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Chemoprevention in lung carcinogenesis – An overview

Nico van Zandwijk *

Department of Thoracic Oncology, Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Plesmanlaan 121, 1066 CX, The Netherlands

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Abstract

Lung cancer ranks among the most commonly occurring malignancies and is currently the leading cause of cancer-related death worldwide. This is due to its late diagnosis and relative resistance to standard oncological treatment approaches. The heavy burden of lung cancer and its treatment resistance have elicited an intense interest in the promising approach of chemoprevention. Chemoprevention is defined as a pharmacologic intervention to suppress or reverse the carcinogenic process and the lung is one of the most studied sites for cancer chemoprevention. This review, with a short update on pulmonary carcinogenesis, will summarize the available knowledge of chemoprevention trials and agents with a preventive potential in the 'lung field'.

Keywords: Chemoprevention; Carcinogenesis; Lung cancer; Tobacco smoking; Early detection; Lung cancer screening; Premalignancy; Vitamins; Antioxidants; Small molecules

1. Introduction

With around 1.2 million new cases each year, lung cancer is one of the most commonly occurring malignancies [1]. Despite all efforts in conventional treatment of lung cancer by surgery, radiotherapy and chemotherapy, the survival of lung cancer has seen only minor improvements over the last 25 years. The main reason for this lack of progress is related to the fact that lung cancer is a conglomerate of diseases that elicit symptoms at a relatively late stage. Thus, in most cases the initial diagnosis is made when the disease has significantly advanced and is beyond the stage of cure.

In many countries, the most important public health response for lung cancer has been focused on tobacco control, which has recently been reinforced by epidemiological evidence that smoking cessation even into the middle age is associated with a major reduction of the lung cancer incidence [2–4]

However, even if all goals of national anti-smoking efforts are met, a large number of former smokers will remain at risk for a significant number of years as is manifest in the US where former smokers account for about half of the new cases of lung cancers [5,6]. In addition, there is an alarming increase of tobacco consumption in developing countries and current smokers in the western world seem to harbor an increased risk [4,6]. It is clear that apart from eliminating or reducing the exposure to known carcinogens, emphasis should be placed on early diagnosis and treatment of preneoplastic or preinvasive lesions including chemoprevention. In this review, attempts are made to place topics germane to the understanding of lung cancer chemoprevention into their appropriate context. To that end, a critical discussion of current and prospective approaches using agents is preceded by a description of specific issues relevant to lung carcinogenesis.

* Tel.: +31 2051 22958; fax: +31 2051 22572.
E-mail address: zandwijk@nki.nl.

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2. Detection of "early" cancer

Spiral computer tomography (SCT) is acknowledged as the most sensitive diagnostic method for the detection of small intrapulmonary nodules [8,9]. A growing number of observational studies in subjects at risk suggest that a large majority of lung cancers can be detected as Stage IA cancers in a program of annual screening [10]. There is now a critical need for research to show that SCT is efficient and safe when used as a screening tool for individuals at increased lung cancer risk [11]. An additional issue is the follow-up studies of completely resected non-small cell lung cancer (NSCLC) patients. They are essential to describing second cancers that occur in the order of 1–3% per year [12]. The tendency to develop multiple independent lung cancers in persons chronically exposed to tobacco smoke carcinogens has been termed 'field cancerization' [13]. It reflects the clonal emergence of independent foci of carcinogen-induced cancer cells from different areas of the respiratory tract. With the growing number of large trials evaluating spiral CT lung cancer screening underway, there is a complementary opportunity to conduct chemoprevention research in these same cohorts. Examination of sputum from high-risk persons has been another active area of research. Hypermethylation of p16, an early step in lung along with other molecular targets have been detected in the bronchial epithelium of chronic smokers [14,15]. As p16 methylation status can be detected with high sensitivity in exfoliated cells, this is considered a potentially valuable tool for early detection [16]. Other investigators have proposed morphometric and proteomic changes in sputum from high-risk individuals as markers for early detection [17,18]. Additionally, the definition of high-risk individuals has been narrowed. In work from the University of Colorado Lung SPORE (Specialized Program of Research Excellence) COPD, adding the selection criteria of a $FEV_1 < 0.75$ to a 30+ pack-years smoking history in a cohort of 1798 persons, resulted in a lung cancer incidence as high as 4.6% [19]. While a growing fraction of new lung cancers are being detected in the peripheral airways, the growing capabilities of specialized endoscopic techniques such as fluorescence bronchoscopy, endobronchial ultrasound and optical coherence tomography are still expected to provide useful new information in the evaluation of the central bronchial tree [20].

