Chemoprevention of lung cancer: from concept to reality

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Abstract

Among all cancer, lung cancer is the leading cause of cancer death in the western world. The poor lung cancer survival figures argue powerfully for new approaches to control this disease such as chemoprevention, that has been defined as the use of agents that reverse, suppress or prevent lung carcinogenesis.

Over 80% of lung cancers are attributed to tobacco and carcinogens from cigarette smoke form the unquestionable link between nicotine addiction and lung cancer.

Confoundingly epidemiological studies show that not more than 15% of heavy smokers will ultimately develop lung cancer. The fact that 85% of heavy smokers will not develop lung cancer points to differences in susceptibility. Dietary and genetically determined factors seem to play an important role in modulating individual susceptibility and are closely linked to the chemoprevention approach.

Developments in tumor biology and chest radiology have given rise to optimism regarding to earlier diagnosis and improved possibilities for the management of preneoplastic – and early invasive lesions. The current article summarizes the chemoprevention efforts in lung cancer during the last two decades and identifies novel strategies for chemoprevention studies in high-risk individuals based on the developments in molecular targeted therapies and developments in response evaluation by using intermediate biomarker endpoints.

Key words: lung cancer, chemoprevention, carcinogenesis.

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Introduction

Lung cancer is the leading cause of cancer death in the western world. Despite several decades of intensive research in the thoracic oncology area, the prognosis for the patients with lung cancer is still poor, and less than 15% of the patients survive 5 years after the primary diagnosis. This grim outlook for lung cancer patients is mainly due to the lack of measure for early diagnosis and insufficient treatment possibilities for systemic disease. More than 2/3 of the patients present with regional or metastatic disease at time of primary diagnosis.

Chemoprevention, defined as the use of naturally occurring or synthetic agents to prevent, inhibit or reverse the process of carcinogenesis, is a relatively new approach to cancer prevention that has a precedence in other areas of medicine such as cardiology, in which cholesterol–lowering and platelet anti-aggregating agents are administered to prevent coronary heart disease in individuals with elevated risk. This concept has been studied in lung cancer but so far without significant success as is summarized below. However recent developments in biology and radiology have given new possibilities for early detection of preneoplastic and early neoplastic lesions. Along with these developments new avenues for chemoprevention are emerging [1]. It is within the scope of this article to describe the basis of chemoprevention and to point to the future perspectives based on recent developments in biology, bronchoscopic techniques and radiology. One concept of these new strategies is to already consider the carcinogenic process as a disease and clinical manifest lung cancer as the 'end stage' of the disease.

Tobacco carcinogenesis

Cigarette smoke contains a mixture of carcinogens, including a small dose of polycyclic aromatic hydrocarbons (PAH's) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) among other lung carcinogens, tumor promoters, and co-carcinogens [2].

