

Chemoprevention of lung cancer—from biology to clinical reality

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Lung cancer is the commonest cause of cancer death in developed countries and throughout the world. Cigarette smoking is the main risk factor for lung cancer and ex-smokers today comprise ~50% of all new lung cancer cases. Chemoprevention builds on the concepts of field of cancerization and multistep carcinogenesis and can be defined as the use of natural or chemical compounds to prevent, inhibit or reverse the process of carcinogenesis. So far, chemoprevention studies in lung cancer have failed to reduce lung cancer mortality. New developments in biotechnology have made it possible to define more accurately high-risk populations, make earlier diagnosis possible, and allow more specific targeted therapies to be developed. Both the development and validation of biomarkers, for the selection of high-risk study populations and for response evaluation in chemoprevention studies, are important for the faster turnover of studies evaluating new agents. This article reviews the current status and describes the perspectives for new approaches in the chemoprevention of lung cancer.

Key words: chemoprevention, cyclooxygenase 2, epidermal growth factor receptor, lung cancer, lung cancer biology, retinoids

Introduction

Lung cancer is the commonest cause of cancer death in developed countries and throughout the world. Worldwide, the estimated number of new lung cancer cases in 2002 is 1.2 million (12.3% of all new cancer cases). Over 90% of these new cases will die as a result of the disease [1]. The death rate for lung cancer exceeds the combined total for breast, prostate and colon cancer in developed countries [1].

Cigarette smoking is the main risk factor for lung cancer, accounting for ~90% of cases in men and 70–85% of cases in women [2]. Genetic risk factors contribute to an individual's susceptibility to lung cancer, which is illustrated by the fact that <16% of long-term smokers will develop lung cancer [3]. Life style factors (e.g. diet) are also thought to be important in the modulation of risk [3]. Unfortunately, effective clinical tests to assign risk are lacking. Smoking cessation programs are crucial, but ex-smokers continue to have a higher risk of developing lung cancer >40 years after cessation compared with never-smokers [4]. Ex-smokers comprise nearly 50% of all new lung cancer cases in developed countries, indicating a strong need for a search for new means of early diagnosis in lung cancer and chemoprevention in this high-risk group [4]. However, an important issue for these approaches is the appropriate selection of an optimal high-risk population.

In 1993, Sporn pointed out that carcinogenesis is the disease and not cancer [5]. For comparison, screening and treatment of cervical dysplasia has led to a remarkable decrease of cervical cancer incidence and mortality [6]. Premalignant lesions that affect other organs have been identified and treated (e.g. carcinoma *in situ* of the bladder, colon polyps and prostate intraepithelial neoplasia) [7]. Treatment of these precancerous lesions appears to be of value for cancer prevention (Table 1).

Chemoprevention trials for lung cancer have been carried out in phase III clinical trials, studying >70 000 patients for over a decade with mostly negative results (Table 2) [8–17]. This underlines the necessity to find new concepts for chemoprevention trials in a more cost-effective and time-efficient manner. In the above-mentioned studies, smoking history has been chosen as the selection criteria for the study population and lung cancer incidence or mortality as the end point. According to published data, smoking history alone might not be selective enough and there is a need to identify more specific criteria to define the optimal high-risk study population. Another scientific goal is to validate intermediate end points in order to achieve short-term studies that include fewer patients. These concepts are discussed in this article.

Chemoprevention can be defined as the use of natural or chemically synthesized compounds to prevent, inhibit or reverse the process of carcinogenesis. Rapid improvement in the understanding of the molecular and biological basis of lung carcinogenesis raises new possibilities for the chemoprevention of lung cancer. Combined with advances in endoscopy techniques, such as laser-induced fluorescence endoscope (LIFE) bronchoscopy, and developments in biotechnology and radiology [18], there are new pos-

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Table 1. Chemopreventive agents used in previous studies

	Mechanisms	Agents
Antimutagens	Inhibition of carcinogenic metabolites	<i>N</i> -Acetyl-cysteine
	Reduction of reactive carcinogenic intermediates	Dithiole-3-thiones (Oltipraz)
	Induction of deactivating phase II enzymes	β-Carotenes Vitamin B ₁₂ , folic acid
Antiproliferatives	Induction of cell differentiation	Retinoids
	Reduction of growth rate	NSAIDs
	Induction of apoptosis	Glucocorticoids
	Inhibition of angiogenesis	
Antioxidants	Scavenge reactive oxygen species	<i>N</i> -Acetyl-cysteine
	Scavenge free radicals	Vitamin E
		Dithiole-3-thiones (Oltipraz)

NSAID, non-steroidal anti-inflammatory drug.

Table 2. Randomized lung cancer chemoprevention trials with retinoids

Trial authors [ref.]	Agents	Study group	No. of patients		End points		Effect (RR; <i>P</i> value)
			Treatment	Placebo	Treatment	Placebo	
Trial I							
No. of lung cancers							
ATBC study [8]	BC/Vit.E	Smokers	14 560	14 573	474	402	Harmful/negative (1.18; 0.01)
CARET [9]	BC + RA	Smokers, asbestos	9420	8894	218	170	Harmful (1.28; 0.02)
Hennekens et al. [10]	BC	Smokers	11 036	11 035	1.273	1.293	Negative (0.93; 0.65)
Trial II							
Reduction of sputum atypia							
Arnold et al. [11]	Etretinate	Sputum a.	71	67	NS		Negative
McLarty et al. [14]	BC + RA	Sputum a.	378	377	NS		Negative
Decrease in MI							
Lee et al. [12]	Isotretinoin	Metaplasia	41	45	5.1%	10.5% ^a	Negative
Kurie et al. [13]	Fenretinide	Metaplasia	41	41	7.1% ^a	4.9%	Negative
Trial III							
SPT							
Pastorino et al. [15]	RP	NSCLC	150	157	18	29	NS
EUROSCAN [16]	NAC/RP	NSCLC + HNC	1290	1283	115	93	Negative
Lippman et al. [17]	Isotretinoin	NSCLC	589	577	76	71	Negative

^aNot significant.

BC, β-carotene; HNC, head and neck cancer; I, primary prevention; II, secondary prevention; III, tertiary prevention; MI, metaplasia index; NAC, *N*-acetylcysteine; NS, not significant (*p* = 0.44); NSCLC, non-small-cell lung cancer; RA, retinoic acid; RP, retinyl palmitate; RR, relative risk; sputum a., sputum atypia; SPT, second primary tumor; Vit.E, vitamin E.

sibilities to diagnose intraepithelial neoplasia and lung cancer at much earlier stages and new treatment possibilities are emerging [18].