3. Lung carcinogenesis

In addition to the concept of field cancerization discussed above, the idea that carcinogenesis evolves in a stepwise fashion is also fundament to chemoprevention. We now understand that there are a series of molecular steps in the development of a cancer including the accumulation of progressive changes from preinvasive histological changes to an invasive neoplastic process [21]. The earliest events of this process are thought to be the mutations, deletions, or polysomy in the chromosomes of epithelial cells chronically exposed to carcinogens. It is not clear that these genetic modifications initially translate into morphological changes [22]. Additional molecular events are necessary to induce the full malignant phenotype including independent cellular proliferation and the competence to invade a basement membrane. It has been proposed that multiple (10–20 or more) genetic events may be essential for the development of a fully, invasive lung cancer [23].

The original description of field cancerization by Slaughter and co-workers [13] showing multiple foci of epithelial hyperplasia hyperkeratinization, atypia and also carcinoma *in situ* occur in otherwise normal appearing epithelium, adjacent to cancers of the oropharynx suggested that the carcinogen exposure has widespread effects throughout the entire epithelial surface of the organ. The diffuse histological changes observed, suggested that the development of malignancy in the proximal aerodigestive tract is a predictable consequence of the dose of carcinogen. This then had an impact on different cell populations as a function of anatomy and the properties of the tobacco smoke carcinogens. The same pattern of heterogeneous, multifocal histological changes of the bronchial epithelium in relation to tobacco smoke were very well documented in reports by Auerbach and colleagues [24]. Field cancerization can thus be seen as a concept to describe the diffuse damage elicited by chronic exposure to carcinogens, and forms the basis for understanding the observation that patients who survive a first cancer in this region are subsequently susceptible to the development of a second primary tumour [25,26].

4. (Epi)Genetic changes during lung carcinogenesis

The most common chromosomal abnormalities in lung cancer are allelic deletions or loss of heterozygosity of tumour suppressor gene sites. For example, it has been shown that highly specific deletions in the short arm of chromosome 3 occur during hyperplasia, the earliest stage of carcinogenesis recognized so far [27]. Similar evidence exists for deletions of the short arm of chromosome 9 and 17 and the long arm of chromosome 13 (retinoblastoma gene/RB) [28–30]. One of the genes on chromosome 3 is the fragile histidine triad (FHIT) gene that is also lost very early in the carcinogenic process [31]. Similarly to the *p53* gene on chromosome 17, this gene is thought to have a tumour suppressor function [32]. Both deletions of chromosome 3 and 9, have been associated with smoking and they remain detectable many years after smoking cessation [31,33]. The

p53 protein has been called the ''guardian of the genome'' and acts as a transcription factor in control of G1 arrest and apoptosis (programmed cell death). Phosphorylation of the retinoblastoma (RB) gene is reduced by p53 thereby halting the cell cycle at checkpoint G1/S and enabling DNA repair or apoptosis. Mutations of *p53* are more frequent in small cell lung cancer (SCLC) (70–100%) than in NSCLC (45–75%) and an increase of *p53* mutations has been noted during the carcinogenic process [29,34–36].

RB protein is the main effector of G1 arrest if DNA is damaged. Expression of RB is lost in 80% of SCLC and only in 15% of NSCLC [37]. However, inactivation of RB is also common in NSCLC. This is caused by loss of the CDK inhibitor p16INK4a, which negatively controls CDK–cyclin activity by overexpression of cyclin D1. 'Silencing' of this last gene may be caused by mutation/deletion or by promoter methylation [38,39]. Recently, an inverse correlation has been found between p16INK4a expression and the expression of the polycomb-group (onco)gene BMI-1, providing an alternative mechanism to down regulate p16 expression [40].

Other genes with potential tumour suppression function include RASSF1A at chromosome 3, encoding a protein that is able to heterodimerize with Nore-1 a RAS effector [41]. In NSCLC RASSF1A, is inactivated by hypermethylation [42,43]. The retinoic acid receptor-beta (RARβ), also mapped on chromosome 3, is a nuclear receptor with vitamin-A dependent transcriptional activity [44]. Down regulation of RARβ occurs early during lung carcinogenesis (hypermethylatin) and is related to the occurrence of lung cancer in experimental animals [45–47].

On chromosome 11 another gene with potential tumour suppressor activity, *TSLC1* (tumour suppressor gene in lung cancer) has been identified [48]. The gene encodes a membrane glycoprotein of the immunoglobulin superfamily (Ig-CAM) and is frequently down regulated in NSCLC [49,50].

