Molecular Detection of Early Lung Cancer

Adi F. Gazdar, John D. Minna

Lung cancer is the most common cause of cancer death in the United States, and most cases follow long-term use of tobacco products (1,2). The first objective in lung cancer control is to prevent persons from starting to smoke and to help those who smoke to quit. Nevertheless, in the United States today, there are 48 million smokers, most of whom find it difficult to quit, with new smokers rapidly replacing the quitters (3) and smokers who die from their disease. In addition, about 40% of all new lung cancers are occurring in former smokers, who remain at high risk for developing lung cancer for many years after they stop smoking (4). Under current standard methods of diagnosis and treatment, less than 15% of patients with lung cancer will survive their disease (2). Most patients who achieve long-term survival are those with non-small-cell lung cancers (NSCLC), who have surgical resection of early stage invasive tumors without metastases (stage I) or tumors with metastases limited to the adjacent ipsilateral lymph nodes (stage II). Patients with preinvasive and microinvasive cancers that are found by cytologic examination of sputum have high survival rates (>90%) after surgical removal or localized therapy (2), but they constitute less than 1% of newly diagnosed cases (5).

Thus, we need ways to aid smoking cessation, ways to prevent the development of lung cancer in current and former smokers, and new methods for the very early detection of lung cancer. There has been intense study of molecular abnormalities involving dominant and recessive oncogenes in clinically evident lung cancers (6). In addition, many of these abnormalities develop in preneoplastic lesions and in histologically normal epithelium of the respiratory tract (7,8). Can these research data be “translated” into clinical applications by using molecular abnormalities as biomarkers for the detection of early stage lung cancers or identification of those individuals at highest risk? In this issue of the Journal, Ahrendt et al. (9) describe their experience with molecular methods for the detection of early stage lung cancer.

Since the 1930s, cytologic examination of sputum has been used for the diagnosis of advanced and early lung cancer [reviewed in (10)]. Sputum samples contain exfoliated cells shed from the oropharynx and larger respiratory passages (as well as macrophages, inflammatory cells, and other nonepithelial cells). Cytologic examination of sputum, especially multiple samples, is helpful for the detection of central tumors arising from the larger bronchi (e.g., squamous cell and small-cell carcinomas). Exfoliated cells from peripheral tumors, such as adenocarcinomas, arising from the smaller airways (small bronchi, bronchioles, and alveoli), especially those less than 2 cm in diameter, can be detected only occasionally in sputum samples. This is particularly important because adenocarcinoma has become more prevalent than squamous cell lung cancer, a trend that is occurring worldwide (11–13). For all lung cancers, although the average specificity of sputum cytology studies for lung cancer diagnosis is high (98%), their sensitivity is low (65%) (14). Most of these studies excelled at detecting centrally occurring squamous cell or small-cell lung cancers; therefore, the actual sensitivity for detecting peripheral adenocarcinomas may be even lower.

Enthusiasm for the use of cytologic analysis of sputum or chest x-rays as techniques for early detection of lung cancer was dampened by several negative screening studies done several years ago that failed to show that screening and subsequent resection reduced lung cancer mortality (15,16). These studies, which detected about four cases of lung cancer per 1000 persons screened per year, actually did detect early stage lung cancer, but surgical resection of tumors in this screened population did not reduce mortality. This situation occurred because microscopic metastatic disease must have been present at this early stage. Thus, we have to be able to diagnose lung cancers before they can be found by “conventional” technologies. This is in marked contrast to the success of mammography as a screening tool, which has revolutionized the early detection and treatment of breast cancer. Ironically, all of the lung cancer-screening studies excluded women, so we do not know if the negative results applied only to men. Lung cancer has now passed breast cancer as the biggest cancer killer of women, supporting the necessity to include both sexes in clinical trials of lung cancer. Radiographically, there are new advances in spiral computed tomography scanning that are currently being studied as screening tools (17).