The chemoprevention principles are built on the concepts of field of cancerization and multistep carcinogenesis. Field cancerization is characterized by diffuse injury of an epithelial surface as the result of long-term carcinogenic exposure [19]. Genetic alteration throughout the respiratory epithelium is the result of exposure

to the carcinogens in cigarettes and to radon or other long-term carcinogenic insults. This 'preconditioned' epithelium can give rise to cancer at multiple points. Studies of the airways of lung cancer patients show that extensive hyperplasia and dysplasia occur throughout the bronchial epithelium, accompanied by aneuploidy. These multiple lesions are not usually genetically distinct from the patient's tumor and presumably arise independently. These findings support the idea that the entire upper aerodigestive

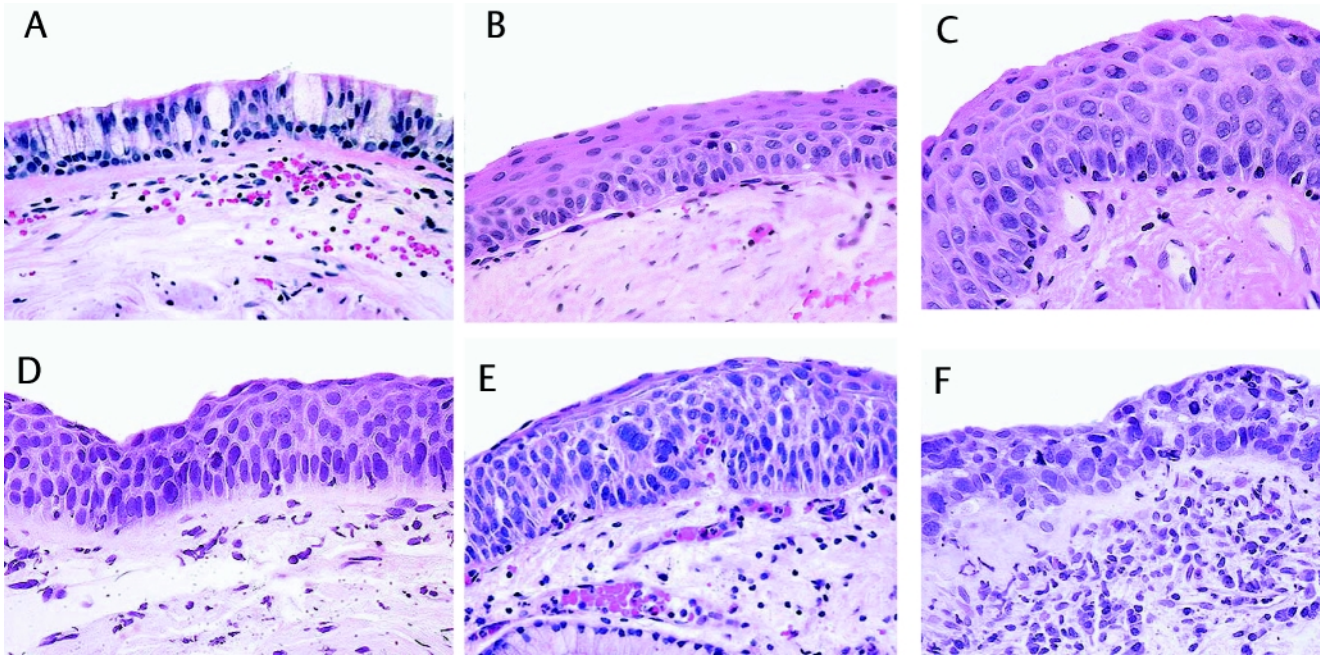


Figure 1. Histology of bronchial epithelium. Grades of dysplasia in bronchial epithelium. (A) Normal two-layered epithelium; (B) squamous metaplasia; (C) mild dysplasia; (D) moderate dysplasia; (E) severe dysplasia; (F) carcinoma *in situ*.

tract is at risk of developing genetic alterations as a result of long-term carcinogen exposure. Genetic changes detected in pre-malignant lesions in one region of the field, translate into an increased risk of cancer development throughout the entire field.

Contributing factors to the genetic predisposition associated with increased lung cancer risk are polymorphisms in enzymes that affect carcinogen activation (P450) and detoxification (glutathione *S*-transferase), DNA repair genes, inactivation of the p53 tumor suppressor gene and activation of dominant oncogenes. The interaction of host susceptibility and exposure to carcinogens leads to variation in cancer susceptibility and presentation.

The concept of multistep carcinogenesis was derived from pathological observations that mucosal changes in the airways, including hyperplasia, metaplasia, dysplasia and carcinoma *in situ* (CIS), precede or accompany invasive squamous carcinoma [20] (Figure 1). Hyperplasia, squamous metaplasia and mild dysplasia have generally been considered as reversible and not premalignant. Alterations in oncogene and tumor suppressor gene expression and chromosome structure known to be associated with malignant transformation are often present in morphologically normal epithelium of smokers and occur with increasing frequency through varying degrees of dysplasia to CIS, where they are universally present.

Chemoprevention studies are based on the hypothesis that interruption of the biological process involved in carcinogenesis will reverse or inhibit the carcinogenic process and reduce cancer incidence, and—similar to the approach for other organs—effective treatment of bronchial dysplasia should reduce lung cancer.

This biological basis of chemoprevention has provided the framework for the design and evaluation of new chemoprevention trials. Molecular epidemiological and genotyping risk assessment

models are encouraged to provide a more sensitive and specific design for the chemoprevention trials.

The purpose of this article is to review the current status and to describe the perspectives for new approaches in the chemoprevention of lung cancer.

What did we learn from previous studies?

Retinoids and carotenoids

Consistent evidence suggests that high intake of fruit and vegetables are associated with a reduced risk of lung cancer [4]. Therefore, it has been suggested that micronutrients and macronutrients present in our diet may act as cancer inhibiting substances. Retinoids are natural and synthetic derivatives of vitamin A (retinol). Carotenoids are a family of conjugated polyene molecules, found largely in fruit and vegetables, that act as antioxidants; certain carotenoids also serve as precursors to retinol.

Retinoids are potent regulators of gene expression and signal their cellular effects through nuclear retinoid receptors. Two classes of nuclear retinoid receptors—retinoic acid receptor RAR and retinoid X receptor RXR—have been identified, each of them having at least three subtypes (α , β and γ , respectively). The receptors are ligand activated and following the binding to retinoids, the retinoid target genes become transcriptionally activated or repressed. The target genes regulate cell growth, differentiation and death (apoptosis) [21].

