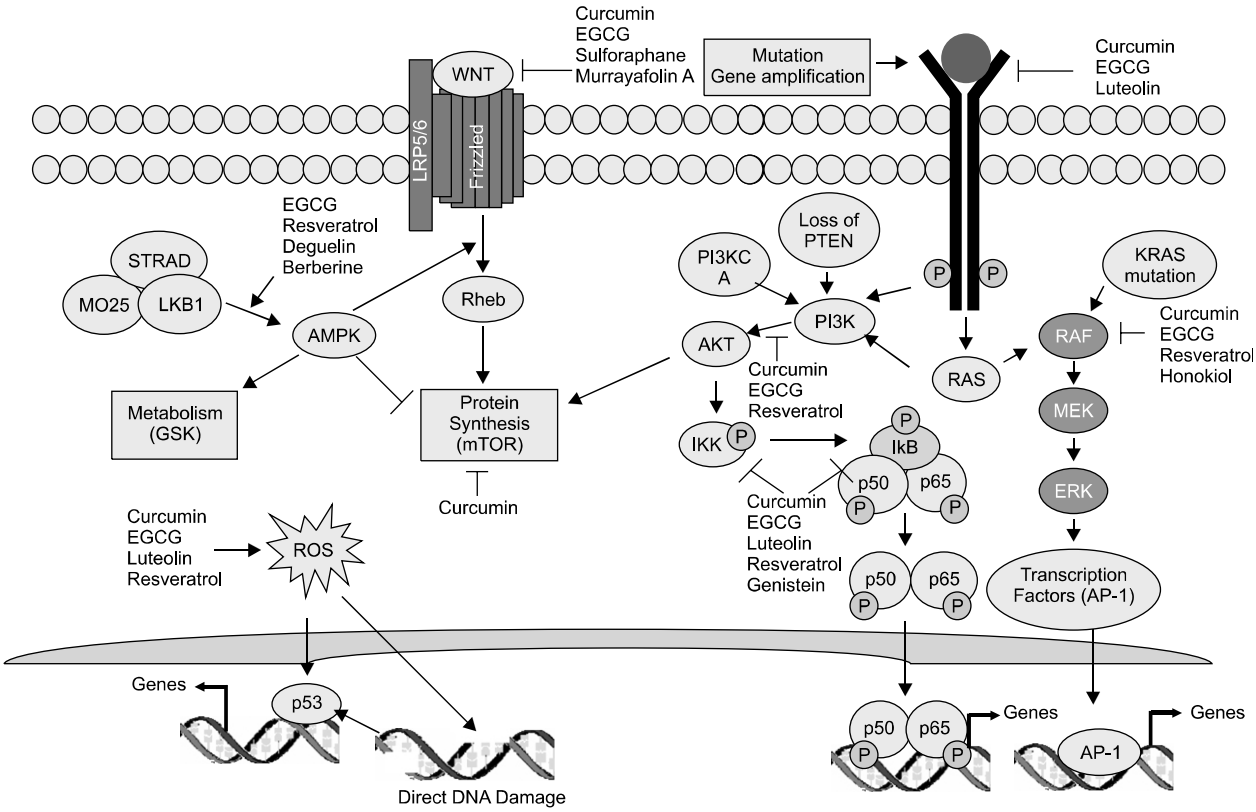

Figure 2. Molecular pathways affected by natural agents. EGCG: epigallocatechin-3-gallate.

