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LUNG CANCER CHEMOPREVENTION

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Lung cancer is the leading cause of cancer related death in the United States and worldwide in both men and women. In 1999, more than 171,600 new cases of lung cancer are estimated in the United States alone and 158,900 lung cancer patients will die of the disease this year (1). Five-year survival rate for lung cancer is about 14% (15% for whites and 11% for blacks) in the United States (1). This poor survival rate is considered the best result worldwide since the average survival in Europe is 8%, the same as that of developing countries (2). Cigarette smoking is the major cause of lung cancer and smoking cessation can reduce the risk for lung cancer development. However, genetic damages caused by cigarette smoking can remain for a long period of time and may be responsible for the higher incidence of lung cancer in former smokers (3). In fact, about half of the lung cancer patients recently diagnosed in the United States are former smokers (4), indicating that lung cancer incidence remains high even after smoking cessation. Since there are currently 45 million active smokers and 45 million former smokers in the United States, reducing lung cancer incidence is an important social and economic problem not only in current smokers but also in former smokers in this country. The full understanding of the underlying biology of lung tumorigenesis is the key to develop novel diagnostic, prognostic, preventive, and therapeutic strategies, which will eventually eliminate the deadly disease.

An active approach to reduce lung cancer incidence and mortality is through chemoprevention which prevents the development of cancer in individuals or populations at increased risk (5). Our group has a long history of using chemopreventive agents to prevent cancer development of head and neck premalignancies and to prevent development of second primary tumors in patients with previous head and neck squamous cell carcinoma (HNSCC). We have also explored the use of chemopreventive agents in reversing morphologic and genetic abnormalities associated with cigarette smoking and carcinogenesis in the lung. To develop an optimal chemopreventive strategy, it is important to understand biological processes in airway during early tumorigenesis and the role of carcinogens. Development of subclinical intermediate endpoints is crucial for conducting effective lung chemoprevention trials. Additionally, it is desirable to assess lung cancer risk and chemoprevention effect without using tissues from lungs. The establishment of a unique system for biology-based chemoprevention trials may allow us to address these issues.

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MECHANISMS OF LUNG CARCINOGENESIS

The concept of field cancerization was introduced in 1953 by Slaughter et al., and was based on the fact that the majority of epithelial surface of the aerodigestive tract is likely to be exposed to many of the common carcinogens, such as tobacco products, and thus has an increased risk of both lung cancer and HNSCC development (6). Numerous clinical, histopathological, and molecular biological observations support this concept. Clinically, it has been shown that patients with lung cancer or HNSCC may present with multiple primary tumors or develop second primary tumors in "defect" field (7, 8). Furthermore, histopathologic abnormalities are observed in the lungs of patients with lung cancer as well as in current smokers (9). Similarly, in the larynx histopathologic abnormalities have been found in multiple epithelial sites of the organ in almost every current smoker or former smoker but is rare in nonsmokers (10). Biologically, multiple distinct genetic and cytogenetic alterations can be identified in different regions of oral epithelium obtained from patients with oral premalignant lesions or HNSCC (11, 12). Like the oral cavity, multiple genetic abnormalities have also been identified in the epithelial cells of the lungs in current smokers and former smokers. Using microsatellite analysis, clonal genetic changes at critical chromosome regions containing tumor suppressor genes have been frequently detected in the lungs of smokers even without history of lung cancer (13, 14). Importantly, many of these genetic changes were found in epithelial areas without definitive histopathologic abnormalities and some of these alterations persisted for many years after smoking cessation (13). These results suggest that epithelium in the respiratory tract exposed to carcinogens contains clonal genetic alterations important in early lung tumorigenesis and may be the basis for an increased risk of cancer development in smokers. It may be hypothesized that the presence of "defect" field in oral cavity reflects the presence of a similar "defect" field in lungs and therefore may predict risk of cancer development both

in oral cavity and lungs. Consequently, the improvement of the "defect" field may predict a reduced risk of cancer development.