Based on the hypothesis that the reduced risk of lung cancer associated with a high intake of fruit and vegetables is due to β -carotene and other antioxidants, epidemiological studies have verified that intake or serum concentration of β -carotene and

cancer risk are inversely related [4]. Experimental models together with these epidemiological data formed the rationale for the use of retinoids or carotenes in cancer prevention. Added support for a retinoid-based clinical chemopreventive approach came from the successful treatment of premalignant lesions (oral leukoplakia, cervical dysplasia [6] and xeroderma pigmentosum). In addition, clinical trials have shown that retinoids are active in reducing some second primary cancers (e.g. second aerodigestive tract tumors in patients with resected head and neck cancers).

Randomized controlled trials with retinoids

Randomized controlled trials have been conducted in three lung cancer chemoprevention settings: primary prevention (healthy high-risk smokers), secondary prevention (pre-malignant lesions) and tertiary prevention [second primary tumors (SPT), SPT in previously treated patients] [8–17] (Table 2).

Three phase III studies were completed involving primary prevention (Table 2): the α -Tocopherol, β -Carotene (ATBC) Study [8], the β -Carotene and Retinol Efficacy Trial (CARET) [9] and the Physicians Health Study [10]. In the ATBC trials, the selected patients were 50–69 years of age and current smokers (five or more cigarettes per day at entry); in the CARET study, patients had been exposed to asbestos 15 years before randomization or were current or former smokers with at least 20 pack-years (pack years = number of packs cigarettes per day \times number of years smoked). The Physicians Health Study recruited male US physicians, 40–84 years of age without any history of cancer, myocardial infarction or stroke; only 11% of the study population were smokers. None of these trials showed a reduction in lung cancer incidence or mortality. In the ATBC study, 876 new cases of lung cancer were diagnosed (Table 2), yielding an increased relative risk of 1.18 among subjects (all of them current smokers) in the treatment arm. In the CARET study, there were 388 new cases of lung cancer, yielding an increased relative risk of 1.28 among the treated patients. Separating the study population in current and former smokers, the relative risk was increased to 1.42 for current smokers and decreased to 0.80 for ex-smokers. In the Physicians' Health Study, 170 new cases of lung cancer were diagnosed, for a relative risk of 0.93 among men taking β -carotene (non-significant). The data from these three studies indicated that smokers (current and ex-smokers analyzed together) who received high-dose β -carotene supplementation had an increased risk for lung cancer (the increase in the lung cancer incidence and mortality in the ATBC study and CARET were 18% and 8%, and 28% and 17%, respectively).

Four phase IIb trials (Table 2) were conducted in smokers with metaplasia or sputum atypia for secondary prevention and all have been negative [11–14]. These trials evaluated α -tocopherol, β -carotene, retinal, retinyl palmitate or isotretinoin in smokers. Only smoking cessation correlated with a significant reduction in squamous metaplasia and cell proliferation [13] and isotretinoin plus smoking cessation further reduced metaplasia, but so far neither metaplasia nor sputum atypia are established intermediate end points for chemoprevention trials.

The consistently positive results of short-term retinoid studies in head and neck chemoprevention contributed substantially to the

rationale for testing retinoids in lung cancer prevention studies. There are three available phase III studies [15–17] for tertiary prevention (Table 2). These studies were designed to determine whether vitamin A or its analogs could prevent secondary primary cancers (SPC) in patients with completely resected lung cancers or head and neck cancers. These patients were previously shown to have a high risk of SPCs. The two most recent studies [16, 17] failed to confirm the positive experiences from studies in head-neck tumors and showed no reductions of SPCs or tumor recurrences in contrast to the much smaller preliminary study of Pastorino et al. [15]. In this small European study, vitamin A administration improved the time to SPTs but produced no benefit in terms of overall survival.

So far, all randomized controlled chemoprevention trials testing retinoids, β -carotenes or α -tocopherol defined their target population by using smoking history, preneoplastic changes of the bronchial epithelium or cancer history. Another critical issue is the selection of study end points. In primary prevention, lung cancer incidence and mortality with a long study time has been the gold standard. In the secondary prevention setting, bronchial metaplasia or sputum atypia were selected as intermediate end points, but metaplasia has been reported to be a spontaneously reversible lesion and neither of them are validated intermediate end points.

Despite these critical points, the use of retinoids has not been effective and has possible harmful effects in the chemoprevention of non-small-cell lung cancer (NSCLC), especially in current smokers. In order to find an explanation for these results, studies of the interaction between the products of cigarette smoking and high blood concentrations of retinoids or β -carotenes have been performed. For example, the studies of Wang et al. and Liu et al. showed that ferrets, in the same way as humans, absorb β -carotene into the bloodstream and transport it to the lungs as well as to other tissues, whereas mice and rats almost completely convert β -carotene to retinoids in the intestine and liver and therefore would transport little to the lungs [22, 23]. The large amounts of β -carotene in lung tissue in combination with cigarette smoke are broken down into oxidative metabolites [24, 25]. One possible explanation of the harm seen in the chemoprevention trials can be a procarcinogenic effect of the toxic oxidative carotene metabolites. But results from Aroro et al. indicate that β -carotene is sensitive to cigarette smoke oxidation but does not lead to prooxidant effects in human bronchial epithelial cells [26]. They rather have a direct effect on the nuclear receptors and the retinoid signaling pathway. The oxidative metabolites induce cytochrome P450 enzymes, lowering the serum levels of retinoid acid and down-regulating RXR and RAR β . Nicotine by itself inhibits RAR β expression via methylation and induction of orphan receptor TR3 (a subfamily of transcription factors belonging to the nuclear receptor superfamily). RAR β is a potent inhibitor of the proliferation-signaling protein AP-1 and a promoter of apoptosis, so down-regulation of the different nuclear receptors, as well as defects in the RA/RAR β -regulated genes, results in retinoic acid resistance and enhanced mitogenic activities and cell proliferation.

In a study looking at lung precursor lesions in the free resection margins of patients undergoing surgery for lung cancer or non-

cancerous diseases, there was a linear increase in the expression of RXR- α and RXR- γ from never-smokers to dysplasia and *in situ* carcinoma and a decrease in RAR- β protein expression from the first to the last group. Methylation of the RAR- β promoter and loss of heterozygosity (3p, which contains the gene locus for RAR- β) are likely to be the important mechanisms.

Several synthetic receptor-selective retinoids have proved to be more potent than retinoic acid in inhibiting cell growth in lung cancer models [27]. However, their value *in vivo* has yet to be proven in controlled randomized trials. Finally, there are indications that new routes of administration, such as inhalation, may provide an effective way of prescribing retinoids [28].

