Lung Cancer Chemoprevention

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Lung cancer is the leading cause of cancer death in the United States, and the majority of diagnoses are made in former smokers. Although avoidance of tobacco abuse and smoking cessation clearly will have the greatest impact on lung cancer development, effective chemoprevention could prove to be more effective than treatment of established, advanced-stage disease. Chemoprevention is the use of dietary or pharmaceutical agents to reverse or block the carcinogenic process and has been successfully applied to common malignancies other than lung (including recent reports on the prevention of breast cancer in high-risk individuals). Despite previous studies in lung cancer chemoprevention failing to identify effective agents, our ability to define the highest-risk populations and the understanding of lung tumor and premalignant biology continue to make advances. Squamous cell carcinogenesis in the bronchial epithelium starts with normal epithelium and progresses through hyperplasia, metaplasia, dysplasia, and carcinoma *in situ* to invasive cancer. Precursor lesions also have been identified for adenocarcinoma, and these premalignant lesions are targeted by chemopreventive agents in current and future trials. Chemopreventive agents can currently only be recommended as part of well-designed clinical trials, and multiple trials have recently been completed or are enrolling subjects.

Keywords: lung cancer; chemoprevention; premalignant dysplasia; biomarkers; clinical trials

Lung cancer is now the leading cause of cancer death in both men and women in the United States, as well as being the leading cause of cancer death worldwide (1). The 5-year U.S. survival rate for lung cancer is a discouraging 15%, and although there has been an interval improvement in survival, the survival advances realized in other common malignancies have not translated to lung cancer. One reason for the discouraging survival statistics is that the majority of patients present with late-stage disease. With advances in biologically targeted therapeutic agents, treatment of advanced lung cancer should continue to improve (2), but effective chemopreventive agents are sorely needed. Approximately 90% of lung cancer cases are attributable to tobacco smoking, and smoking cessation is clearly the most effective intervention to reduce lung cancer risk. Former smokers still carry a significant risk and, in the United States, the majority of lung cancers continue to be diagnosed in former smokers (3).

Similar to many solid organ tumors, lung tumorigenesis results from a series of genetic and epigenetic alterations in pulmonary epithelial cells. The World Health Organization classification

for lung cancer recognizes distinct histological lesions that can be reproducibly graded as precursors of non-small cell lung cancer (NSCLC) (4). Clinical experience has shown that chemopreventive agents may have dramatically different results in current and former smokers (5), and many trials either exclude current smokers or analyze these subjects separately.

PRINCIPLES OF CHEMOPREVENTION

Chemoprevention is defined as the use of dietary or pharmaceutical interventions to slow or reverse the progression of premalignancy to invasive cancer (6). Lung carcinogenesis can involve 20 to 30 years, and more recent studies have appropriately chosen to evaluate the effects of treatment on premalignant lesions or inhibition of the carcinogenic progression. Chemoprevention studies are further subdivided into three distinct areas (primary, secondary, and tertiary). Primary chemoprevention measures the development of cancer in a high-risk population (for instance, current or former smokers who have COPD), whereas secondary chemopreventive studies examine the development of cancer in subjects with precursor lesions (for example, severe dysplasia on an endobronchial biopsy or atypical adenomatous hyperplasia on a transthoracic needle biopsy). Tertiary chemoprevention studies examine the development of lung cancer in subjects with a previous tobacco-related aerodigestive cancer.

Development of agents follows the standard progression of phased clinical trials. A biomarker is defined as a characteristic that is objectively measured or evaluated as an indicator of a pathogenic process or a response to a therapeutic intervention. The development and validation of biomarkers is particularly important for lung cancer prevention studies as invasive cancer progresses over many years, and longitudinal studies with the development of lung cancer as an endpoint take considerable time to complete. The National Cancer Institute and Food and Drug Administration have developed general strategies for developing chemopreventive agents. This typically involves the initial *in vitro* studies, progressing to animal tumorigenesis studies, and then concluding with phased human trials. Animal models allow for more thorough preclinical testing that should result in only the most promising agents progressing to human trials. Murine adenocarcinoma models have many similarities to human adenocarcinoma in terms of histology, mutations, and gene expression patterns (7), and more recently a squamous cell murine model has been described wherein animals develop endobronchial dysplasia and carcinoma *in situ* (8).