ACCUMULATION OF MOLECULAR ALTERATIONS IN LUNG TUMORIGENESIS

The multistep genetic process in cancer development has initially been illustrated in a model of colorectal cancer known as the "Vogelgram" (15). Modern molecular technology has facilitated a rapid and effective identification of mutations, deletions, and amplifications of critical genes in the colorectal carcinoma model. A similar progression process may also exist in many other types of human cancers. Several conceptual features that may be involved in the tumor progression process have been illustrated in this model. First, early in the colorectal tumor development, mutational activation of an oncogene, K-ras, coupled with predominant mutational inactivation of a tumor suppressor gene, APC, are the earliest detectable events of genetic alterations. The term "gatekeeper" has been used to describe a crucial molecule, such as APC, which is essential in preventing cells from becoming neoplastic in a particular cell type. Second, alterations of multiple genes are required for the development of an invasive tumor, while fewer alterations suffice for the development of benign tumors. Third, the genetic alterations often occur according to a preferred sequence, suggesting not only that a total number of accumulated genetic changes is important for the transformation of a cell from normal to malignant phenotype but the order of specific genetic alterations may also be important in the tumorigenic process (15, 16).

A statistical analysis based on the age-specific mortality rates of different types of human cancer indicates that usually three to 12 critical genetic mutations might have taken place before most clinically diagnosable tumors form (17). This assumption is likely underestimating alteration events required for the development of human cancers because the model did not consider the rates of mutations and other types of genetic alterations are accelerated in the multistep tumorigenic process. Epithelial cells in the oral and respiratory tract are exposed to various carcinogens, such as cigarette smoking, and accumulate genetic damages in the carcinogenic process. Many genetic alterations have been identified with high frequencies in human lung cancer in the past two decades. p53 tumor suppressor gene was found mutated in about 50% of non-small cell lung cancers (NSCLC) and 80% small cell lung cancers (SCLC) (18-20). pRB is inactivated frequently in SCLC (21) but rare in NSCLC. Mutations of K-ras proto-oncogene were found in about 30% of adenocarcinomas of the lung (22) but less frequent in other types of lung cancers. Inactivation of p16 tumor suppressor gene was seen in more than 70% of NSCLC (23). Genetic alterations in several other candidate tumor suppressor genes and proto-oncogenes, such as DPC4, FHIT, c-MYC, and cyclin-D1, were also found in subsets of lung cancers (24-27). Because one of the two alleles of a tumor suppressor gene is often inactivated by deletion, loss of heterozygosity (LOH) has been widely used to determine minimally deleted chromosome regions where tumor suppressor genes locate. LOH has been identified frequently in a number of chromosomal loci, such as the short arms of chromosomes 3 and 9, in lung and head and neck cancers (28-32).

It is believed that lung cancer also develops through a multistep process similar to that of colorectal cancer. Although it is difficult to identify definitive preneoplastic lesions that precedes the lung cancer development, bronchial metaplastic and dysplastic lesions are thought to be precursors of squamous cell carcinomas of the lung. Indeed, recent studies of bronchial dysplastic lesions from patients with lung cancer revealed specific genetic alterations, such as K-ras gene mutations and loss of chromosome 9p and 3p regions where critical tumor suppressor genes have been identified (33-35). We and others have found that LOH, particularly at 3p and 9p regions, are present in bronchial epithelium of current smokers and former smokers, even in histologically normal appearing bronchial epithelium, suggesting inactivation of certain critical tumor suppressor genes occurs in very early stages of lung carcinogenesis (13, 14). Other abnormalities, such as p53 mutations and loss of 18q, however, appear occurring mainly in the histologically later stages of the process (31, 36).

The FHIT gene is located at 3p14.2 , a region frequently lost in multiple tumor types. Loss of FHIT protein expression has been found to occur frequently in multiple tumor types including NSCLC . Despite the early controversy whether FHIT is a true tumor suppressor, recent data from knockout animal experiments and transfection experiments indicate FHIT does play an important role in tumorigenesis. Using an adenovirus vector containing FHIT, Ji et al. demonstrated that using the adenovirus-vector to infect lung cancer cells lacking FHIT could inhibit lung