Looking at the side-effects described in randomized trials [8–17], there was a statistically significant increase in yellowing of the skin using β -carotenes and dryness of the skin or mucous membranes using retinoids. Other significant treatment-related toxic effects included arthralgia, nausea or dyspepsia, headache and hypertriglyceridemia. There is also evidence that a high vitamin A intake is associated with increased bone fragility and risk of fracture.

Vitamin E (α -tocopherol)

α -Tocopherol (AT) is an antioxidant, scavenging reactive oxygen species and free radicals, and protecting against oxidative damage. Similar to carotenes, epidemiological and dietary studies suggest a potential preventive role for vitamin E [4]. In the only published, controlled randomized trial—the ATBC study [8]—vitamin E supplementation had no effect on lung cancer incidence (risk ratio, 0.99; Table 2). The higher mortality due to hemorrhagic stroke among the participants who received α -tocopherol was possibly related to known effects on platelet function. However, in the same study, there was an association between blood levels of α -tocopherol and incidence of lung cancer [29]. A 19% reduction of lung cancer incidence was observed in the highest versus the lowest quintile of serum α -tocopherol [relative risk, 0.81; 95% confidence intervals (CI) 0.67–0.97]. α -Tocopherol was found to be more protective in younger men with fewer years of smoking, suggesting that high levels of serum α -tocopherol, if present during the early critical stages of carcinogenesis, may inhibit lung cancer development.

Selenium

Epidemiological studies suggest that selenium (Se) has anticarcinogenic capacity and plays a role in cellular defense against oxidative stress [30]; results of these studies have shown an inverse association between Se status and lung cancer. A recent update of the Nutrition Prevention of Cancer Trial [31] indicated that Se supplementation did not significantly decrease lung cancer incidence in the full population, but a decrease among individuals with baseline plasma selenium in the lowest tertile was observed (hazard ratio, +0.42; 95% CI 0.18–0.96; $P = 0.04$). There is an ongoing randomized phase III trial to determine the effectiveness of selenium in preventing the development of secondary primary lung tumors in patients with previously resected stage I NSCLC, comparing the incidence of specific cancers, mortality from cancer

Table 3. List of possible candidates for chemoprevention

EGFR inhibitors	Angiogenesis inhibitors
COX-2 inhibitors	Cell-cycle inhibitors
Lipoxygenase inhibitors	Proteasome inhibitors
Prostacyclin analogs	mTOR inhibitors
Farnesyltransferase inhibitors	Protein kinase C inhibitors
Ras inhibitors	Demethylation agents
Rexinoids	DFMO combination
PPAR γ agonists	Budenoside

EGFR, epidermal growth factor receptor; DFMO, difluoromethylornithine; PPAR γ , peroxysome proliferator-activated receptor gamma; mTOR, mammalian target of rapamycin.

and overall survival of participants treated with selenium versus those treated with placebo (ECOG-E5597).

Future directions

New classes of chemopreventive agents

Advances in molecular biology have led to a better understanding of the important pathways necessary for lung cancer development. Antibodies and small molecules have been developed to target specific proteins and block many of these important signaling pathways, thus leading to a new era of therapeutic possibilities (Table 3).

The erbB family of receptors [erbB1 (EGFR), erbB2 (HER2/neu), erbB3 and erbB4] is an important growth factor family of receptors in epithelial cancers. These receptors are activated after ligand binding and a downstream cascade of biological and physiological reactions occurs. This cascade culminates in cell proliferation, differentiation and apoptosis [32] (Figure 2). Several classes of agents block these signaling pathways at different levels [33]. Monoclonal antibodies, such as C225 (Cetuximab; Erbitux®, Merck KGaA Pharmaceuticals, Darmstadt, Germany), that prevent ligand-dependent activation and small molecules such as ZD1839 (Gefitinib; Iressa®, AstraZeneca Pharmaceuticals, Macclesfield, UK) and OSI-774 (Erlotinib; Tarceva®, OSI Pharmaceuticals, Melville, NY), that selectively inhibit the intracellular tyrosine kinase domain of erbB1 (EGFR), are well tolerated after chronic administration and have shown regression of advanced head and neck cancers and NSCLC, even after failure of multiple chemotherapeutic agents [34].

Immunohistochemical studies of bronchial preneoplasia have demonstrated significant expression of erbB1 (EGFR) and erbB2 [32] indicating the importance of these growth factor receptors in lung carcinogenesis. All of the erbB family of receptors heterodimerize with each other [35]. ErbB2 is the preferred heterodimeric partner for erbB1, erbB3 and erbB4. Such erbB2-containing heterodimers induce potent mitogenic signals. Simultaneous interruption of both erbB2 and erbB1 is an appealing therapeutic strategy. Dual erbB1–erbB2 tyrosine kinase inhibitors, such as the small molecule GW572016, have recently been identified and preclinical