Activation of oncogenes can be elicited by a variety of mechanisms including mutation, amplification, chromosomal rearrangement as well as by epigenetic events such as change in DNA methylation status. Of the large number of oncogenes identified to date in lung carcinogenesis, RAS, c-MYC, epidermal growth factor receptors ErbB1(EGFR), and ErbB2 (HER2/NEU), exert their effect by tyrosine kinase activity. The RAS family of genes encode 21 kDa proteins that bind to GTP forming a RAS-GTP complex, which act by inducing proliferation signals through a number of transcription factors including c-FOS, c-JUN and c-MYC. K-ras mutations which are preferentially detected in non-squamous lung cancers, are associated with exposure to carcinogens from tobacco smoke [51,52]. c-MYC is important for cellular proliferation and is also involved in the induction of apoptosis in normal cells. In lung cancer, this last pathway is frequently deregulated. Oncogenic activation of MYC occurs in 20% of SCLC and 10% of NSCLC in association with genetic amplification and is being regarded as an early event in carcinogenesis [53]. EGFR and HER2/NEU are both involved in the transduction of growth signals and are overexpressed in a large percentage of NSCLC. This overexpression is associated with disease progression, metastatic growth and poor survival [54,55].

As already discussed, there is a growing awareness that lung cancer develops in a stepwise fashion as the result of multiple genetic events. Although the particular events and their sequence(s) leading to the development of lung cancer are not yet fully elucidated, it is hoped that strategic disruption of one or more of these pre-invasive steps may be fundamental to defining successful chemoprevention strategies.

5. Genetics, diet and gender influence on lung cancer susceptibility

Carcinogens from tobacco smoke form the unquestionable link between smoking and lung cancer. Epidemiological studies show that approximately 15% of heavy smokers will ultimately develop lung cancer. The fact that 85% of heavy smokers will not develop lung cancer has been cited to suggest that there may be important differences in lung cancer susceptibility in the population.

From a broader perspective, it is important to understand that some of the confusion may result from the very significant competing risks that exist for a heavy smoker with cardiovascular disease, chronic obstructive pulmonary disease as well as the other lethal tobacco-related diseases. According to Sir Richard Peto and colleagues [56], every other heavy smoker dies of smoking. On the other hand more and more evidence is accumulating that genetic and epigenetic factors are responsible for modulating individual susceptibility to lung cancer or other consequences of tobacco exposure [57,58]. Carcinogens from cigarette smoke like benzpyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) require metabolic activation before they can 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 metabolising enzymes (activation) and also glutathione transferases (detoxification enzymes) are known to be polymorphic. Approximately 40–50% of the human population has a so-called null genotype that is modestly associated with lung cancer [47]. '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 [59–61] (Fig. 1).

Dietary factors may represent one of the most powerful epigenetic factors influencing lung cancer susceptibility. In case-control studies, defective detoxification and repair of genetic damage have been associated with increased individual susceptibility to lung cancer, while certain food constituents seemed to afford a degree of protection to individuals with limitations in their detoxification capacity [62,63]. In the light of these gene–environment and gene–diet interactions, it is not surprising that smoking has been found to interact synergistically with a family history of lung cancer [64,65].

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 the association between a high intake of fruits and vegetables and a reduced risk of lung cancer [66]. Much effort has been expended to identify the specific components of these foods that may be responsible for lowering 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 subset analysis performed on the data from the Skin Cancer Prevention Trial [67,68].

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, 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 [63]. Allyl sulphur compounds present in onions and garlic were able to induce apoptosis suggesting that it may be interesting to evaluate them as chemoprevention agents [69,70]. An increasing number of studies support the premise that green tea polyphenols may be useful as preventive agents for NSCLC [71–73].

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 [74].

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 [63,75].

Finally, it is important to note that a number of cohort studies have suggested that females are more susceptible to the carcinogenic effects of tobacco smoke than men [76,77]. A recent CT screening study also pointed to females as being more susceptible [78]. These observations have been confirmed by the smoking analysis of a large chemoprevention study revealing significantly less exposure to tobacco (pack-years) before occurrence of the lung or head and neck cancer in females in comparison with males [79]. Explanations for this difference may be sought in detoxification capacity and/or hormonal factors.