tumor formation in nude mice and injection of the virus vector could reduce sizes of already formed lung tumors in the animal model (41). The mechanism of the effects is massive apoptosis caused by a high level of Fhit expression. Their recent data further indicate that the apoptosis in lung tumor cells induced by introducing the FHIT-adenovirus might be via upregulation of a death (DR4 and DR5) signaling pathway and caspase cascade as well as inhibition of tumor angiogenesis (Ji, L. personal communication). We recently analyzed Fhit protein expression in primary lung tumors from patients with stage I NSCLC and bronchial biopsy specimens from chronic smokers without lung cancer. We found that Fhit expression was absent or markedly reduced in about half of the primary tumors and in 23% of bronchial biopsy specimens from chronic smokers (42). The loss of Fhit, located at 3p14, expression was associated with bronchial metaplastic changes, patients' metaplasia index (MI), and current smoking status (42). Recently, several genes with high homology to p53 tumor suppressor gene were identified including p40/p51/Np63/p73L located at chromosome 3q27 (43-46). Due to the transcription variants of the gene, different names were given in literature. p63 but not Np63 contains a transcriptional activation domain and was shown to suppress colony formation in cancer cell lines and to transcriptionally activate p21 in a fashion similar to the p53 tumor suppressor gene (45). Hibi et al. studied p40/p73L expression in lung cancer and HNSCC and found that the gene was overexpressed in all squamous cell carcinomas tested (47). They further examined the copy number of the gene by using FISH analysis and found the region of the gene located was amplified in all these tumor cells tested and this amplification correlated with the gene expression and protein expression, suggesting that amplification plays an oncogenic role in lung and head and neck squamous cancer development (47). Normal differentiated cells have a defined lifespan and a limited number of cell divisions. Bronchial epithelial cells are renewable and usually shed in a few weeks. To allow an accumulation of multiple genetic and epigenetic abnormalities in these cells for malignant transfromation, immortalization is crucial. Human chromosomes are capped at each end by telomere, a structure essential in the regulation of cell lifespan (48, 49). This specific structure prevents chromosomes from degradation and fusion (50). Telomerase, an RNA-dependent DNA polymerase, is believed to be the enzyme that maintains and elongates telomeres (51). Indeed, telomerase activity has been detected in germline cells but not in the majority of postnatal somatic tissues or in somatic cells grown in culture (52-54). However, telomerase has been found re-activated in the majority of human cancers including 80-85% of lung cancers (55, 56) and may be required in early tumorigenic process (57, 58). It has been reported that telomerase activity in lung cancer is associated with cigarette smoking and poor prognosis (59, 60). This concept is supported by a recent elegant study in which a multistep tumorigenic model was established by introducing telomerase as well as several other critical tumor suppressor genes or oncogenes into a single cell (61).

It is now widely accepted that DNA methylation plays an important role in regulating gene expression and development. Experiments carried out in mammalian cells provide strong evidence to support that DNA methylation is sufficient to inactivate a gene and that demethylation is sufficient to activate it. In normal cells about 80% of CpG sites in the genome are methylated while most of CpG-rich promoter regions are not methylated (62, 63). Accumulating data suggest that disregulated DNA methylation in the genome may be one of the initial abnormalities in tumorigenesis. Decrease of global level of methyl cytosines was found to begin early in colon tumorigenesis in experimental animal models as well as in human tumorigenesis (64, 65). It is possible that this widespread demethylation may activate certain genes and play an important role in early tumorigenesis. In fact, activation of MAGE genes, which encode tumor specific antigens, is found in many types of human cancers and this activation is due to the CpG hypomethylation in the promoter regions of these genes (66-68). While most of CpG islands located at promoter regions are normally unmethylated, some of these promoters may be de novo hypermethylated during carcinogenic process. Our earlier study of p16 tumor suppressor gene has firmly demonstrated that de novo DNA hypermethylation is an important mechanism to inactivate the gene in multiple tumor types (69). A body of evidence now supports the importance of de novo DNA hypermethylation in inactivation of a considerable number of tumor suppressor genes in tumorigenesis (70). These common abnormalities occurred in the early process of lung tumorigenesis may be detected in specimens obtained from individuals participating clinical chemoprevetion trials. The presence of these abnormalities represents an increased risk of developing lung cancer and therefore may be used to assess level of the risk and efficacy of chemopreventive agents.

PROCEDURES FOR BRONCHIAL TISSUE COLLECTION

Bronchoalveolar Lavage (BAL) fluid

Bronchoalveolar lavage is a fluid sample of protein and cellular contents from alveoli and distal airways. It is obtained by wedging the flexible bronchoscope into a subsegmental bronchus of the right middle lobe. Aliquots of 20 ml of 0.9% sterile saline are sequentially infused and aspirated until 20 ml of lavage are obtained. The lavage is to be stored at -80 C immediately after removing cellular components.