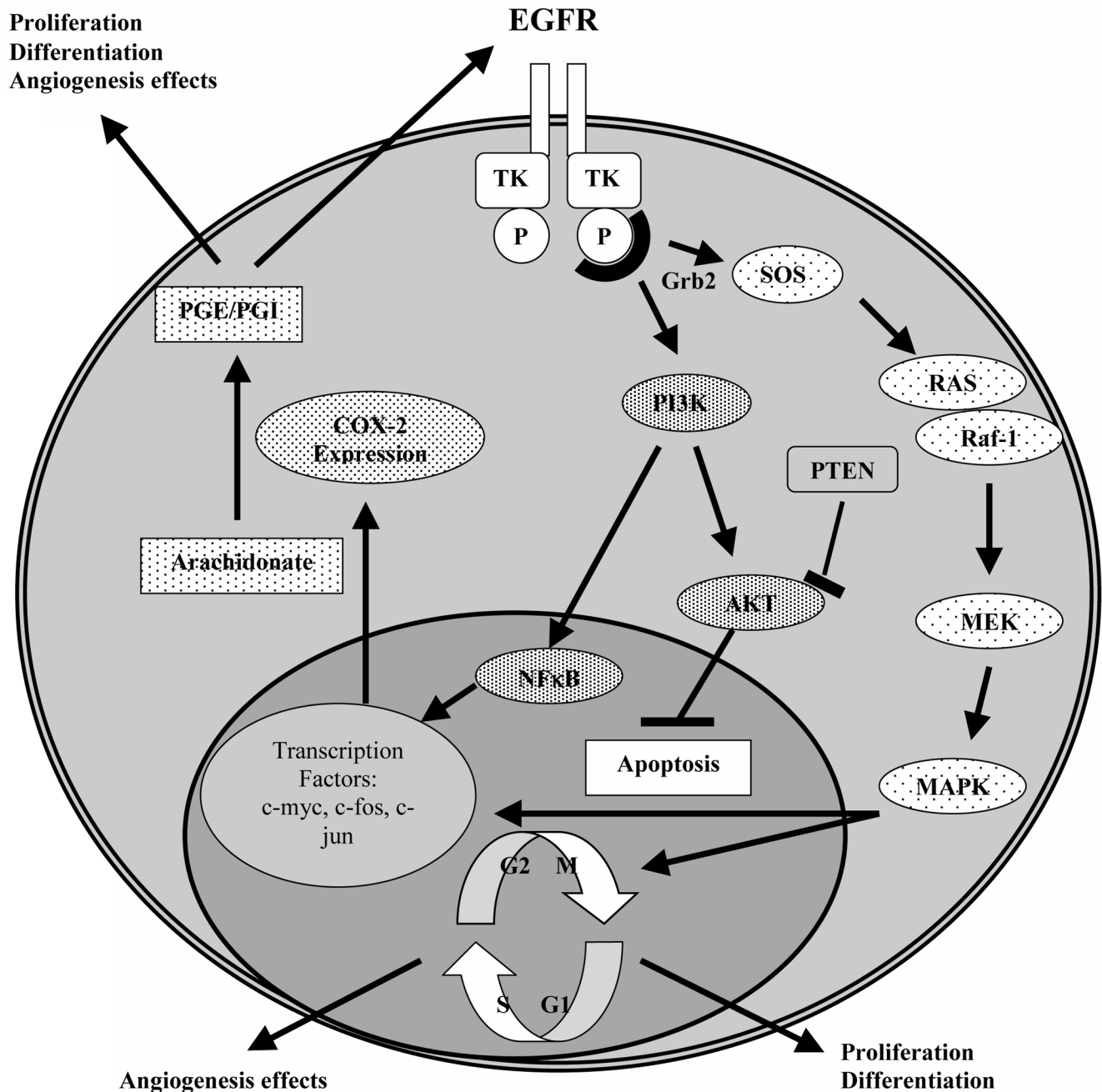


Figure 2. Cell signaling pathways linking epidermal growth factor (EGFR) and cyclooxygenase 2 (COX-2). EGFR activation leads to the initiation of a signaling pathway that includes the molecules Grb-2, SOS (Son of Sevenless), Ras, Raf and finally activates mitogen-activated protein kinase (MAPK). This results in activation of a group of nuclear transcription factors (c-myc, c-fos, c-jun). They initiate transcription of genes involved in the regulation of cell proliferation and differentiation and also induce transcription of COX-2. A second link involves the nuclear transcription factor kappa B (NFκB). EGFR ligand binding leads to activation of phosphati-dylinositol-3-kinase (PI3K) which in turn activates the downstream serine/threonine kinase, AKT, promoting cell survival. PI3K is involved in the activation of NFκB and the result is increased COX-2 transcription and expression.

studies indicate potent inhibition of erbB1 and erbB2, leading to tumor growth arrest and apoptosis [35]. Clinical data about the safety and tolerance of these agents must be available before these drugs can be used in chemoprevention studies.

Activation of the erbB1 leads to downstream ras activation. The *ras* gene is mutated in ~40% of NSCLC cases and this leads to

farnesylation and activation of the ras protein [33]. The farnesyl transferase inhibitors (FTI) were designed to block ras activation and the downstream signaling pathway. They also block other signal proteins that require farnesylation, such as Rho and raf. In phase I studies, the dose-limiting toxicities of FTIs have been myelosuppression, neurological complications, nausea, vomiting,

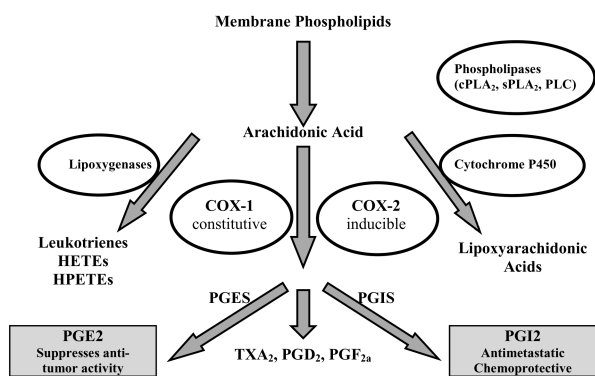


Figure 3. The arachidonic acid pathway. COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroxyperoxyeicosatetraenoic acid; PG, prostaglandin; PL, phospholipase; TXA₂, thromboxane A₂.

diarrhea and fatigue. Other significant toxicities included skin sensitivity and rash, but in general, these agents were well tolerated at doses that generated pharmacologically significant plasma concentrations [36].

The eicosanoid pathway

Cyclooxygenase (COX) catalyzes the synthesis of prostaglandins (PGs) from membrane arachidonic acid (Figure 3). The first step in the synthesis of PGs is the hydrolysis of phospholipids to produce free arachidonate, a reaction catalyzed by phospholipase A₂ [37]. Molecular oxygen is then added to arachidonic acid in a reaction catalyzed by the cyclooxygenase activity of COX. This reaction produces an unstable product, PGG₂. PGG₂ is rapidly converted to PGH₂ by the peroxidase activity of COX. PGH₂ is the common precursor for all other prostanoids (e.g. prostaglandins and thromboxanes), in reactions catalyzed by specific synthetases. Several of these terminal pathway molecules in the COX and lipoxigenase (LOX) pathways, such as PGE₂ and 5-, 8- and 12-LOX, appear to promote carcinogenesis and metastases, while other products, such as PGI₂, 15-LOX-1 and 15-LOX-2, promote differentiation and apoptosis.

There are two isoforms of COX, COX-1 and COX-2. COX-1 is a housekeeping gene, constitutively expressed in most tissues and mediating normal physiological functions. In contrast, COX-2 is undetectable in most normal tissues but is induced by inflammatory and mitogenic stimuli.

Several lines of evidence suggest that COX-2 is important in carcinogenesis [38]. First, increased amounts of COX-2 are commonly found in both premalignant tissues and malignant tumors, including cancers of the head and neck, esophagus and lung, reflecting the effects of oncogenes and growth factors [39]. Importantly, wild-type and not mutant p53 suppressed COX-2 transcription. Second, genetic and pharmacological studies showed extensive evidence that COX-2 is mechanistically linked to the development of cancer [40]. Third, tobacco specific carcinogens, such as the β -adrenergic receptor agonist 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), releases arachidonic acid, up-regulates COX-2 expression and stimulates proliferation in lung adenocarcinoma cell lines [41]. There is also increasing evidence that nicotine may induce COX-2 expression. COX-2 appears to be

implicated in tumorigenesis in a number of different ways such as the inhibition of apoptosis and the enhancement of angiogenesis. It has also been shown that COX inhibitors, such as indomethacin, contribute positively to the induction of an immune response in NSCLC.