To improve the sensitivity of sputum examination as a population-screening tool for the detection of early lung cancer, several approaches are currently under development: immunostaining of abnormal epithelial cells, computer-assisted image analysis of exfoliated sputum cells, polymerase chain reaction (PCR)-based assays to detect epigenetic changes in dominant and recessive oncogenes, and genetic epidemiology markers to more precisely define the population of current or former smokers likely to get lung cancer. A heterogeneous nuclear ribonucleoprotein, A2/B1, has shown promise as a marker of lung cancer (18,19). In a pilot study of archived preneoplastic sputum specimens, overexpression of A2/B1 more accurately detected preclinical lung cancer than standard cytomorphology (18,19). Malignancy-associated changes refer to subvisual or nonobvious changes in the distribution of DNA in the nuclei of histologically normal cells due to the presence of preinvasive or invasive cancer in their vicinity (20). These changes can be quantitated by computer-assisted image analysis, and they were found to be

Affiliation of authors: Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas.

Correspondence to: Adi F. Gazdar, M.D., Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas TX 75235-8593 (e-mail: gazdar@simmons.swmed.edu).

See “Note” following “References.”

© Oxford University Press
present in the opposite lungs of 86% of lung cancer subjects (21). In a retrospective analysis of sputum cytology slides, malignancy-associated changes alone correctly identified 74% of the subjects who later developed lung cancer (22). Of the many molecular changes that have been described in lung cancers, some can be detected in corresponding sputum samples. In a retrospective study, Mao et al. (23) found that point mutations in the p53 (also known as TP53) and ras genes in sputum samples preceded the clinical diagnosis of lung cancer. The identical mutation present in the primary tumor was also detected in at least one sputum sample obtained up to 1 year before cancer diagnosis. Microsatellite alterations are a form of genomic instability resulting in altered sizes of one or both alleles of polymorphic microsatellite markers (24). Identical alterations have been found in lung cancers and corresponding sputum samples demonstrating minimal atypia (25).

The limitations of sputum examination have led to the development of invasive procedures for lung cancer diagnosis, including bronchoscopic examination, fine-needle aspiration of peripheral nodules, transbronchial, open lung or mediastinal biopsies, and the bronchoalveolar lavage (BAL) technique used by Ahrendt et al. (9). Fiberoptic bronchoscopic examination, usually by white light, may be used for the detection of invasive and noninvasive lung cancers and their precursor lesions. Examination by fluorescent light greatly increases the sensitivity of detection of noninvasive cancers and dysplastic lesions (26). Pathologic diagnosis is obtained after examination of bronchial biopsy specimens or cytologic specimens. Although direct bronchoscopic examination of the peripheral lung is not possible, cells exfoliated from the peripheral airways may be present, especially in samples of properly obtained BAL samples (27). BAL involves the infusion and reaspiration of a sterile saline solution in distal segments of the lung via a fiberoptic bronchoscope. The predominant cell types in most BAL samples are alveolar macrophages and lymphocytes, and the relative number of epithelial cells (normal or abnormal) is small (28). However, examination of the cellular or solid components of BAL fluids may aid cancer diagnosis, especially that of peripheral tumors. The overall diagnostic yield in reported case series ranges from 35% to 76% (29,30). Activating mutations of the K-ras oncogene, which occur in a subset of lung adenocarcinomas, can be detected in BAL fluids by sensitive techniques (31). Other molecular changes, such as allelic losses and microsatellite alterations, may be detected in bronchial lavage samples (as opposed to BAL) from smokers (32). It has been suggested that high levels of peptide amidating activity (a marker of neuroendocrine cell differentiation) in BAL fluids may predict increased risk of developing second lung cancers (33).