Proteins and metabolites in the BAL fluid are to be determined whether the candidate genes characterized by cDNA array analysis (see section 3 below) coincide with changes in their expression levels. We also determine global changes of expressed proteins in the BAL using Proteomics analysis.

First Tracheobronchial Washing

First tracheobronchial washings is obtained by infusing 20 to 40 ml of 0.9% sterile saline into the large airway (trachea and main bronchi). This sample contains mostly superficial exfoliated epithelial cells and airway secretions. The first bronchial wash will be obtained prior to biopsies or brushings of the airway. The washings (airway secretions) are to be stored at -80 C immediately after removing cellular components. Proteins and metabolites in the airway secretions are to be determined as mentioned above.

Bronchial Biopsies

Endobronchial biopsies are done under direct vision with a flexible bronchoscope. Biopsies are taken from 6 predetermined sites from the main carina and the carinae of the right upper lobe, right middle lobe, right lower lobe (RB10), left upper lobe, and left lower lobe (LB8). Mucosal regions that appear abnormal are also biopsied and prepared for histologic analysis of H&E stained sections. Evidence of carcinoma in situ or invasive carcinoma disqualifies patients from further participation in the chemoprevention protocol, and the patient is referred for further treatment.

Normal HBE cells are grown from bronchial mucosal biopsy samples resected from fresh surgical specimens. The mucosal layer is sterilely stripped from bronchial specimens, cut into small pieces, and placed on a plastic tissue culture plate containing a thin layer of medium. When normal HBE cells have grown from these tissues into a 60% confluent monolayer population, they are expanded for use in this study. Normal HBE cells are grown on standard plasticware (Falcon Labware, Becton Dickinson and Co., Franklin Lakes, NJ) in Keratinocyte Serum-Free Medium (GIBCO BRL, Gaithersburg, MD) containing epidermal growth factor and bovine pituitary extract at 37C with a pCO2 of 5%.

Bronchial Brushings

Bronchial brushings are obtained using a 3-mm cytology brush. Airway epithelial cells are then harvested. Samples are obtained from the walls of the mainstem bronchi and 6 preselected sites adjacent to endobronchial biopsy regions.

Epithelial cells are to be used for 1) cDNA array analysis to determine differential expression of mRNAs, and 2) in vitro cell cultures to determine differential responses against chemotherapeutic agents.

Tracheobronchial Washing

A second bronchial wash is also collected after bronchial brushes and biopsies are obtained. Twenty to 40 ml of 0.9% sterile saline will be used. This second sample will be rich in epithelial and submucosal cells. The wash will be stored at -80 C after separating cellular components.

IMPORTANCE OF BIOMARKERS IN EARLY CANCER DETECTION AND RISK ASSESSMENT

One of the major obstacles in cancer diagnosis and risk assessment is the lack of effective indicators that can be used to make diagnosis before clinical symptoms appear and accurately measuring individual's risk of cancer development. For effective chemoprevention, we have to select individuals at different risk levels and use different preventive strategies. For an individual with extremely high-risk to develop invasive lung cancer, the use of multiple chemopreventive agents or even cytotoxic agents might be justified. For individuals with intermediate risk, a single chemopreventive agent might be sufficient. Since most of these individuals with risk of developing lung cancer lack a clinical symptom or sign, molecular or genetic measurements (biomarkers), which may occur far earlier than any clinical sign, may play a crucial role in risk assessment. Furthermore, because lung cancer develops over decades and involves accumulation of molecular changes in the tumorigenic process, selection of molecular changes as intermediate endpoints may benefit clinical chemoprevention trials and the assessment of treatment effects.