6. Different strategies for lung cancer chemoprevention

Frequently, chemoprevention approaches have been based on epidemiological and basic biological research and translated into clinical chemical interventions to suppress or reverse the process of carcinogenesis. In one classification, three different categories of chemoprevention have been defined: (1) primary prevention – prevention of cancer in healthy individuals, who are at high risk (*e.g.*, current or former smokers); (2) secondary prevention – prevention of development of cancer in individuals with precancerous lesions (*e.g.*, intraepithelial neoplasia, leukoplakia, dysplasia); and (3) tertiary

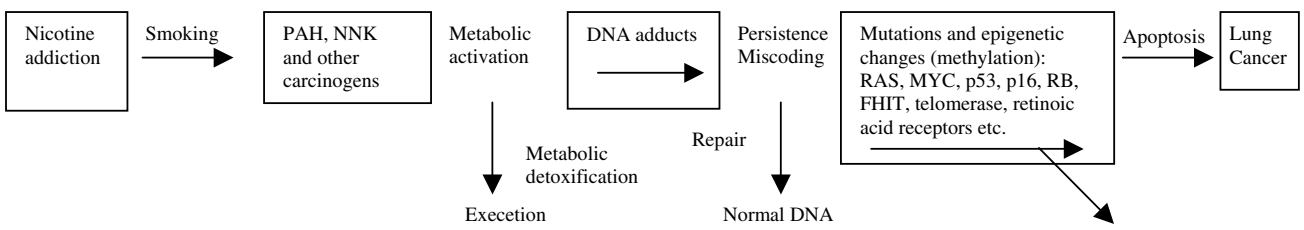

Fig. 1. Association between nicotine addiction and lung cancer via tobacco smoke carcinogens and their induction of multiple mutations in critical genes. PAH, polycyclic aromatic hydrocarbons; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butone. Adapted from Hecht [61].

prevention to prevent second primary tumours in patients who have had a previous cancer [80].

As mentioned earlier, smoking history as defined by pack years is a relatively rough estimate of lung cancer risk and a modified criteria has been proposed for screening studies [81]. To conduct cost-effective chemoprevention studies it is essential to define the optimal risk subset of individuals that share the greatest risk for progression to lung cancer without missing an important fraction of cancers. Strategies combining tobacco exposures with other biological markers, the so-called intermediate endpoint markers have been discussed as a strategy to more efficiently conduct screening in high-risk smoking cohorts [82,83].

7. Specific chemopreventive agents

Hundreds of natural and synthetic agents have shown the potential for chemopreventive activity in experimental systems, but only a handful has been validated in clinical trials. Among the agents studied in clinical trials are NSAIDs, selenium, alpha-tocopherol, calcium, tamoxifen, *N*-acetylcysteine, while retinoids/carotenoids have attracted the most research interest [69].

7.1. Vitamin A

Due to their ability to regulate cell proliferation and differentiation, the derivatives of vitamin A or retinoids have been regarded as a potentially useful class of agents from the very beginning of chemoprevention research [7]. In multiple animal model systems, at different organ sites and with a variety of inducing carcinogens, retinoids were found to be preventive. Retinoids are potent regulators of gene expression and work through an elaborate family of cytoplasmic retinoic acid binding proteins as well as intranuclear retinoic acid receptors [84]. The family of retinoid nuclear receptors is a member of the corticosteroid superfamily, which includes other members such as thyroid hormone receptors. For the nuclear retinoid receptors, the two main types are RAR and RXR and at least three subtypes: alpha, beta and gamma. The receptors are ligand activated following binding to retinoids and work together to regulate cell growth, differentiation, and death. Loss of expression of RARβ has been noticed in the airways of smokers and as mentioned previously, there is increasing evidence that RARβ has tumour suppressive activity [85,86]. The retinoid receptors do not efficiently bind all the different synthetic retinoids currently available and this has been suggested as one explanation for the lack of effect seen in many clinical studies with retinoids so far.

7.2. Retionoids/carotenoids

One of the earliest studies with relevance to lung cancer was a trial with 103 patients with previous head and neck cancer in which the effect of 13-*cis*-retinoic acid (13-*cis*-RA) on recurrence and second primary cancer was studied [87]. The incidence of second primary tumours (SPTs) in the treatment arm receiving 50–100 mg/m^2 of 13-*cis*-retinoic acid was 4% compared to a response rate of 24% in patients receiving placebo. The second trial involved 307 patients with early stage lung cancer, randomized after complete surgical resection to receive either a vitamin A precursor (retinyl palmitate) or no further treatment [88]. Also in this study, there was a significant difference in the frequency of second primaries (12% *versus* 21%). Both studies were carried out in populations at risk for SPTs and are considered for tertiary chemoprevention trials (Table 1). In contrast to these two tertiary chemoprevention trials, several secondary chemoprevention trials, *i.e.*, controlled studies in individuals with premalignant changes in the bronchial tree (sputum) but otherwise 'healthy' have been negative (Table 2).