Carcinogens such as PAH's and NNK require metabolic

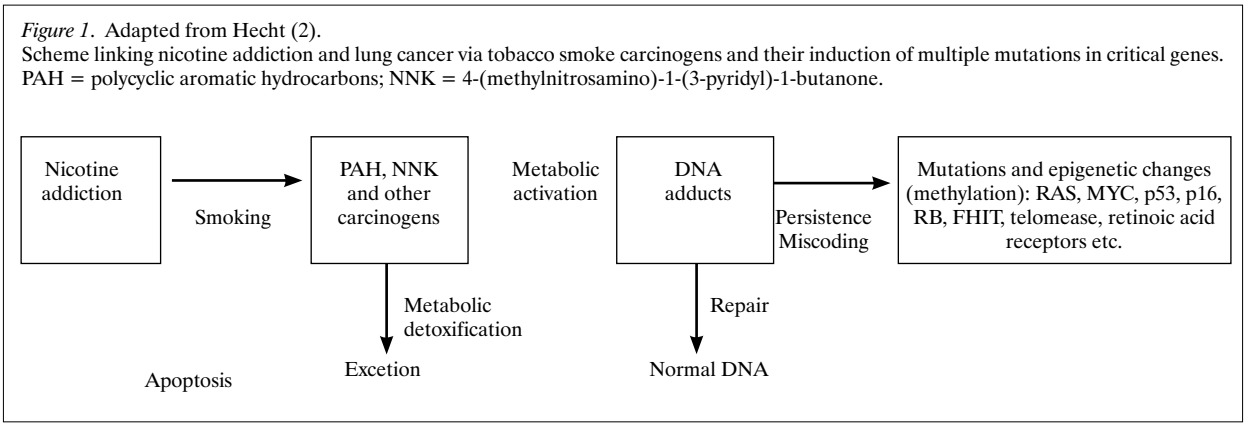

Figure 1. Adapted from Hecht (2).
Scheme linking nicotine addiction and lung cancer via tobacco smoke carcinogens and their induction of multiple mutations in critical genes. PAH = polycyclic aromatic hydrocarbons; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

activation to exert carcinogenic effects. The activation pathways are competing with detoxification pathways and the balance between activation and detoxification is assumed to affect the cancer risk. The genes for the cytochrome P-450 carcinogen metabolizing enzymes (activation) have been found polymorphic and also the glutathione transferases (detoxification enzymes) are polymorphic. Approximately 40-50% of the human population has a so-called null genotype, that has a modest association with lung cancer [3].

Successful metabolic activation will lead to the formation of DNA adducts, which are carcinogen metabolites bound covalently to DNA. If DNA adducts escape cellular repair mechanisms and persist, they may cause miscoding, resulting in a permanent mutation [4,5] (*Fig. 1*).

In recent case-control studies defective repair of genetic damage and increased sensitivity to mutagens have been associated with increased susceptibility to lung cancer [6]. Also the DNA excision repair pathway has been suggested to influence the individual susceptibility for lung cancer [7]. From molecular studies it is known that genetic alterations (scars) persist after the cessation of tobacco use [8]. In this respect it is important to mention the fact that ex-smokers carry an elevated risk of lung cancer for many years and that most of those who now develop lung cancer in the US are ex-smokers [9,10].

In the last 10-20 years our understanding of the molecular and biological basis of lung cancer has significantly expanded. However, we must not forget that traditional epidemiological studies have provided reliable indicators of host susceptibility to tobacco carcinogenesis.

A number of case-control studies have suggested that females are more susceptible to the carcinogenic effects of tobacco smoke than men [11,12]. This observation has been confirmed by the analysis of smoking habits in a population of lung and head & neck cancer patients, that took part in a study aiming at preventing second primary tumors, revealing significantly less exposure to tobacco (pack-years) before occurrence of the first cancer in females in comparison with males [13]. It is not surprising that smoking has been found to interact synergistically with a family history of lung cancer [14,15].

Diet

The relationship between diet and lung cancer has been extensively explored in many ecologic and case-control studies and there are many leads to support an association between a high intake of fruits and vegetables and a reduced risk of lung cancer [16]. Much effort has been expended to identify the specific components of these foods that may be responsible for the lower lung cancer risk. In the majority of studies, attention has focused on the pro-vitamin A carotenoids, particularly beta-carotene because of their antioxidant properties and the importance of vitamin A in cell growth and differentiation.

More recently other micronutrients have been identified as having the potential to decrease lung cancer risk, including vitamin E, selenium, isothiocyanates, allyl sulphur compounds and green tea polyphenols. While Vitamin E (a term that refers to eight natural compounds, including the tocopherols) failed to lower the lung cancer risk in a large randomized (ATBC) trial a protective effect of selenium was found in a Skin Cancer Prevention Trial [17].