Along with the advances in understanding biology and biotechnology, progress has been made in terms of biomarker development and potential clinical applications

in the past few years. It has been shown that rare malignant cells with clonal genetic alterations could be successfully detected in bodily fluids and other clinical specimens. p53 mutations had been successfully used as clonal markers to detect rare tumor cells in urine sediments of patients with bladder cancer (71), in sputum of patients with lung cancer (72), and in saliva and histologically negative surgical margins of patients with HNSCC (73). K-ras mutations have been detected in sputa collected before clinical diagnosis of lung cancer (71) and bronchoalveolar lavage fluid (BAL) from patients with early stage lung cancer (74). Microsatellite analysis has also been used for detecting clonal alterations in body fluids, such as saliva, sputum, urine, and serum (75-77). We have demonstrated that urine sediments using microsatellite analysis alone might detect more than 90% of patients with primary and current bladder cancers (78, 79), which has been confirmed by other independent research groups (80). We are the first to demonstrate that oral leukoplakia is a clonal disease and LOH at critical chromosome regions can predict development of invasive cancer in oral cavity (81) and augment clinical and histopathologic assessment (82). Similar results have been recently reported by other independent researchers (83, 84). Epigenetic alterations specific in tumor cells have also been tested for potential applications in detecting rare tumor cells in bodily fluids or lesion-specific molecules in serum. De novo DNA methylation of promoter regions of tumor suppressor genes has been found in a substantial proportion of lung cancers as well as HNSCC and results in inactivation of these tumor suppressor genes (69, 85, 86). We recently found that hypermethylation of death associated protein kinase (DAP-kinase) is frequent in early stage NSCLC and strongly associated with patients' outcome (87). Belinsky, et al. reported that abnormal methylation of p16 tumor suppressor gene could be detected in sputum of patients with lung cancer as well as in some smokers without lung cancer, suggesting it might be used as a clonal marker (88). In another study, Esteller et al. tested hypermethylation of promoter regions in four tumor suppressor genes in primary lung tumors and the corresponding sera (89). Tumor-specific methylation of at least one of these genes was detected in 15 of 22 (68%) NSCLC tumors but not in any paired normal lung tissue. In the patients whose tumors contained the hypermethylation, 11 of 15 (73%) also had identical abnormal hypermethylated DNA in the matched serum samples, but none of the sera from patients with tumors not demonstrating hypermethylation exhibited a positive serum (89). In our preliminary study, we have found that de novo promoter hypermethylation in several tumor suppressor genes or modifier genes are also common in bronchial as well as buccal brush specimens from former smokers (see preliminary results section). We have revealed that de novo demethylation might also occur in lung tumorigenesis. Expression of MAGE genes located at X chromosome have been shown to be activated in melanoma and other malignancies including lung cancers but not in any normal tissues except germ cells. The activation of the gene expression has been shown the result of demethylation of promoter regions of the genes (68, 90). We have shown in our preliminary study that the genes were not only activated frequently in lung cancers but also in bronchial cells from smokers (see preliminary results section), suggesting that the activation of the genes is an early event in lung tumorigenesis and may play an important role in tumor immunology. These findings raise a possibility to use both de novo hypermethylation and demethylation as markers to increase power of detecting abnormalities in specimens obtained from relatively healthy individuals to evaluate risk as well as therapeutic effects.

LUNG CANCER RISK AND BIOLOGICAL DIFFERENCES BETWEEN CURRENT AND FORMER SMOKERS

Epidemiological studies have singled out cigarette smoking as the number one cause of lung cancer. One of the major variables of smoking risk is duration of the exposure, since the development of lung cancer requires the accumulation of specific genetic alterations over a long period of time. The risk of developing lung cancer is about 15-fold greater in smokers than in nonsmokers. The relative risk of developing lung cancer decreases from about 15-fold to 1.5- to 4-fold in former smokers who have quit for 15 years. Despite this decreased relative risk, recent United States studies show that almost 50% of new lung-cancer cases occur in former smokers. Our group has shown that the metaplasia rate and cell proliferation in bronchial epithelium may be significantly reduced after smoking cessation (91). However, our recent data indicate that some genetic and epigenetic abnormalities might remain in the lung for a long period time despite smoking cessation (13). Results from several large-scale retinoid-based chemoprevention studies suggest that retinoids might not be effective or may even have a harmful effect in current smokers (92, 93), suggesting the presence of differences in biology between current smokers and

former smokers. Therefore, it is crucial to understand biologic basis of tobacco-damaged lungs of current and former smokers in order to develop novel strategies for lung cancer prevention.