A significant challenge in conducting and interpreting these trials is whether the endpoint, such as reversal of histological change, can be measured reliably and further, whether a change in histological status in fact correlates with a significant reduction in the frequency of cancer progression. These issues have been considered in a review of intraepithelial neoplasia (IEN) as a surrogate for drug effect in evaluating chemoprevention drugs [94].

The results of a number of large primary chemoprevention trials with β-carotene, a retinoid precursor in 'healthy' individuals with elevated risk for lung cancer

Table 1
Randomised 'tertiary' chemoprevention trials

No. of individuals	Endpoint	Intervention	Effect	Author	References
103	SPT[a]	Isotretinoin (H&N pts)	Yes	Hong	[87]
307	SPT	Retinyl palmitate	Yes	Pastorino	[88]
2592	SPT	Retinyl palmitate NAC[b]	No	Van Zandwijk	[99]
41 161	SPT	Isotretinion[c]	No	Lippman	[100]

[a] Second primary tumour.
[b] NAC, *N*-acetylcysteine.
[c] Isotretinoin is synonym for 13-*cis*-retinoic acid (13-*cis*-RA).

Table 2
Randomised 'secondary' chemoprevention trials

No. of individuals	Endpoint	Intervention	Effect	Author	References
72	Reversal of metaplasia	Folic acid/ Vitamin B12	Yes/No	Heimberger	[89]
150	Reversal of atypia	Etretinate	No	Arnold	[90]
152	Reversal of metaplasia	Isotretinoin	No	Lee	[91]
755 (asbestos workers)	Reversal of atypia	β-carotene and retinal	No	Mc Larthy	[92]
139	Reversal of metaplasia	4-HPR[a]	No	Kurie	[93]
57	HTERT expression	4-HPR	Yes	Soria	[104]
41	DNA Adducts, Micro-Nuclei (mouth floor)	*N*-acetylcysteine	Yes	Van Schooten	[121]
101	Reversal of dysplasia	Anethole dithioleethione (ADT)	Yes	Lam	[124]
112	Reversal of dysplasia	Budesonide	No	Lam	[127]

[a] 4-HPR, *N*-(4-hydroxyphenyl) retinamide.

Table 3
Randomised 'primary' chemoprevention trials

No. of individuals	Endpoint	Intervention	Effect	Author	References
29133	Lung cancer incidence	Vitamin E, β-carotene	No	ATBC prevention study group	[68]
18314	Lung cancer incidence	Vitamin A, β-carotene	No	Omenn	[95]
22071	Lung cancer incidence	β-carotene	No	Hennekens	[96]

became available in the 1990s, including the alpha tocopherol beta carotene (ATBC) and the beta carotene and retinol efficacy (CARET) trials [68,95]. The ATBC trial accrued 29133 Finnish male smokers and tested the effects of dietary supplementation of β-carotene and α-tocopherol. Against all expectations, this trial did not show any protective effect from either α-tocopherol or β-carotene. On the contrary, β-carotene was associated with a significant increase in lung cancer incidence (18%) and mortality (8%). The detrimental association of β-carotene was confirmed by the CARET study, involving 18314 smokers, former smokers or aerospace workers exposed to asbestos. CARET revealed a 28% higher rate of lung cancer and 17% higher overall death rate in those participants taking β-carotene (Table 3).

The findings of ATBC and CARET trials have clearly been a shock for the chemoprevention community. On the other hand, they also confirmed the unquestionable importance of large-scale controlled (randomized) studies. Since the detrimental effects of β-carotene have not been observed in the Physicians Health Study involving 22071 mainly non-smoking physicians [96], it has been hypothesized that cigarette smoke in the lungs, which is highly oxidising, may interact with β-carotene, yielding unstable by products that could have pro-oxidant activity [97]. An experimental study in ferrets exposed to cigarette smoke and β-carotene confirmed that a negative interaction existed [98]. Thus, the importance of smoking cessation is once more emphasized and smokers should avoid β-carotene supplementation. In addition, this trial experience demonstrated the obligate need to do the necessary early clinical development including defining a safe chemopreventive dose in a Phase I trial as well as to define pharmacodynamic efficacy *in vivo* as part of a Phase II trial. On such a foundation, a Phase III trial has a much more credible scientific basis and allows for a more predictable prevention drug development process.