The relationship between non-steroidal anti-inflammatory drug (NSAID) use and lung cancer has been examined in several epidemiological studies. These studies suggest that aspirin and NSAID ingestion may reduce the risk of developing lung cancer. Pre-clinical data have shown the chemopreventive effects of NSAIDs for lung tumorigenesis. In several studies using mouse models, NSAIDs could prevent the development of lung tumors induced by tobacco carcinogens by inhibiting COX-2 and by inducing apoptosis in the premalignant cells. Encouraged by these data, treatment with COX-2 inhibitors (celecoxib) for chemoprevention is already under clinical investigation, such as in the NCI-G01-1966 trial, with celecoxib for primary prevention in high-risk tobacco smokers. Although the question remains: what is the optimal dose of celecoxib for chemoprevention.

Recent data from transgenic mouse studies at the University of Colorado Cancer Center indicate that high levels of prostaglandin synthetase (PGIS), that lead to increased amounts of prostacyclin (PGI₂), result in reduced tumorigenicity in mice exposed to lung cancer carcinogens (Figure 3). A chemopreventive approach is to maintain a high level of PGI₂ in high-risk patients by giving an oral PGI₂ analog (iloprost). This and related agents are approved for use in the treatment of several conditions, including thromboarteritis obliterans, primary pulmonary hypertension and others. Iloprost is currently under investigation in a phase I/II chemoprevention study at the University of Colorado Cancer Center. The primary objective of that study is to see if iloprost can reverse premalignant histological changes. The secondary end point is to examine the effect on the Ki-67 proliferative index.

5-Lipoxygenase (LOX) is a key enzyme in the metabolism of arachidonic acid to leukotrienes. Increasing evidence suggests that LOX-catalyzed metabolites have an impact on the development and progression of cancers [42]. Compared with normal tissues, significantly elevated levels of LOX metabolites have been found in lung, prostate, breast, colon and skin cancers. LOX-mediated products elicit diverse biological activities important for neoplastic cell growth, influencing growth factor and transcription factor activation, oncogene induction, stimulation of tumor cell adhesion and regulation of apoptosis. Agents that block LOX's catalytic activity may be effective in preventing cancer. Pharmacological agents inhibiting the LOX-mediated signaling pathways (e.g. zafirlukast) are already being used in the treatment of inflammatory diseases, such as asthma, arthritis and psoriasis. Preclinical studies have demonstrated that lipoxygenase inhibitors may have benefits as preventive agents of lung tumorigenesis [43] and should be studied in human trials.

The combination of COX and LOX inhibitors could also be an interesting chemopreventive approach. Preclinical data indicated that inhibition of the COX pathway can be answered with shunting into the LOX pathway. Interestingly, there are efforts being made to develop dual inhibitors, able to block both the COX and LOX metabolic pathways. These dual inhibitors possess a wide

Table 4. Lung cancer chemoprevention trials with intermediate biomarkers

Trial [ref.]	No. of patients	Biomarker	Intervention (versus placebo)	Result
Heimbürger et al. [54]	73	Sputum atypia	Folate + B ₁₂	Increased reversal of atypia in treated subjects ($P = 0.02$) ^a
Arnold et al. [11]	138	Sputum atypia	Etretinate	No difference of atypia in treated subjects ($P = 0.45$)
McLarty et al. [14]	755	Sputum atypia	BC + retinol	No significant reduction in sputum atypia after treatment
Van Poppel et al. [55]	114	Frequency of micronuclei in sputum	BC	27% lower micronuclei count in the treatment group (95%CI 9% to 41%)
Lee et al. [12]	86	Bronchial metaplasia	Isotretinoin	No significant metaplasia index change in the treatment group ($P = 0.37$)
Kurie et al. [13]	82	Bronchial metaplasia	Fenretinide	No significant decrease in metaplasia index ($P = 0.79$)
Lam et al. [46]	112	Bronchial dysplasia	ADT	No difference in improvement of histology but significantly lower progression in ADT group (8% versus 17%, $P < 0.001$)
Soria et al. [56]	57	Expression hTERT	Fenretinide	Statistically significant reduction of hTERT expression in treated subjects ($P = 0.01$)

^aReanalysis of these data found no significant difference between the placebo and treatment groups.

ADT, anethole dithiolethione (5-[*p*-methoxyphenyl]-1,2-dithiole-3-thione); B₁₂, vitamin B₁₂; BC, β -carotene; hTERT, human telomerase reverse transcriptase catalytic subunit.

range of anti-inflammatory and anti-proliferative activity and appear to be almost exempt from gastric toxicity, which is the most troublesome side-effect of COX inhibitors [44].

Recent data indicate that COX inhibitors can inhibit signaling via the EGF receptor (Figure 2). In addition, EGF has been shown to stimulate COX-2 expression, whereas EGFR and HER2 inhibitors markedly decrease COX-2 activity. A combined blockage of both pathways (COX-2 and EGFR) has already been studied with positive results in colon cancer prevention [45]. Such an approach could also be attractive for lung cancer.

The dithiolethiones are a class of organosulfur compounds, including oltipraz (5-[2-pyrazinyl]-4-methyl-1,2-dithiol-3-thione) and anethole dithiolethione (ADT; 5-[*p*-methoxyphenyl]-1,2-dithiole-3-thione). Epidemiological studies associate a diet high in vegetable consumption with a lower risk for many cancers [3]. These vegetables contain many potential cancer-protective substances including dithiolethiones. In animal models, the administration of these compounds protects rodents from the development of lung and stomach tumors in response to alkylating agents. The dithiolethiones appear to act via different mechanisms, including the inhibition of cell replication, and predominantly by increasing the expression or activity of phase II enzymes, such as glutathione *S*-transferase (GST). In addition, ADT increases the intracellular level of glutathione and displays free radical scavenger properties. Lam et al. performed a randomized phase IIb study to determine the effects of ADT in smokers with bronchial dysplasia, identified by LIFE bronchoscopy [46]. One hundred and twelve current or former smokers were randomly assigned to receive placebo or ADT at 25 mg orally thrice daily for 6 months. The primary end point was response defined by improvement in histology. No response difference was seen between the two groups. However, as an undefined end point, the progression rate was statistically

significantly lower in the ADT group (8%) than it was in the placebo group [17%; $P < 0.001$, difference in progression rate, 9% (95%CI 4% to 15%)]. This trial suggests that in smokers, ADT is potentially an effective chemoprevention agent for lung cancer. This is the first phase IIb lung cancer chemoprevention trial to use bronchial histology as the primary intermediate end point biomarker.