Although there are no approved chemopreventive agents for lung cancer, recent studies have proven that agents like oral iloprost can improve endobronchial dysplasia in former smokers (9).

Definition of High-Risk Groups

Multiple requirements must be met for clinically effective chemoprevention. First, an adequately high-risk population must be readily identifiable. Second, effective agents with a tolerable side

(Received in original form July 8, 2011; accepted in final form August 11, 2011)

Supported by Department of Veterans Affairs Merit Review Program and the National Institutes of Health (Colorado SPORC in Lung Cancer).

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Proc Am Thorac Soc Vol 9, Iss. 2, pp 52–56, May 1, 2012

Published 2012 by the American Thoracic Society

DOI: 10.1513/pats.201107-038MS

Internet address: www.atsjournals.org

effect profile must be available given that annual risk for cancer development is small. Subjects enrolled in tertiary chemoprevention trials may have a previous history of a tobacco-related cancer (lung or head and neck), and therefore more toxicity may be tolerated. This is a particularly important group of patients as the risk of developing a second primary lung cancer after resection can be as high as 2 to 3% per year (10), and most second primary tumors in head and neck patients are lung cancer. Third, endpoints to the clinical trials must be identified, defined, and validated in terms of demonstrating reduction in cancer development. For tertiary trials this can be cancer incidence, but for secondary prevention trials intermediate biomarkers must be present and have an acceptable risk of progression. For lung cancer, there is no current gold standard biomarker, and histology is used in a similar paradigm to the development of other epithelial cancers. Histologic changes may ultimately not prove to be the best biomarker, and advances in genomics, proteomics, and molecular imaging studies increase our understanding of lung tumorigenesis.

One in nine smokers eventually develops lung cancer (11), and epidemiologic studies have been able to more accurately identify high-risk populations. The development of COPD (as evidenced by airflow obstruction on spirometry) and lung cancer have a common genetic basis, as current and former smokers with airflow obstruction have a significant increase in lung cancer incidence (12, 13). Bach and colleagues developed and validated a model of lung cancer risk based on age, sex, and tobacco smoke and asbestos exposure history (14). Clinical experience has shown that significant variation in risk within smokers is evident, with 50% of lung cancers occurring in the highest-risk quartile and only 8% in the lowest-risk quartile (14). Risk of developing lung cancer in the upper quartile is up to 1.5% per year (14). Therefore, high-risk groups for lung cancer can be better identified on the basis of age, smoking history, exposure to asbestos, pulmonary function, and family history. A more recent report also showed an association of spiral computed tomography-detected emphysema and risk of developing lung cancer (odds ratio, 3.56; 95% confidence interval, 2.21–5.73) (15). This association was maintained after controlling for airflow limitation. Ongoing genetic testing should further clarify and aid in better defining lung cancer risk in the near future and improve the ability to identify even higher-risk subpopulations.

PREVIOUS TRIALS

No agents have been validated as effective for lung cancer chemoprevention (16). The only intervention that has been shown to be effective in reducing risk of lung cancer is smoking cessation, demonstrated prospectively in the Lung Health Study. In this large study, smokers were assigned to smoking cessation intervention programs or no smoking cessation intervention, with a resultant 55% reduction in lung cancer risk observed in successful quitters (17). Early lung cancer chemoprevention trials were based on epidemiological data, which suggested diets high in vitamin A reduced risk (18). Three large trials in primary prevention evaluated β -carotene plus retinol, β -carotene and/or α -tocopherol, or β -carotene alone. Three other trials investigated secondary prevention strategies by administering retinyl palmitate, retinyl palmitate and/or N-acetylcysteine, or 13-*cis* retinoic acid (5, 16, 19, 20). These trials were also disappointing. No protective effect against lung cancer was observed, and two trials suggested these agents could increase the risk of lung cancer in current smokers (19, 20). Only smoking cessation correlated with a significant reduction in squamous metaplasia (21). Additionally, a metaanalysis of the large β -carotene trials (Alpha-Tocopherol, Beta-Carotene; Carotene and Retinol Efficacy Trial; Physician's Health; and Women's Health) found an increased risk of lung

cancer in current, not former, smokers who received β -carotene (odds ratio, 1.21; 95% confidence interval, 1.09–1.34) (22). Thus, high doses of a single antioxidant vitamin do not appear to be a viable chemopreventive strategy.