Isothiocyanates are non-nutrient compounds in cruciferous vegetables that can influence P-450 enzyme levels and enhance detoxification. A recent series of newly diagnosed lung cancer cases had significantly lower isothiocyanate intake when compared with controls. In this study also glutathione-S-transferase (GST) (null) genotype and smoking were associated with increased lung cancer risk, suggesting that smokers with low intake of isothiocyanates and a null GST genotype carry an extra risk [18]. Allyl sulphur compounds that may induce apoptosis, and green tea polyphenols have also been mentioned as potential preventive agents for NSCLC [20,21].

In addition attention has focused on cooking practices. Increased lung cancer risk has been noted as a consequence of high intake of heterocyclic amines, which are produced when meats are cooked at high temperatures [21].

Overall, the epidemiological data support the hypothesis that a different intake of dietary compounds could modulate the risk of lung cancer. The confirmation of this hypothesis, however, can only be provided by carefully designed prospective trials that balance for smoking behavior and ideally also take diet-gene interactions into account as in the recent isothiocyanate study [18,22].

Field Cancerization and Multi-step Carcinogenesis

The rationale for chemoprevention is based on two principles of tumor biology common to most epithelial can-

cers: field cancerization and multi-step carcinogenesis. Field cancerization was first described by Slaughter et al in 1953, who identified epithelial hyperplasia hyperkeratinization, atypia and also carcinoma in situ in grossly normal appearing epithelium adjacent to cancers of the oral cavity [23]. These histologic changes throughout the oral epithelium suggested that the development of malignancy in the aerodigestive tract is not a random event but rather the result of diffuse changes that are present throughout the epithelium. The existence of diffuse premalignant changes in the aerodigestive tract confirms the observation that patients who survive a first cancer in this region are subsequently susceptible to the development of a second primary tumor [24].

Specific genetic alterations seem to be involved. For example, it has been shown that highly specific deletions in the short arm of chromosome 3 occur during hyperplasia, the earliest stage on the way to lung cancer [25]. Similar evidence exists for deletions of the short arm of chromosome 9 and 17 [26]. One of the genes on chromosome 3 is the fragile histidine triad gene. Similarly to the p53 gene on chromosome 17 this gene is thought to have a tumor-suppressor function [27].

Although the series of events eventually leading to the development of lung cancer are not yet completely elucidated, it is generally accepted that lung cancer develops in a stepwise fashion as the result of multiple genetic events. Suppressing one or more of the pre-invasive steps may impede the development of cancer.

Retinoids

Due to their ability to regulate cell proliferation and differentiation retinoids represent a potentially useful class of drugs in the chemoprevention field. In multiple animal model systems at different organ sites and with a variety of inducing carcinogens retinoids were found to be preventive [28].

A randomized phase II study with cis-retinoic acid in heavy smokers suggested however that smoking cessation was more important than the actual prevention with retinoids [29].

Several randomized clinical chemoprevention trials have been completed in the 1990s. Among these the Alpha Tocopherol Beta Carotene (ATBC) and the Beta Carotene and Retinol Efficacy (CARET) trials [30,31]. The ATBC trial accrued men, all heavy smokers and tested the effects of dietary supplementation of beta-carotene and alpha-tocopherol. Against all expectations this trial did not show any protective effect from either alpha-tocopherol or beta-carotene. On the contrary, beta-carotene was associated with a statistically significant increase (18%) in lung cancer incidence and mortality (8%). The detrimental effect of beta-carotene was confirmed by the CARET study, also involving smokers. CARET revealed a 28% higher rate of lung cancer and 17% higher overall death rate in those participants taking beta-carotene. The findings of ATBC and CARET have clearly been a shock to the prevention community but they have shown the unquestionable importance of large-scale controlled (randomized) studies. Since the detrimental effect of beta-carotene was seen primarily in smokers, it has been hypothesised that cigarette smoke in the lungs, which is highly oxidizing, may interact with beta-carotene, yielding unstable by-products that could have pro-oxidant activity. A recent experimental study in ferrets exposed to cigarette smoke and beta-carotene suggests that such an interaction exists [32]. Smokers should therefore avoid beta-carotene supplementation and the importance of smoking cessation is once more emphasized. An explanation for the lack of preventive effects of retinoids in the human situation is provided by the observation that the nuclear retinoid receptor beta, which act as transcription factor, was found to be suppressed in preneoplastic bronchial lesions and NSCLC's [33]. Promoter methylation and allelic loss have been thought to be responsible for this suppression [34].

