(2) Patients with discrete mediastinal lymph node enlargement and contralateral FNA-demonstrated disease.

FDG PET might provide information concerning the likelihood of involvement of the node as well as possible metastatic disease. The false positive rate for PET in the mediastinum is 15 to 20% and the false negative rate for patients with enlarged lymph nodes is 15 to 25%. Thus, performing a needle aspiration technique either by EUS or EBUS is warranted. Given the false negative rate of 20 to 30%, finding a negative FNA should be followed by a mediastinoscopy. Patients with adenocarcinoma, large cell carcinoma or other histologies that are more likely metastatic, primary lesions larger than 2-3 cm in largest dimension, centrally located tumors, tumors that have clinical N1 disease, those with FDG-PET SUV of >7 or multiple abnormalities on PET or in those that have serum CEA level >5 ng/L are all considered risk factors for potential N2 or N3 disease and necessitate FNA and if negative, mediastinoscopy. In patients with peripheral primary tumors with no lymph node enlargement on CT, no other risk factors and no primary tumor FDG uptake (<2.5) have a mediastinal disease prevalence of less than 3%.

In summary, patients who have enlarged or suspicious appearing mediastinal nodes on CT and/or those with FDG-PET or PET/CT uptake, an EUS and/or EBUS can be performed to confirm the presence of disease rather than assuming malignant involvement. A thorough evaluation of the mediastinum should be performed. If metastatic disease is not found, then mediastinoscopy should be performed as the negative predictive value of FNA is insufficient to rule out the presence of metastasis. If it is found, then induction therapy can be used. For those patients with a negative mediastinum on PET and CT and if the lesion is peripheral, in the outer third of the lung field and the SUV of the primary is less than 2.5, then mediastinoscopy is unlikely to provide further helpful information. For more adenocarcinomas and large cell cancers or central or lesions larger than 4 cm or clinically N1 patients or in whom the SUV of the primary is larger than 7, then the prevalence of mediastinal involvement is higher, increasing the error of CT and PET. The negative predictive value in these cases is not sufficient to eliminate the probability of disease in the mediastinum. Prospective trials evaluating the use of TEMLA should be performed to assess its ability in providing a survival advantage, but it does appear to provide superior staging information. Future methods of genetic analysis of the primary tumor as well as genetic and marker analyses of the lymph nodes, bronchial washings, bone marrow and serum might provide greater prognostic information than simple histological examination of the mediastinal lymph nodes. If this is the case, mediastinoscopy might be unnecessary.

Session M15: Genetics and Epigenetics in Lung Carcinogenesis

M15-01 Integrating epigenetic and genetic biomarkers for early lung cancer detection

Belinsky, Steven A.

Lovelace Respiratory Research Institute, Albuquerque, NM, USA

Lung cancer is the leading cause of cancer-related death in the U.S. and is projected to reach epidemic levels in the world during the 21st century. Chemotherapy has been largely ineffective in producing complete responses or cures in advanced disease so that over 85% of patients with lung cancer eventually succumb to the disease. Mortality could be reduced greatly by identifying persons at high risk for cancer and developing effective interventions to impede or reverse respiratory carcinogenesis. Key to the success of this strategy is the identification of highly efficacious preventive interventions and biomarkers that can both identify persons at the earliest stages of lung cancer development and monitor the efficacy of interventions.

The silencing of genes through promoter hypermethylation is now recognized as a major and causal epigenetic event that occurs during lung cancer initiation and progression. Genes silenced by methylation are involved in all aspects of normal cellular function that include control of cell proliferation, differentiation, and death. The involvement of gene methylation in carcinogenesis has led to studies focused on establishing the utility of methylation as a biomarker in screening for cancer risk, prevention, treatment, and prognosis. The development of the methylation-specific PCR (MSP) assay has facilitated these studies because it allows for the assay of methylation of specific genes in biological fluids such as sputum where epithelial cells comprise only a fraction of the cellular content.

In a small proof-of-concept study, we detected p16 or MGMT gene promoter methylation in sputum up to 3 years prior to the diagnosis of SCC. The evaluation of cancer-free individuals who were at risk for lung cancer because of smoking and/or exposure to radon through uranium mining revealed methylation of the p16 and MGMT genes in 15% and 25% of sputum samples, respectively. The most striking difference seen between lung cancer cases and controls was the detection of both methylated genes in 48% of sputum samples from cases but only in 3% of controls. These findings were the first to suggest that aberrant gene methylation could be highly sensitive as molecular markers in population-based screening for early detection of lung cancer.

The well-documented field carcinogenesis seen in lungs of smokers stemming from exposure of the entire respiratory tract to inhaled carcinogens within cigarette smoke presents an obstacle to the early detection of lung cancer. The generation of multiple, independently initiated pre-malignant lesions throughout the lungs of people with a long history of smoking likely accounts for detecting methylation of genes such as p16 that is inactivated in the earliest stages of pre-invasive disease. We hypothesized that the use of promoter methylation as a biomarker for early detection of lung cancer would require a panel of genes whose presence in sputum confer a high enough sensitivity and specificity for distinguishing very advanced dysplasia or early lung cancer from the large “at risk” population. This hypothesis was tested in Specific Aim 1 of this project by conducting a nested case-control study of incident lung cancer cases from an extremely high-risk cohort (developed through the Colorado Lung SPORE) for evaluating promoter methylation of 14 genes in sputum. Controls (n=92) were cohort members matched to cases (n=98) by gender, age, and month of enrollment. The comparison of proximal sputum collected within 18 months to >18 months prior to diagnosis showed that the prevalence for methylation of gene promoters increased as the time to lung cancer diagnosis decreased. Six of 14 genes were associated with a >50% increased lung cancer risk. The concomitant methylation of three or more of these six genes was associated with a 6.5-fold increased risk and a sensitivity and specificity of 64%. These studies have now been extended to evaluate new cases and controls as well as 20 additional candidate genes. These results will be presented. In addition, studies are ongoing to assess methylation of a gene panel in sputum obtained from the prevalent Stage I lung cancer case that is generally asymptomatic.
for disease. These early stage lung cancers represent the cases whose identification by the sputum-based methylation assay should benefit from surgical resection. A case-control study is underway comparing methylation of an 8-gene panel in sputum from prevalent Stage I lung cancer cases to cancer free controls from the Lovelace Smokers cohort. Results indicate a sensitivity and specificity of 75% and 81%, respectively for case identification.