2. Epigenetic changes in gene regulation

Under normal circumstances, epigenetic mechanisms of gene regulation play a major role in stem cell maintenance and the imprinting of the second female X-chromosome. In lung cancer, it is well established that epigenetic events occur early on in carcinogenesis[70-72]. Silencing of tumor suppressor genes by methylation of CpG islands in the promoter regions of genes or alterations of the histone code have been well established in lung cancer for critical cell cycle genes such as p16, DNA repair genes such as MGMT and hMLH-1, apoptosis inducers such as DAPK and genes involved in the ras-(RASSF1) and the wnt-signaling pathways (SFRP 1, 2, 4, 5, APC, LKB1). In a cell culture model, siRNA knockdown of DNMT1 prevented smoke carcinogen-induced transformation of normal human bronchial epithelial cells[73] and combined pharmacologic inhibition of DNMTs and HDACs prevented the formation of murine lung cancer[74]. In humans, early clinical trial data suggest that the combination of the HDAC inhibitor etinostat and the DNMT inhibitor 5' azacytidine may be an active combination for the treatment of lung cancer. The main constituent of green tea, EGCG[24] and the soy isoflavone genistein[75,76] have also been shown to inhibit DNMT at concentrations that can achieved *in vivo*, thus making these two agents very attractive candidates for lung cancer chemoprevention. In addition, genistein may inhibit HDACs[77].

3. EGFR-RAS-MAPK pathway

Activating mutations in the EGF signaling pathway, either in EGFR itself[67,68] or in its downstream targets k-ras and b-raf, highlight its relevance for lung cancer carcinogenesis[78]. Inhibition of this pathway with the EGFR-TKIs erlotinib and gefitinib is particularly effective for the treatment of NSCLC in EGFR-mutated tumors[79], but also shows activity in EGFR wildtype tumors as long as no additional k-ras or b-raf mutations are present[80]. EGFR overexpression in bronchial dysplasia is frequent, implicating it as an early event in lung cancer carcinogenesis[81]. EGCG[31], curcumin[82] and luteolin[83-85] have all been shown to inhibit EGFR signaling.

4. PI3Kinase-AKT-mTOR pathway

It has been estimated that Akt is one of the most frequently activated protein kinases in human cancer. The PI3Kinase-Akt-mTOR pathway acts downstream from receptor tyrosine kinases such as EGFR, IGFR, c-met and ERBB3. Activation of the Akt pathway has been recognized as a major mechanism of acquired resistance to EGFR-TKIs in NSCLC. Two physiologic inhibitors provide additional checks and balances in this pathway: PTEN acts upstream from Akt and is frequently inactivated in NSCLC in part due to promoter hypermethylation; and the TSC1/TSC2 complex prevents activation of mTOR. Alterations in these genes are associated with tuberous sclerosis and lymphangioleimyomatosis, which form benign tumors but have not been conclusively associated with lung cancer. Many phyotochemicals have been shown in cancer cell lines to inhibit AKT-mTOR signaling such as EGCG[86,87], curcumin[88-90], resveratrol[91,92], genistein[93,94], pomegranate[95] and lycopene[96].

5. Wnt-signaling pathway

The wnt-signaling pathway plays a crucial role in embryonal development and cancer. While the predominant oncogenic pathway in colorectal cancer, recent research has also shown a major contribution to pulmonary carcinogenesis. The pathway is tightly regulated through a network of wnt-antagonists, many of which have been shown to be abnormally regulated through epigenetic means in NSCLC[97]. Activation of the wnt-signaling pathway has also been associated with an increased rate of brain and bone metastasis in lung cancer[98]. The phytochemicals EGCG[99-101], curcumin[102-104], murrayafoline A, an alkaloid isolated from the glycosmis stenocarpa root[105], and broccoli-derived sulforophane[106], have been shown to inhibit wnt-signaling in cancer models[106]. EGCG inhibits wnt-signaling both through epigenetic means as well as through upregulation of the transcriptional repressor HBP-1[100].