From a chemopreventive point of view, the future challenge is to find the most optimal targeted therapy (or combination of therapies, Table 3) with respect to the fact that certain requirements for feasibility and low toxicity need to be met in such a category of patients who are at high risk for developing lung cancer, but have not yet developed cancer.

Intermediate markers for response evaluation

Considerable research is focusing on the identification of biomarkers as surrogate or intermediate end points in place of overt cancer in cancer chemoprevention trials (Table 4). Identification and validation of such markers is important as it would allow smaller trials of shorter duration than when using cancer as the end point. This intermediate biomarker concept is used as well in the management of other diseases. For example, cholesterol quantitation is used to indicate the progression of atherosclerosis as a surrogate in determining the risk of myocardial infarction.

The use of intermediate biological end points is often referred to as reversal of premalignancy by successful chemoprevention. Several requirements need to be fulfilled by a potential intermediate marker: it should be closely involved in the process of carcinogenesis so that modulation of expression correlates with the course of the disease; it should have different expression levels in normal versus preneoplastic tissue and expression/evaluation

should be easily reproducible. Before integration into routine use, potential markers need to be validated in prospective clinical trials.

Promising markers include morphologic changes of the bronchial epithelium, as well as cytogenetic and molecular changes. Research is focusing on intraepithelial neoplasia, a premalignant condition exemplified by colorectal adenomas or cervical intraepithelial neoplasia. To develop a more dependable outcome than a pathological judgement on a single biopsy, the metaplasia index—a semiquantitative method to characterize the degree of metaplasia in a number of bronchial biopsies—has been introduced [47]. The level of metaplastic/dysplastic changes in the bronchial mucosa seems to be dependent on the selected study population rather than differences in histopathological interpretation. In a study from the M.D. Anderson Cancer Center (participants with >20 pack-years and having quit smoking for ≥ 1 year), metaplasia was the most prevalent change, while only a few patients had dysplasia [47]. In a study from the University of Colorado Cancer Center, >50% of high-risk patients (>30 pack-years, chronic obstructive pulmonary disease, sputum atypia) had moderate dysplasia or worse [48]. In the latter randomized study, it was shown that LIFE bronchoscopy improves the detection of preneoplastic bronchial lesions significantly compared with traditionally used white-light bronchoscopy. Thus, the inclusion of LIFE bronchoscopy is recommended for the evaluation of chemoprevention studies.

An intermediate marker for chemoprevention studies of preneoplasias must be reproducible. Because metaplasia can spontaneously reverse, while dysplasia rarely does, dysplasia should be a superior intermediate marker for treatment response. Importantly, morphologic criteria for dysplasia have been defined in the recent World Health Organization (WHO) classification [20]. This WHO classification of bronchial preneoplastic changes (Figure 1) has been found to be highly reproducible by a panel of lung cancer pathologists [49]. However, more data are needed to determine the prognostic implications of the different levels of epithelial changes in the bronchi of high-risk individuals and sparse data are available for the natural course of the different levels of bronchial dysplastic changes.

Biological/genetic markers, such as Ki67, MCM2, p53, epithelial growth factor receptor (EGFR) and HER2, have been studied by immunohistochemistry in bronchial biopsies [50] (Figure 4). These markers need to be validated as useful intermediate biomarkers in clinical-pathological studies before they can be routinely used as intermediate end points for chemoprevention studies. Furthermore, pathway-specific, ‘downstream’ activated proteins need to be evaluated as surrogate markers for the biological effect of the different chemopreventive agents (Figure 2). New markers are under development, such as microchip gene array and proteomic evaluation of multiple proteins. So far it is too early to predict their usefulness as intermediate biomarkers in chemoprevention studies.

Of importance for future studies is the validation of the different candidates for intermediate biomarkers. Such validation studies are most properly studied first in nested case–control studies in untreated high-risk cohorts. Samples for such studies require care-

ful follow-up and a sufficient number of cases (i.e. individuals who later develop lung cancer) and controls (individuals who do not develop lung cancer). At the University of Colorado a high-risk cohort study [>30 pack-years and chronic obstructive lung disease (COPD)] was started in 1993 and includes >3000 individuals. Patients with sputum atypia are followed annually with sputum cytology. Biomarker validation of tumor suppression gene methylation is currently ongoing in a nested case–control study [51].

Optimal target population for chemoprevention studies

One of the challenges for future studies is to define the optimal high-risk study population for chemoprevention studies. Different populations have been studied in primary, secondary and tertiary chemoprevention studies. The delineation of the optimal high-risk populations is not well defined but several studies have shown that it is possible to identify high-risk persons [18]. Most trials selected the study populations based on smoking history [8, 9, 11–13]. Other studies included risk factors like radon exposure, obstructive lung disease, prior resected stage I cancer and family history [3]. Preliminary data from the University of Colorado high-risk cohort study, including a population with a history of more than 30 pack years and COPD (defined by spirometry), showed an accumulated risk of developing lung cancer of almost 20% after 10 years [51]. Several studies demonstrated a clear dose–response relationship between the development of lung cancer and the degree of exposure to cigarette smoke, measured in pack-years. However, there is strong evidence that smoking duration and the age at which smoking began is more important than the number of cigarettes per day. The relative risk of developing lung cancer among male smokers with 20 pack-years is 11.59 when they have smoked 20–29 cigarettes a day (approximately one package) for 20–29 years, but 29.66 when they have smoked 10–19 cigarettes a day (approximately half a package) for 40 years. These data support the use of a smoking history of at least 30 pack-years and an average consumption of at least 20 cigarettes a day to identify high-risk smokers. Preliminary data from the University of Colorado high-risk cohort study showed a significantly lower level of morphological changes in former smokers compared with current smokers [52].

Another method of defining high-risk smokers is to use intermediate markers, such as preneoplastic changes in the bronchi. In an autopsy study, sections were taken from the bronchial tree from 445 men who did not die of lung cancer [19]. Advanced histological changes occurred far less frequently in non-smokers than in cigarette smokers and increased in frequency with amount of smoking [19]. However, the relationship between preneoplastic changes and smoking is most likely dependent on individual (genetic) factors.