The identification of genetic determinants for gene methylation could augment the gene methylation marker panel being developed for early detection. The hypothesis being tested is that diminished DNA repair capacity (DRC) is associated with increased methylation index detected in sputum from persons at risk for lung cancer. A nested, case-control study was conducted with members of the Lovelace Smokers Cohort. Cases and controls were defined as cohort members with ≥ 3 or 0 genes methylated in sputum, respectively. The mutagen sensitivity assay was used to assess DRC. Lymphocytes from cases and controls were treated with bleomycin and chromosome breaks counted for 100 metaphases. A striking difference in DRC capacity (p < 0.001) between cases and controls was seen. Details of this study will be presented. These studies demonstrate a strong association between reduced repair capacity for double-strand DNA breaks and methylation index in sputum. Studies are in progress to identify specific gene haplotypes that predict for reduced DNA repair capacity and can add to the predictive power of our gene methylation panel for early detection of lung cancer. (Support by P50 CA58184 and U01 097356).

M15-02 Genetics and Epigenetics in Lung Carcinogenesis, Thu, Sept 6, 10:30 - 12:00

Molecular pathogenesis of lung cancer and its precursor lesions

Gazdar, Adi F.

UT Southwestern Medical Center, Dallas, TX, USA

Molecular pathogenesis of lung cancer and its precursor lesions

From histological and biological perspectives, lung cancer is a complex neoplasm. It can be divided into centrally arising tumors arising from the major bronchi and their divisions (squamous cell and small cell carcinomas) and peripherally arising adenocarcinomas (mainly adenocarcinomas and large cell carcinomas). In the bronchi the stem cell is thought to be the basal cell (for squamous cell carcinomas) and neuroendocrine cells (for small cell carcinomas). A peripheral airway cell has been identified in the mouse, and apparently is the common origin for clara cells and type 2 pneumocytes which are believed to be the precursor cells for bronchioles and alveoli respectively. Although the sequential preneoplastic changes have been defined for centrally arising squamous carcinomas of the lung, they have been poorly documented for the other major forms of lung cancers, including small cell lung carcinoma and adenocarcinomas. There are three main morphologic forms of preneoplastic lesions recognized in the lung: squamous dysplasias, atypical adenomatous hyperplasia (AAH), and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia. However, these lesions account for the development of only a subset of lung cancers. Of interest, many changes associated with peripheral adenocarcinomas including KRAS and EGFR mutations have been demonstrated in AAH lesions. Some oncogenes such as EGFR may be activated by both mutations and/or amplification. Preliminary studies indicate that mutation occurs first, followed by preferential amplification of the mutant locus. Several studies have provided information regarding the molecular characterization of lung preneoplastic changes, especially for squamous cell carcinoma. These molecular changes have been detected in the histologically normal and abnormal respiratory epithelium of smokers. They indicate that molecular changes appear very early in the lengthy preneoplastic process, even before histologically detectable changes. Many histologically detected preneoplastic lesions are very small in size (1-5 mm). The small size and difficulties of identifying such lesions complicates their study. Fluorescence endoscopy aids the identification of such lesions, although most are fixed in formalin and paraffin embedded, rendering molecular studies more difficult. Approaches to the study of small preneoplastic lesions include microdissection of the epithelium, morphometric studies, allelic losses, gene mutations and amplifications, protein expression by immunostaining or in situ hybridization, gene expression at RNA or protein levels etc. The advent of CT screening approaches for early detection of lung cancer has led to the identification of small ground glass opacity (GGO) type of lesions, some of which are AAH lesions. Thus these lesions can be biopsied or removed and information about their natural history obtained.

The natural history of preneoplastic lesions is complex, and cancers may appear at sites previously biopsied and having minimal or no morphologic changes. Thus preneoplastic lesions may be indicators of smoke damaged epithelium with a greater propensity to progress to high grade lesions or invasive cancer, although the latter may not arise directly from morphologically identified lesions. Global approaches to studying preneoplasia include microarray expression data and comparative genomic hybridization. These techniques clearly demonstrate progressive changes from normal epithelium to dysplasia to CIS. CIS is also somewhat different from invasive squamous carcinomas. In addition, the bronchial epithelium of smokers and never smokers demonstrate many changes.

A novel method to study preneoplasia is the introduction of tumor associated genes into bronchial immortalized preneoplastic cell cultures. Bronchial epithelial cultures are immortalized by infection with CDK2 and hTERT. Such cells are immortal but retain minimal cytogenetic and molecular changes. After introduction of oncogenes such as mutated KRAS or p53 knockdown, or both, they develop morphologic and biologic features suggestive of preneoplastic respiratory epithelium. These altered cell lines demonstrate squamous dysplastic changes and the ability to invade the underlying matrix. However they usually lack tumorigenicity in mice. They appear to be useful models for studying preneoplasia.

Advances in biology are providing insights into the complex and lengthy preneoplastic process. These advances may impact on prevention measures and identification of individuals at greatly increased risk.

Selected references