The results of EUROSCAN, a large tertiary chemoprevention study designed to assess the effects of retinyl palmitate and the anti-oxidant *N*-acetylcysteine (NAC) became available in 2000. The early positive experience with retinyl palmitate and the promise of the antioxidant NAC could not be confirmed in a population of almost 2600 patients with early stage head and neck cancer or lung cancer, who received a 2-year supplementation with retinyl palmitate and/or NAC following treatment with curative intent [99]. SPTs or tumour recurrences were not reduced in these studies. A similar result has been obtained in the US NCI intergroup trial with a daily dose of 25 mg of 13-*cis*-retinoic acid in 1486 Stage I NSCLC patients. 13-*cis*-Retinoic acid did not improve the rate of SPTs or mortality [100]. Subgroup analyses suggested that 13-*cis*-RA acid might have been associated with a short time to disease progression in individuals who manifested metastatic disease. The subset of patients belonging to the category of never smoked was associated with a more favorable outcome. However in contrast to the β-carotene studies, there was no evidence of any increase in the number of new lung cancers occurring in the overall group or any subgroup that received 13-*cis*-retinoic acid. From this perspective, the results from the β-carotene studies are very different from the results of the intergroup trial but neither type of trial suggested a chemopreventive benefit. Both EUROSCAN and the NCI intergroup trial underline the importance of large confirmatory studies and strengthen the importance of smoking cessation, *i.e.*,

participants, who had permanently stopped smoking, had a better survival than those who continued smoking. As mentioned above, explanations for the lack of preventive effects of retinoids in these studies are provided by the observation that RARβ is frequently suppressed in preneoplastic bronchial lesions and the distinct patterns of binding of different retinoids to nuclear receptors. A recent retrospective study on methylation status and occurrence of SPTs in completely resected NSCLC patients revealed an association between RARβ2 hypermethylation and the development of SPTs in former smokers only. In current smokers, hypermethylation was associated with a protective effect, which points to our difficulties in understanding the biological significance of retinoids in lung cancer [101,102].

7.3. New retinoids and alternative routes for delivery

N(4-hydroxyphenyl) retinamide (4-HPR), in spite of its inability to bind directly to nuclear receptors, showed some preventive activity in experimental animals and was also active in lung cancer cell lines by inhibiting growth and inducing apoptosis [103]. In a group of smokers, 4-HPR was able to modulate telomerase expression [104]. This is an interesting polar, synthetic retinoid that may mediate its principal anti-cancer effect through mechanisms that are independent of vitamin A receptor biology [105].

Recently another retinoid, 9-*cis*-retinoic acid (9-*cis*-RA) has been tested in the chemopreventive setting. 9-*cis*-RA has a high affinity for both RAR and RXR receptors and was able to modulate the suppression of RARβ in ex-smokers. In contrast, 13-*cis*-RA is a prodrug form of all trans retinoic acid such that 13-*cis*-RA cannot directly bind to nuclear RA receptors and has been reported to not substantial change RARβ expression [106,107]. This indicates that 9-*cis*-RA is indeed a candidate for additional studies.

All-*trans* retinoic acid (ATRA) binds only to the RARs but it has a short half-life and is not the best candidate for lung cancer prevention studies. On the other hand, the discovery that ATRA can induce differentiation and clinical remission in patients with acute promyelocytic leukaemia has shown the potential of this approach [108]. In this context targretin or bexarotene, which has been occasionally associated with a favorable outcome in patients with advanced NSCLC is undergoing further clinical evaluation [109].

In light of issues with oral drug metabolism, there are concerns that chemopreventive agents when taken orally may not result in drug dose delivery to the respiratory epithelium in sufficient concentrations to mediate favorable nuclear retinoic acid receptor interactions. This is another reason why new routes of chemoprevention drug administration such as the inhalational route may provide an effective and safe way of prescribing retinoids as it confines dose exposure to the target organ [110]. As with anti-microbial or asthma therapeutics, the use of aerosolized delivery technologies may permit higher drug levels to be administered with minimal toxicities by preferentially targeting the cancer field. Aerosolized 13-*cis*-retinoic acid was effective in inhibiting tobacco-carcinogen related lung nodules in experimental animals. Lung tissues from animals receiving retinoic acid showed upregulated levels of nuclear RA receptors compared to lung tissues obtained from animals receiving placebo [111–113]. A pilot report of aerosolized delivery of retinyl palmitate directly onto the respiratory epithelium showed that most patients had a significant improvement in histological status as documented by serial autofluorescence bronchoscopy in the absence of any discernable side effects [114]. The pharmacological benefit of direct drug delivery strategies in the airway has strong theoretical appeal as an approach to improving the therapeutic benefit of lung cancer chemoprevention and more research in this area is needed. Indeed, a recent report showed that administration by aerosol of difluoromethylornithine and 5-fluorouracil was able to prevent experimental tumours in hamsters [115].