Phase III chemoprevention trials evaluating isotretinoin, vitamin A/N-acetylcysteine, or selenium in patients with a prior lung or head and neck cancer resected for cure have been completed and required fewer patients, but none have shown a reduction in lung cancer incidence (20, 23, 24). Other completed trials that have failed to yield positive results include: (1) anethole dithiolethione (an organosulfur compound that increases glutathione-S-transferase and additional phase II enzymes) did not reverse bronchial dysplasia (25); and (2) inhaled budesonide in smokers with dysplasia did not induce histologic regression (26). Phase II intermediate endpoint trials are currently being conducted and these trials have emerged as one strategy for prioritizing agents for longer and more expensive phase III chemoprevention trials containing a lung cancer endpoint.

PRECLINICAL STUDIES

One area of considerable advancement in chemoprevention is in preclinical testing. Historical chemoprevention studies would have benefited from animal studies. Mice develop pulmonary adenomas that progress to adenocarcinomas in response to a number of agents, including ethyl carbamate, nicotine-derived carcinogens, or tobacco smoke exposure (27, 28). In addition, investigators have developed a number of transgenic models in which viral oncogenes or transforming ras mutants are selectively and conditionally expressed in lung tissue (29). The murine tumors developed in these models have many similarities to human adenocarcinoma, ranging from specific markers to gene expression patterns (27, 30). Models of squamous cell lung cancer have also been developed, and the N-nitroso-tris(chloroethyl)urea model also induces dysplastic lesions that are similar to those found during bronchoscopy and can therefore be used to evaluate one proposed surrogate endpoint in preclinical studies (8, 31).

RECENT TRIALS

There are a number of chemoprevention trials currently being conducted, and the majority of these are phase II trials based on epidemiologic and preclinical studies.

COX Inhibitors

Alterations in eicosanoid production have been associated with many types of cancer, including lung cancer. Inhibition of cyclooxygenase (COX-1 and COX-2, PGH₂ synthase) activity decreases eicosanoid production and prevents lung cancer in animal models (32). A large number of COX-2-dependent genes are involved in lung tumorigenesis (reviewed in Reference 33) and COX-2 can be up-regulated in carcinoma *in situ* and many NSCLCs. One recent study evaluating celecoxib and the proliferative marker Ki-67 in current and former smokers found a decrease in Ki-67 at higher celecoxib doses (34).

Iloprost

PGI₂ is a PGH₂ metabolite with antiinflammatory, antiproliferative, and potent antimetastatic properties. Preclinical studies in transgenic mice with selective pulmonary prostacyclin synthase overexpression showed significantly reduced lung tumor multiplicity and incidence in response to either chemical carcinogens or exposure to tobacco smoke (35, 36). Iloprost, a long-lasting oral prostacyclin analog, also inhibits lung tumorigenesis in wild-type mice (37). These studies formed the basis of a National

Cancer Institute–sponsored double-blind, placebo-controlled clinical chemoprevention trial in which subjects at high risk for lung cancer received oral iloprost or placebo. The primary endpoint for the trial was endobronchial histology, and this recently published trial showed 6 months of oral iloprost significantly improved endobronchial dysplasia in former smokers (9). Additional studies using the iloprost samples are now in progress.

Selenium

The use of selenium for cancer prevention has been an area of considerable interest, as it improves cellular defense against oxidative stress (38). The Nutritional Prevention of Cancer trial tested selenium for the prevention of nonmelanoma skin cancer, and although it did not prevent skin cancer, the subjects in the trial exhibited a 44% decrease in lung cancer incidence (38). Further analysis of the trial determined that selenium supplementation decreased lung cancer incidence only in the tertile with the lowest baseline plasma selenium levels (39). The SELECT (Selenium and Vitamin E Cancer Prevention Trial) trial for prostate cancer chemoprevention has established lung cancer as a secondary endpoint, but results reported in abstract form did not show a benefit.