The role of sputum atypia as a risk factor for the detection of bronchial preneoplasia and development of lung cancer is a subject currently under investigation. In the Colorado High-risk Study, 55% of individuals with sputum atypia had high-grade dysplasia at bronchoscopy [48]. Subjects with moderate or worse atypia on sputum had an increased odds ratio of 3.2 for developing lung cancer compared with individuals in the high-risk cohort without sputum atypia [52]. By adding DNA hypermethylation of

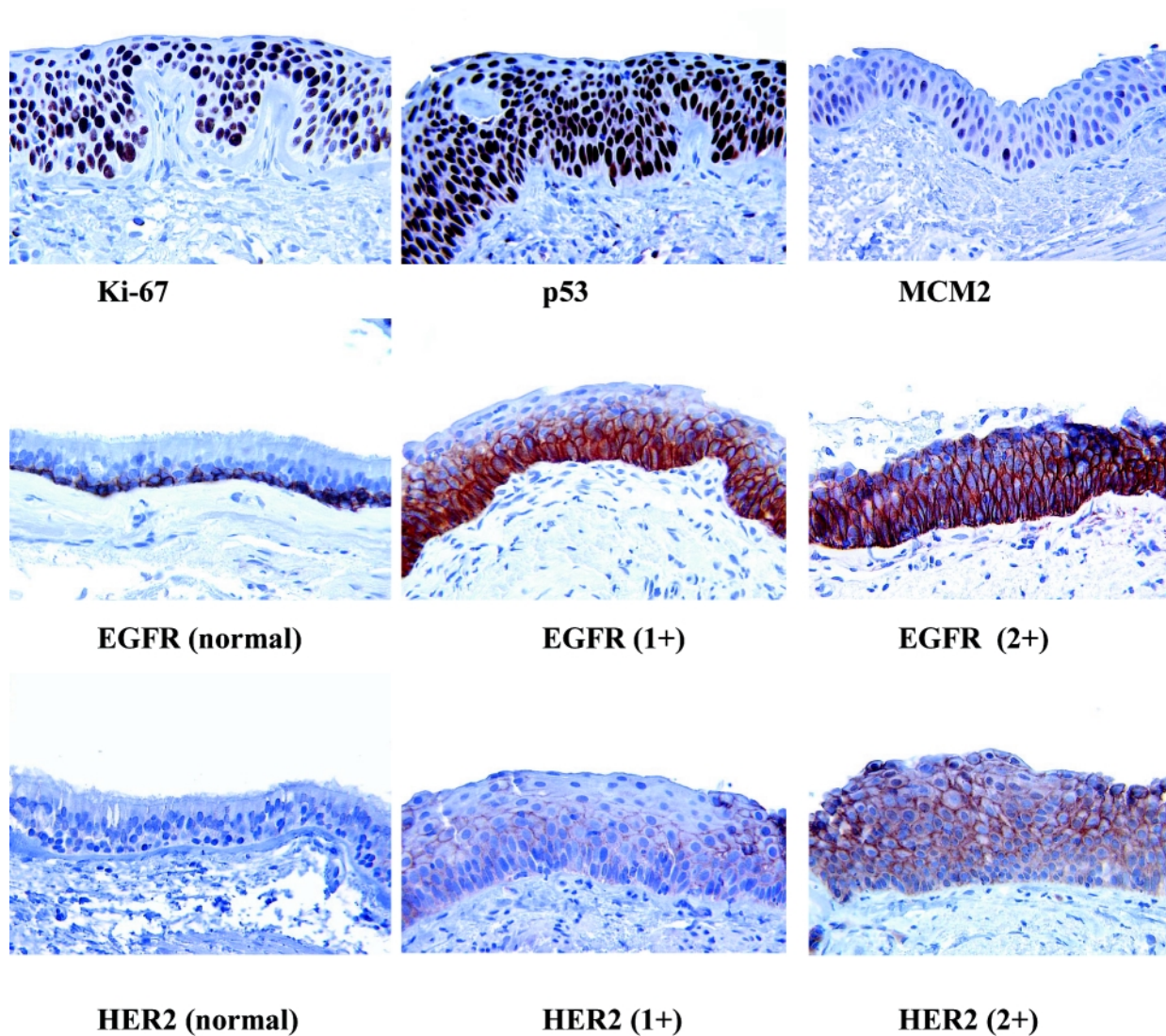


Figure 4. Potential immunohistochemical biomarkers. Immunohistochemistry on paraffin-embedded sections with antibodies for Ki-67, p53, MCM2, EGFR and HER2. Marker expression for Ki-67, p53 and MCM2 is the average percentage of cells (in a sample of 400 cells from each biopsy) that express the marker. EGFR and HER2 are in these cases evaluated from 0 to 2. Normal bronchial histology have EGFR/HER2 expression mostly in the basal cell layer (0), and the expression increase by increased dysplastic changes to half thickness of the epithelium (1+) or expression throughout the full thickness of the epithelium (2+).

seven genes in the sputum to the sputum atypia, preliminary data from 33 cases and 33 controls have shown that the risk increased to 10.2 [51]. These findings need to be verified in a larger study population.

Patients who previously had curative treatment for a primary lung or head and neck cancer have an increased risk of a second primary cancer of the aerodigestive tract (1–3.4% per year [53]). These patients are good candidates for intermediate marker-based chemoprevention studies with histology as a primary end point. Ongoing chemoprevention studies in this high-risk population will elucidate the role of several biomarkers as markers for chemopreventive effect and/or prediction of outcome. They are also an excellent group for randomized trials with second cancers as the primary end point, as described above.

With the development and utilization of low-dose spiral computed tomography for screening and early detection of lung

cancer, new challenges have evolved to characterize the subcentimeter small lesions. So far, very little is known about the biology of these lesions. Many of the detected lesions are characterized as atypical adenomatous hyperplasia (AAH) and are considered as premalignant lesions. A chemopreventive treatment approach of the AAH lesions should be considered when sufficient biological information is available. However, because these lesions are relatively rare, it seems crucial to establish a registry for patients with these small lesions, so careful follow-up can give a clinical/biological profile to distinguish between those patients who develop invasive cancer and those who do not.

Conclusions

The continuing magnitude and severity of the lung cancer problem make it imperative to enhance smoking cessation campaigns and

to make progress in early detection and chemoprevention. With the expanded understanding of the molecular and biological mechanisms of lung cancer development, new specific targets for prevention are being identified. The identification of appropriate high-risk patient groups, i.e. a high-risk study population where more than one in five patients will develop lung cancer, is crucial and will in the future enable smaller studies to be designed. When lung cancer incidence is the primary end point, large trials will accrue subjects over many years. It is important to integrate the growing biological knowledge in a rational timeframe. The design of successful chemoprevention trials in the future requires identification and validation of intermediate end points that are sufficiently predictive of lung cancer development.

Lessons from the treatment of advanced lung cancer and the increased understanding of important cellular signaling pathways point out that inhibiting these different regulatory cascades might prevent/reverse lung carcinogenesis. Because of the complexity of the signaling network, combinations of targeted therapies might be an interesting possibility to be tested in chemoprevention studies. However, it is important that such studies are well designed in order to gather as much clinical/biological information as possible for future chemoprevention studies.

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