One of the major challenges in the lung chemoprevention trials is the lack of reliable and practical endpoints. Ideally, cancer incidence should be used as an endpoint to test chemopreventive agents. However, since the lifetime risk of lung cancer for a heavy smoker is about 10% and the development of lung cancer requires several decades of carcinogen exposure, it is not practical to use cancer as an endpoint due to the requirement of a large number of cohorts, a long experimental period, and huge budget for testing each regimen. Some morphologic changes such as bronchial metaplasia and dysplasia have been used as study endpoints and will probably continue to be used as intermediate endpoints to evaluate preventive efficacy for awhile due to the lack of validated alternatives. However, the diagnosis of these morphologic changes is somehow subjective and requires experienced pathologists. More importantly, the association between these changes, particularly metaplasia, and lung cancer development has not been firmly established. In fact, many of these morphologic changes could be the result of inflammation or cellular responses to other stimuli. As we have discussed above, the basis of lung tumorigenesis is the accumulation of molecular alterations in a long multistep process. We and others have demonstrated that some of the genetic and molecular alterations important in lung tumorigenesis could be frequently identified in bronchial epithelium of smokers without evidence of lung cancer. Because these alterations are involved in the early lung tumorigenic process, they could potentially be used as intermediate endpoints in evaluating the effects of promising chemopreventive agents.

Some of the promising biomarkers have been integrated into clinical chemoprevention trials. For example, RAR, whose expression is commonly lost in oral premalignant lesions as well as bronchial epithelium of smokers without lung cancer, has been used as an intermediate endpoint in our ongoing retinoid-based chemoprevention trials (U19 CA68437, Lung SPORE P50 CA70907). LOH at regions harboring critical tumor suppressor genes such as 3p14, 9p21, and 17p13 was frequently detected in the lungs of smokers and has also been considered as a secondary endpoint in the ongoing lung chemoprevetion trials. However, it is just a beginning of exploring the potential implication of molecular markers in cancer risk assessment and chemoprevention studies. Many issues remain to be resolved. The precise role of each molecule used as a biomarker in the multistep tumorigenesis must be fully understood. A panel of biomarkers rather than a single one must be developed to reflect carcinogenic pathways and biologic properties of cells at different stages of carcinogenic processes. If these goals can be achieved, potential efficacy of candidate agents for preventing cancer development may be quantified. Another major obstacle in evaluating efficacy of lung cancer chemoprevetion strategy is the selection of biologic materials for assessing intermediate endpoints. Although bronchial biopsies, brushes, and lavage specimens can provide bronchial epithelial cells from distinct areas of the airway for various morphological and biomarker examinations, the sample collection requires bronchoscopy. Sputum is a logical source to obtain epithelial cells of the airway for the determination of morphologic and molecular changes in lungs. However, sputum is often non-representative and contains minimal cells from the lungs with significant variable number of contaminating cells. It is also difficult to obtain epithelial cells from distal lungs, particularly in former smokers. Despite the difficulties, we and others continue to explore the possibility of using sputum in lung cancer risk assessment. Ideally, biomarkers associated with high-risk of lung cancer/lung premalignancies and detectable in serum should be used to assess lung cancer risk and to evaluate therapeutic/preventive effects. This is an area need to be explored and we will conduct experiments using supports from other mechanisms. Should promising biomarkers be identified through these studies, they could be integrated into our biomarker-based chemoprevetion trials. Another approach is to use surrogate tissues in the airway such as oral epithelial cells to determine potential effects of chemopreventive agents in the lungs. Development of cancer in the oral cavity and in the lungs, particularly squamous cell carcinoma, has many similarities. Both types of cancers develop as a result of the exposure to similar carcinogens such as tobacco, and they share many common genetic and epigenetic alterations. If preventive drug-related molecular modulations (measured by biomarkers) prove to be similar, we may possibly use buccal epithelial cells, which can be easily and inexpensively obtained through a simple brushing, to replace bronchial samples for biomarker analyses in lung chemoprevention trials.