Ahrendt et al. (9) utilized four molecular markers (frequently abnormal in lung cancers) in a series of 50 consecutive, resected, early stage invasive NSCLC tumors and in corresponding BAL fluids obtained at the time of resection. The molecular markers included p53 mutations (detected by sequencing and gene chip technology in tumors and by a plaque hybridization assay in BALs), K-ras mutations (detected by a ligation assay and by a sensitive mutant-enriched PCR technique), the methylation status of the CpG island of the p16 gene (detected by a methylation-specific PCR method), and microsatellite alterations (detected with a panel of 15 markers). With the possible exception of the test for microsatellite alterations, all of the tests had relatively high sensitivities and could detect mutant cells in the presence of a large excess of normal cells. The frequencies of these changes in the tumors ranged from 27% (for K-ras mutations) to 56% (for p53 mutations). As expected, p53 mutations were more frequent in central (predominantly squamous) tumors, and K-ras mutations were more frequent in peripheral (predominantly adenocarcinoma) tumors. When an abnormality was detected in a tumor, identical molecular changes were present in 14%–63% of the corresponding BALs. The least sensitive assay was the detection of microsatellite alterations, perhaps because of heavy contamination with nontumor cells. In tumors with one or more abnormalities (86% of all tumors), at least one assay was positive in 53% of the BAL samples. Abnormalities in BAL samples were more frequent with centrally located tumors than with peripheral tumors (100% versus 29%; \( P = 0.032 \)). Tumor size also influenced the finding of molecular abnormalities in BAL fluids, and few changes were found in fluids from stage IA tumors (i.e., tumors smaller than 3 cm in diameter without metastases). Unfortunately, this is precisely the group of NSCLCs that one wants to detect, since these patients have a 5-year survival rate of 61% (34).

Can the very interesting findings of Ahrendt et al. (9) be used for routine clinical diagnosis and screening? In an editorial written in 1994 (35), David Sidransky, the senior author of the article by Ahrendt et al., asked, “how long can we afford to wait” before we implement molecular screening as a clinical tool? Unfortunately, we will have to wait longer. In the article by Ahrendt et al., their specificity was high (nearly 100%), since, with the exception of microsatellite alterations, they always found the same genetic change in the BAL sample as in the tumor. However, their sensitivity was lower, and in only 53% of tumors that contained molecular lesions were the same abnormalities detected in corresponding BAL fluids. Specifically, the tests were least helpful in the group of patients in whom improved diagnostic abilities are most needed—those with small, peripherally located tumors. Also, because of the multiple tests performed, Ahrendt et al. were unable to perform routine cytopathologic analysis of their BAL specimens. Thus, we cannot directly compare the results of molecular testing with the current “gold standard” diagnostic test. If, in fact, Ahrendt et al. had been able to detect molecular fingerprints of all or most of the tumors in the corresponding BAL specimens, including those with a negative cytologic examination, the authors’ methodology would be ready for expanded testing, despite the laborious and invasive nature of BAL and the skill, labor, time, and expense involved. With further experience and improved throughput methodology, modified versions may remove the above-mentioned obstacles. Thus, sensitivity needs to be improved, and enrichment of the minority epithelial cell compartment of BAL specimens may assist in this regard. In addition, we need to know the results of testing of subjects at increased risk (current and former smokers without lung cancer or survivors of a previous cancer of the upper aerodigestive tract) and subjects with chronic lung diseases, as well as results from healthy never-smoking subjects. Only molecular tests found to be abnormal in the tumors were applied to the corresponding BAL specimens. Several of the molecular abnormalities tested (and others including allelic losses) have been found to be present in the nonmalignant epithelium of smokers (8,36–38). For these reasons, the true sensitivities and specificities of the molecular methodologies cannot be determined.

Despite these shortcomings, the findings reported by Ahrendt
et al. (9) should provide the stimulus for further advances, eventually leading to large multi-institutional studies. In addition, the combination of molecular testing with newer diagnostic approaches, such as low-dose spiral computed tomography (17), quantitative microscopy (22), or genetic epidemiology, should be investigated.

REFERENCES


Note

Supported by a Public Health Service Specialized Program of Research Excellence (SPORE) grant 5P01CA79097 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.