PPAR γ agonists

Prostacyclin analogs like iloprost selectively increase peroxisome proliferator–activated receptor (PPAR) γ activity both in nontransformed epithelial cells and in NSCLC. In human NSCLC cell lines, activation of PPAR γ by pharmacological agents, or by molecular overexpression, strongly inhibits transformed growth, as assessed by colony formation in soft agar (40, 41). The thiazolidinediones are oral PPAR γ agonists, and lung cancer incidence in patients with diabetes on PPAR γ agonists was decreased 33% when compared with patients with diabetes on non-PPAR γ -modulating medications (42). Thiazolidinediones have also been shown to induce 15-hydroxyprostaglandin dehydrogenase (15-PGDH), the enzyme that inactivates the antiapoptotic and immunosuppressive PGE₂ by conversion to 15-keto prostaglandins (43). PPAR γ overexpression chemoprevents murine lung cancer (37), and the PPAR γ agonist pioglitazone has been shown to chemoprevent lung cancer in the N-nitroso-tris, chloroethylurea model (44). There is a current chemoprevention trial at the Denver Veterans Affairs Medical Center evaluating pioglitazone in high-risk current and former smokers.

FUTURE TRIALS AND ENDPOINT BIOMARKERS

Advances in our understanding of the early events in lung tumorigenesis have led to the discovery of important targeted pathways for chemopreventive agents. Future trials must build on the results of preclinical testing, and the identification and validation of intermediate endpoint biomarkers should allow for a more rapid evaluation of novel agents. The chemoprevention world can take a cue from lung cancer therapeutics, and it may be possible in the future to determine altered pathways in premalignant lesions (for example, PI3K activity [45]) and to place these subjects on targeted (or “personalized”) chemoprevention.

Future trials will also need to be based on improved biomarkers of both risk and response. Validated intermediate endpoints will allow for shorter trials that do not have cancer as an endpoint, but these intermediate biomarkers need to meet the following requirements: expression correlates with disease course, expression is different between normal and premalignant tissue, and evaluation must be reproducible. Examples of biomarkers being evaluated are Ki67, p53, PI3 kinase, epidermal growth factor receptor expression, and gene methylation. These biomarkers must ultimately be validated in prospective clinical trials.

Mammalian Target of Rapamycin Inhibitors

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that mediates the akt signaling pathway. Human lung cancers, as well as dysplastic endobronchial lesions (46), have been shown to exhibit activation of the akt/mTOR pathway. Tobacco-specific carcinogens also activate this pathway (47, 48), and mTOR inhibitors (rapamycin and sirolimus) can successfully induce cell cycle arrest. Phase I studies evaluating the mTOR inhibitors are in the planning stage.

Inositol

There is increased activation of the PI3 kinase pathway in the bronchial airways of smokers with airway dysplasia or lung cancer (45), and this activation may precede the development of cancer. Oral inositol inhibits the PI3 kinase pathway, and studies are currently evaluating whether inositol can prevent lung cancer in current or former smokers with endobronchial dysplasia.

Combinatorial Agents

Due to the potentially large number of genetic and metabolic derangements in premalignant lesions, combinations of agents may prove to be the most effective route to chemoprevention. This could largely be directed by genetic or proteomic abnormalities detected in the dysplastic lesions, and similar to personalized lung cancer chemotherapy, chemoprevention may also be directed based on specific “signatures” (gene expression, proteomics, etc.) being present in biopsy specimens from high-risk individuals. The most effective chemoprevention may depend on abnormalities found in biologic specimens.

CONCLUSIONS

Despite advances in lung cancer chemotherapy, there is a pressing need for effective lung cancer chemoprevention beyond smoking cessation. The understanding of molecular changes present in premalignant lesions continues to progress, and this has resulted in more targeted single agent prevention studies. Additionally, higher-risk subjects can now be identified (including tobacco history, occupational history, airflow limitation, and family history) and trials can be enriched for these subjects. The lung chemoprevention field still needs validated secondary endpoint biomarkers, and the current trials are evaluating histology from lung biopsy specimens, along with targeted markers in specific pathways. Eventually, phase III trials with lung cancer as the primary endpoint are needed before chemoprevention agents can be recommended for widespread use in high-risk populations. These trials are difficult, expensive, and time-consuming, and the above-outlined strategies should assist in prioritizing agents for phase III trials. Information from epidemiology, cell biology, preclinical models, and phase II intermediate endpoint trials all may be useful to inform choices of agents for definitive phase III chemoprevention trials. It is clear that we must continue to promote successful smoking cessation and tobacco control. Lung cancer chemoprevention research will advance more quickly as the number of subjects involved in clinical trials increases, and at the current time subjects should only be encouraged to use chemopreventive agents in the context of a clinical trial.

Author disclosures are available with the text of this article at www.atsjournals.org.

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