CYCLOOXYGENASE (COX)-2 INHIBITORS AND THEIR POTENTIAL CHEMOPREVENTIVE EFFECTS

Cyclooxygenase is the key regulatory enzyme for prostaglandin synthesis and is transcribed from two distinct genes, COX-1 and COX-2. COX-1 is expressed constitutively and is thought to be important in maintaining cellular homeostasis while COX-2 is induced by a wide variety of factors. COX-2 expression levels are elevated in multiple types of human cancers including lung cancer as well as premalignant lesions (94-98), suggesting its role in early tumorigenesis. It has been shown that COX-2 expression can be up-regulated by cigarette carcinogens such as benzo[a]pyrene in normal and transformed oral epithelial cells (99) and an increased expression of COX-2 associated with rat lung tumorigenesis induced by the tobacco-specific carcinogen nitrosamine 4-(methylnitrosamino-4-(3-pyridyl)-1-butanone (NNK) (100). Inhibition of COX-2 activity has been found to regulate cell proliferation, antiangiogenesis, and increase apoptosis in certain tumor types including lung cancer (101-103). The roles of COX-2 in tumor angiogenesis and/or immune responses have also been suggested (104, 105). Suppression of intestinal polyposis was observed in COX-2 knock out animals (106) and suppression of colon adenocarcinoma formation by COX-2 inhibitor, Celecoxib, in an animal model (107, 108). A large body of evidence has suggested that inhibition of COX-2 could be an important strategy for preventing cancer.

SUMMARY

A major obstacle in the lung cancer chemoprevention trials is the lack of reliable intermediate endpoints to assess efficacy. It is very difficult to use lung cancer incidence and survival, a gold standard, as an endpoint in these trials because of the requirement of a large number of cohorts, long study duration, and extremely high cost in such study design. Therefore, the identification and utilization of intermediate endpoints may significantly enhance our ability to conduct lung chemopevention trials. Currently, bronchial metaplasia index, dysplasia, and proliferation markers have been used in some of the lung cancer prevention trials. RAR- expression has been used to determine effects of retinoids in reversing the mRNA expression of the gene. However, most of these parameters may be heavily influenced by factors not related to lung carcinogenesis and others may only monitor the activation of drug-associated pathway rather than reduced cancer risk. Furthermore, the functional pathways of most chemopreventive agents are complex and may not be fully understood at the time of clinical trials. Therefore, the intermediate endpoints currently used may not be able to accurately assess efficacy of the agents. Clearly, novel intermediate endpoints are badly needed and a panel of parameters may be required to more precisely evaluate treatment effects. Many molecular abnormalities which are important in lung cancer development and progression can be identified in very early stages of lung carcinogenesis, even in lung epithelial cells without obvious morphologic abnormalities. Because lung cancers develop through the accumulation of multiple molecular abnormalities, the reduction of cells with such abnormalities or preventing the creation of new abnormalities by chemopreventive agents is logically predictors of a reduced risk of lung cancer development. As a result, these abnormalities can be measured and used as biomarkers for intermediate endpoints of chemoprevention trials.

For lung cancer chemoprevention, target cohorts are the population of heavy smokers with a higher risk to develop lung cancer. We now know that heavy smokers have defects in their epithelial field of the airway where numerous small clones with molecular abnormalities exist. If an agent or agents can reduce lung cancer risk, they should be able to reduce the number of these abnormal clones or the size of these clones regardless the mechanisms and functional pathways of the agents. Our long term goal is to develop a risk estimate model based on a panel of molecular markers to evaluate efficacy of different chemopreventive agents. Because the target populations of chemoprevention are those at higher risk but not necessary to have clinically measurable lesions or abnormalities and development of clinically measurable disease may take a decade or longer, it is crucial to develop subclinical parameters which can be used as intermediate endpoints. Administration of Celecoxib, a cyclooxygenase-2 (COX2) inhibitor the frequencies or sizes of the clones with genetic/epigenetic alterations. These intermediate endpoints are being analyzed using a panel of biomarkers consisting of deletions at critical chromosomal regions, epigenetic changes (DNA methylation), expression of telomerase catalytic subunit, Fhit protein expression level, and Ki-67- a marker of epithelial proliferation.

Because lung is an inner organ, obtaining tissues from lung is difficult and invasive. It is challenge and one of the rate-limiting factors in conducting lung

chemoprevention clinical trials. The degree of invasiveness in the work up of patients at risk can be initially assessed by historical risk factors. Additional non-invasive studies (buccal brushings, serum samples) may provide the information needed to determine those who will require bronchoscopy of more invasive work up. These analyses will constitute the basis for establishing the specific chemoprevention strategy.

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