Recently a new class of retinoids has been identified that seems to be more effective in growth inhibition and induction of apoptosis of lung cancer cell lines [35]. This observation together with the favorable early clinical experience with bexarotene, a retinoid-X-receptor selective retinoid, in NSCLC patients [36] and the negative impact of loss of retinoic acid receptor beta in stage I NSCLC patients [37] indicates that the retinoid receptors will remain a target for lung cancer chemoprevention studies. We must however avoid the premature start of large and costly comparative chemoprevention trials i.e. before validation studies with intermediate markers of carcinogenesis have shown an unequival positive outcome.

Second Primary Tumors

One of the earliest studies with relevance to lung cancer was a trial in 103 patients with previous head & neck cancer in which the effect of 13-cis-retinoic acid on recurrence and second primary cancer was studied [38]. The incidence of second primary tumors was significantly lower in the treatment arm (4% versus 24%). The second trial involved 307 patients with early stage lung cancer, randomized after complete surgical resection to receive either retinyl palmitate or no further treatment [39]. Also in this study there was a significant difference in the frequency of second primaries (12% versus 21%).

The large EUROSCAN study designed to assess the effects of retinyl palmitate and N-acetylcysteine (an aminothiol precursor of reduced glutathione) in almost 2600 patients with early stage head and neck cancer or lung cancer following treatment with curative intent, did not confirm the positive outcomes of the previous studies with 13-cis-retinoic acid and retinyl palmitate. In contrast to expectations this study did not show any benefit for a 2-year supplementation with retinyl palmitate and/or N-acetylcysteine in a population at risk for second primary tumors and tumor recurrences in the upper and lower airways [40].

A similar result was recently obtained in the US NCI intergroup trial with 13-cis-retinoic acid to prevent second primary tumors (SPT) in Stage I NSCLC. 13-cis-retinoic acid did not impove the rate of SPT's or mortality [41]. Subgroup analyses suggested that 13-cis-retinoic acid might have been harmful in smoking patients and beneficial for those patients belonging to the category of never smokers. The EUROSCAN and the NCI intergroup trials underline the importance of large confirmatory trials. Agents long assumed to be helpful may be found to have no preventive effect at all, when tested in a rigorous way.

EUROSCAN also strengthened the importance of smoking cessation: participants who had permanently stopped smoking had a better survival than those who continued smoking.

Intermediate markers

When invasive cancer is taken as an endpoint, as has been done in the aforementioned studies, significant investments in time and money are needed. One approach to avoid the primary evaluation of potential chemopreventive agents in large clinical trials with cancer as primary endpoint is to use intermediate biological endpoints. This is often referred to as reversal of premalignancy. Potential intermediate markers include microscopic changes of the bronchial epithelium, such as metaplasia, or more specific markers, such as cytogenetic or molecular changes. In an effort to develop a more dependable outcome than a pathologic judgement on a single biopsy, the metaplasia index, a semiquantitative method to characterize the degree of metaplasia in a number of bronchial biopsies, was introduced [42].