6. DNA-adduct formation

Tobacco smoke, the major risk factor for NSCLC, contains a large number of carcinogens including polycyclic hydrocarbons. Benz(a)pyrene and its active metabolite benzo(*a*)pyrene-7,8-diol-9,10-epoxide (BPDE) form stable DNA adducts by reacting with guanine bases in the DNA. Carcinogen activation frequently depends on cytochrome p450 pathways. Targeting these and other smoke carcinogens for the prevention of lung cancer is therefore a potentially attractive approach. Chemopreventive strategies currently under investigation aim at increasing the detoxification enzymes which are involved in carcinogen metabolism or inhibition of carcinogen activation. Glucosinolates, a component of cruciferous vegetables, are currently under investigation for their presumed chemopreventive properties. Upon ingestion, these compounds are metabolized to various breakdown products including isothiocyanates, which have been shown to inhibit cytochrome p450-dependent carcinogen activation. The transcription factor Nrf-2 plays a major role in the induction of detoxification enzymes and in the response to oxidative stress. Nrf-2 −/− mice are more susceptible to benz(a)pyrene-induced tumor formation and to oxidative stress[107]. Induction of Nrf-2 expression by either natural or synthetic compounds as a chemopreventive strategy against tobacco smoke-related cancers has received considerable interest. Nrf-2 levels can be upregulated by broccoli-derived sulforaphane[108,109], dibenzyolmethane[110], an ingredient of licorice, and the synthetic drug oltipraz[111]. Oltipraz has been tested in a randomized chemoprevention study for lung cancer but failed to meet its primary endpoint of PAH-DNA adduct formation and its secondary endpoint of decreasing PAH blood levels. Moreover, it caused significant gastrointestinal toxicity[112].

7. Cell metabolism pathway

Unlike normal differentiated cells, most cancer cells rely on aerobic glycolysis (conversion of glucose to lactate regardless of the availability of oxygen) to generate the energy needed for cellular processes. The LKB1-AMPK (AMP-activated kinase) signaling pathway is one of the master regulators of cellular metabolism. In case of adenosine triphosphate (ATP) depletion, the adenylate kinases convert two adenosine diphosphates (ADPs) to one ATP and one adenosine 5'-monophosphate (AMP). However, accumulation of AMP activates AMPK which is dependent on LKB1. Activation of LKB1-AMPK signaling leads to phosphorylation of several downstream targets to improve energy charge in cells and inhibits mTOR signaling, an important pathway for protein synthesis. Activators of AMPK such as metformin, phenformin, and aminoimidazole carboxamide ribonucleotide were reported to inhibit tumor cell growth or prevent tumor development[113-116]. Studies suggest that many natural compounds including EGCG, resveratrol, genistein and curcumin are activators of the LKB1-AMPK signaling pathway which contribute to their antitumor or chemopreventive potential. The apoptotic effect of EGCG on colon cancer is mediated via activation of AMPK signaling[117]. The anti-obesity effects of EGCG, i.e., suppression of hepatic gluconeogenesis or inhibition of adipogenesis, and of genistein are also mediated via AMPK pathways[118,119]. It is now well accepted that inhibition of obesity is associated with reduced risk of cancers. Multiple studies also suggest that resveratrol can activate the AMPK signaling cascade which is associated with its chemopreventive effects[120-122]. In an ovarian cancer cell line, the apoptotic effect of curcumin is mediated via activation of the AMPK-p38 signaling pathway[123]. Other natural compounds modulating the LKB1-AMPK signaling cascade include the dietary flavonoid quercetin[124], ginsenoside[125], caffeic acid[126], and berberine[127].

Conclusions

Smoking cessation and abstinence programs are the single most important preventive strategy against lung cancer. Unfortunately, the lag time until this effect can be observed is measured in decades and many patients with prior tobacco exposure will not benefit. Lung cancer mortality rates remain high with an estimated 75～

80% of all lung cancer patients ultimately succumbing to their disease. Therefore, even a moderately effective chemopreventive compound promises to reduce lung cancer deaths more significantly than all other clinical treatment strategies combined. Large chemoprevention trials in the past have highlighted the challenges of translating exciting epidemiologic, *in vitro* and *in vivo* observations into clinically beneficial results. Better understanding of the molecular events and pathways leading to field cancerization and lung cancer development has dramatically improved our ability to identify promising compounds. The potential role of phytochemicals is particularly exciting given their non-toxic nature, their abundance in our normal food chain and the fact that they generally target many cellular processes which might contribute to lung carcinogenesis.

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