7.4. Vitamin E (alpha-tocopherol)

Although epidemiological and dietary studies have suggested that vitamin E might have preventive potential, the ATBC study did not show any effect on the prescription of vitamin E in a population of mainly smokers. Vitamin E used in conjunction with 13-*cis*-RA and interferon-α seemed to modulate the toxicity of 13-*cis*-RA and this triplet was found to have a significant protective effect on premalignant oral lesions [116,117]. However, it remains to be determined what role, if any, vitamin E has when administered as a single agent or as a modulator of retinoid toxicity.

7.5. Selenium

The trace element selenium that resembles sulphur in its various forms was first associated with cancer protection in the late 1960s [118]. Several years later, epidemiological evidence also pointed to preventive capacities of selenium. A randomized controlled trial to prevent skin cancer in 1312 patients suggested that supplementation with l-selenomethionine was associated with a reduction in the risk of developing lung and prostate cancer [67]. The precise mechanism in which selenium could be mediating a beneficial chemoprotective effect is not known. Confirmation of the clinical results are being sought in a large epidemiological study and an intergroup trial carried out by the Eastern Cooperative Oncology Group [119].

7.6. N-acetylcysteine (NAC)

NAC, which is a cysteine conjugate, has been synthesized to provide a precursor of reduced glutathione, avoiding the toxicities of cysteine. The majority of laboratory investigations, revealing antimutagenic and anticarcinogenic activities of NAC have been carried out by a group of European investigators. NAC is able to detoxify reactive electrophiles and free radicals through conjugation or reduction reactions, stimulate DNA repair and inhibit invasion. Moreover, the formation of carcinogen-DNA adducts was found to be inhibited by NAC in both experimental animals and smoking volunteers [120,121]. In spite of these encouraging results no preventive action of NAC could be detected in the EUROSCAN study. One explanation for this lack of success might be the fact that the follow-up (49 months) in this study had been too short to notice a positive effect of NAC.

7.7. Dithioleethiones

Anethole dithioleethione (ADT) and Oltipraz belong to the dithioleethione class of organosulphur compounds, which have antioxidant, radioprotective and chemopreventive properties [122]. Like NAC and isothiocyanates, these agents also have unique detoxification properties. In animal models of carcinogenesis they exerted chemoprotective properties against the development of lung and other cancers [123]. In a recent phase IIB study, ADT showed an effect on progression of histology but was not effective in changing nuclear morphometry index, which was the secondary trial endpoint. This result suggests that ADT merits further evaluation as a potential chemoprevention agent [124]. Bronchial dysplasia as detected by fluorescence bronchoscopy was used as the primary intermediate endpoint marker, but the results of this analysis failed to show a significant benefit. ADT has been used in clinical practice for drug and radiation induced hyposalivation. Oltipraz has been generally regarded as too toxic to consider for routine chemopreventive purposes.

7.8. Other agents and novel molecular targeted approaches

Chalcones, synthetic flavonoids, have been found to inhibit benzpyrene-induced pulmonary adenoma formation in experimental animals [125]. Dexamethsone and budesonide were found to be effective in a similar model [69,126]. It is therefore not surprising that inhalational corticosteroids, widely prescribed for obstructive pulmonary disease are being studied in phase IIB chemoprevention studies. The first experiences with budesonide in a group of volunteers did not show any consistent preventive pattern in intermediate endpoints [127].

From corticosteroids to other inhibitors of inflammation is a small step. Non-steroidal anti-inflammatory agents (NSAIDs) inhibit the conversion of arachidonic acid to a class of inflammatory mediators known as eicosanoids. The first suggestion that NSAIDs could inhibit neoplastic growth came from epidemiological and population-based studies of patients who were taking such drugs for other purposes [128]. These observations led to an in-depth biological analysis of the role of the cyclooxygenase-2 (COX-2) in carcinogenesis and also prompted further investigations into the potential use of COX-2 and other inhibitors of the eicosanoid pathways in the prevention of solid tumours that express COX-2, such as lung cancer [129–134]. Manipulation of prostaglandin production distal to cyclooxygenase significantly reduced the tumour incidence elicited by exposure to tobacco smoke in mice [135].