However, differences in study population might reflect differences in the level of metaplastic/dysplastic changes in the bronchial mucosa, which needs to be taken into account if morphology is used as intermediate marker. In a study from MD Anderson most of the high-risk patients had metaplasia, but very few patients had dysplasia [43], while in a study population of the University of Colorado Cancer center about 55% of the high-risk population had moderate dysplasia or worse at bronchoscopy [44]. In the Colorado study fluorescence bronchoscopy (LIFE) was shown to improve the detection of preneoplastic bronchial lesions when compared with white light bronchoscopy. Essential is a reproducible classification of (i.e. minimal interobserver variation) of these lesions. Hirsch et al. undertook an interactive study on the Internet that revealed very little interobserver variability among pathologists involved in this area [45]. Another issue is the reversibility of preneoplastic changes. There is increasing evidence that metaplasia is a spontaneous reversible condition whereas dysplasia is not [46,47]. Consequently metaplasia may not be a reliable intermediate marker to study the efficacy of chemopreventive measures. More data from longitudinal analyses are needed to validate the prognostic implication of subtle epithelial changes in the airways. Based on new technologies such as microchip gene array or protein analysis, new markers are under development, but so far it is too early to predict whether these markers will indeed be useful as intermediate endpoints for chemoprevention studies.

New Classes of Chemopreventive Agents

Advances in molecular biology has led to a better understanding and knowledge about important pathways necessary for cancer development, and antibodies and small molecules have been developed to target specific proteins and block several important signaling pathways. Some of these molecules have attracted attention as potential chemopreventive agents opening a new era of therapeutic possibilities.

The identification of the *ErbB*-family (Epidermal Growth Factor EGFR, Her-2/*neu*, HER-3 and HER-4) as a family of receptors has been especially important. These receptors activate, after ligand binding, a cascade of biological- and physiologically reactions eventually leading to cell proliferation and apoptosis. These signaling pathways can be blocked at different levels. Preliminary studies of bronchial preneoplasia have demonstrated significant expression by immunohistochemistry of EGFR and HER-2 [48] and a chemopreventive treatment approach with antibodies or small molecules blocking these pathways seems to be an attractive approach (49). Likewise, activation of the EGFR involves *RAS* activation, which again requires a farnesylation. Thus, treatment with a farnesyl transferase inhibitor (FTI) might block this signaling pathway. In fact in the US a group of centers (Lung Cancer and Biomarker and Chemoprevention Consortium) are currently studying the chemopreventive effects of a combination of an EGFR inhibitor and FTI in a prospective randomized study in persons at risk for lung cancer with reversal of morphological changes and Ki-67 modulation as the primary and secondary endpoint.

The prostacyclin and the eucosonoid pathways are important for tumor function and progression. There are several ways to block prostaglandin synthesis, and COX-inhibitors are under clinical investigation as chemopreventive agents [49,50]. In addition several other molecular targets have been identified and the therapeutic potential is expanding rapidly. Today's challenge is to find the most optimal targeted therapy (or combination of therapies) given the fact that certain requirements for feasibility and low level of toxicity are needed for "healthy" populations at risk for cancer. Reflecting on all the new trials that are needed it cannot be underlined enough how important the need is for reliable intermediate biomarkers and robust models for response evaluation.

Conclusions

The understanding of the molecular and the biological mechanisms of lung cancer development has significantly expanded over the last 20 years. Multiple genetic and biological events have been identified that are considered essential for the carcinogenic process leading to the final malignant phenotype. Some of these lesions have been found closely associated with tobacco smoke exposure, and it has become increasingly clear that there is a wide variation in individual susceptibility to lung cancer. Valid risk markers are now appearing on the horizon, and more recently several novel agents with chemopreventive potential have been identified including molecules that block cellular receptors and important signaling pathways.

It is anticipated that after a period with increasing insight into lung carcinogenesis but without appreciable benefits of the chemoprevention approach in randomized trials, the road is now being paved for more successful studies in high-risk individuals. Current challenges are the identification of the most optimal high-risk population for chemoprevention studies and the validation of intermediate markers.

Considering the continuing lung cancer epidemic worldwide it is clear that we must continue our effort to strengthen the fundamental strategy of avoidance the exposure of carcino-

gens in parallel with the development of additional approaches such as chemoprevention.

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