Other biological pathways of lung carcinogenesis are also providing attractive targets for chemopreventive purposes. As mentioned above, the Erb B family of receptors has been recognized as a potentially important site to halt carcinogenesis [136,137]. Likewise, activation of the EGFR involves RAS activation. Thus, treatment with small molecule tyrosine kinase inhibitors of the EGF receptor, antibodies against the same receptor and/or blocking the RAS signalling pathway by farnesyl transferase inhibitors are new avenues in chemoprevention research [138]. As a result of vastly expanded pharmaceutical development capabilities, a large number of candidate drugs for lung cancer will be introduced over the next few years. Most of these compounds will be considered for application in managing early lung cancer. Rather than providing a lengthy list of candidates that would soon be obsolete, it may be more useful to consider some core principles in evaluating new candidates for lung cancer chemoprevention.

Drug targets for chemoprevention should be directed at molecular pathways that appear to be modulated in the process of carcinogenesis relative to normal pathways. Ideally, there should be mechanistic evidence demonstrating that the process of carcinogenesis is causally linked to the function of that mechanism. In addition, early developmental work should demonstrate that the pathway can be abrogated using clinically achievable dose and schedule of the agent. Further clinical trials should establish that such dose and schedule can be routinely administered without incurring unpleasant or dangerous side effects. There is an emerging sense in the research community developing lung cancer chemoprevention agents that more concerted attention to pharmacology issues in rigorously defining a dose and schedule associated with a robust pharmacodynamic effect is crucial. Many of the early chemoprevention trials proceeded in the absence of any firm

understanding of the molecular pharmacology of the candidate agent. In this setting, it is difficult to make progress since firm conclusions about next steps can not be established. There is an extensive and growing literature about developing new trial structures for chemoprevention development. Much of this literature has been summarized in the American Association of Cancer Research White Paper on the use of intraepithelial neoplasia as a surrogate marker in the development of early cancer drugs [94]. Design of chemoprevention trials will be an ongoing area of dynamic clinical research interest and one of the most critical issues in this regard will be developing an effective approach to designing drug validation trials around the response of disease surrogates.

8. Intermediate markers

When the frequency of development of invasive cancer is used as an endpoint, as has been done in many of the aforementioned clinical studies, significant investments in time and money are needed to permit a critical study size and duration to be realized. The disappointing results of chemoprevention studies so far has been a strong stimulus to invest in new approaches based on new technology and better understanding of essential pathways of pulmonary carcinogenesis. One approach to avoid the premature start of large comparative chemoprevention trials with cancer as primary endpoint is the use of intermediate biological endpoints as surrogates to definitive clinical endpoints. Premalignant lesions are a potential source of intermediate markers and if the disappearance of these lesions correlates with a reduction in cancer incidence, markers of premalignant disease could serve as intermediate endpoints for chemoprevention trials.

9. Conclusions

The rapidly expanding understanding of the molecular and the biological basis of lung cancer has been a strong stimulus for chemoprevention research. In addition, new methods for early detection will result in a much larger number of early stage patients being diagnosed with potentially curative lung cancer. A consequence of this is that many more individuals will be brought to clinical attention where concerns about field cancerization and subsequent primary lung cancers will be problematic. This is a population that would need effective chemoprevention drugs but also represents a growing cohort to participate in the research process to develop such drugs.

An increasing number of molecular and genetic lesions considered essential for the final malignant phenotype have been identified and some of these lesions are directly related with exposure to carcinogens from tobacco smoke. At the same time, an increasing number of studies have pointed to the wide variation of individual susceptibility to carcinogens from tobacco smoke. Despite an overwhelming number of epidemiological and experimental data, no definitive proof for a preventive effect of vitamin A and its analogues against lung cancer has been provided so far. Several explanations for the negative outcomes with retinoids are brought forward and there is consensus that the premature start of large and costly comparative studies with cancer as an endpoint should be avoided and that relatively small comparative studies with surrogate or intermediate endpoint markers should precede.

Potential valuable intermediate endpoint markers have appeared on the horizon and also the target populations for chemoprevention studies are being better defined while several novel agents with chemopreventive potential have been identified.

Thus it is anticipated that after a period with increasing insight into lung carcinogenesis, but without appreciable benefits from the chemoprevention approach in randomized trials, the road is now being paved for more successful studies in high-risk individuals. Considering the continuing lung cancer epidemic worldwide, it is clear that we must continue our effort to strengthen the fundamental strategy of avoiding exposure to carcinogens in parallel with the development of additional approaches such as chemoprevention.

Conflict of interest statement

None declared.

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