

Interphase Cytogenetics of Sputum Cells for the Early Detection of Lung Carcinogenesis

Perspective on Varella-Garcia et al., p. 447

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Abstract

This perspective on Varella-Garcia et al. (beginning on p. 447 in this issue of the journal) examines the role of interphase fluorescence *in situ* hybridization for the early detection of lung cancer. This work is an important step toward identifying and validating a molecular marker in sputum samples for lung cancer early detection and highlights the value of establishing cohort studies with biorepositories of samples collected from participants followed over time for disease development. *Cancer Prev Res*; 3(4); 416–9. ©2010 AACR.

Lung cancer is the leading cause of cancer death in the United States among men and women (1). Estimated deaths from lung cancer in 2009 exceeded those of the next four most deadly cancers combined (colorectal, breast, prostate, and pancreas; ref. 2). The overall 5-year survival rate for lung cancer is less than 15%, largely because of the late stage at diagnosis and the lack of effective therapies for advanced disease (3). The 5-year survival rates for lung cancer diagnosed at its earliest stage, however, approach 70%; therefore, improved techniques to detect lung cancer early and appropriate trials showing that early detection can reduce lung cancer mortality are urgently needed. This early detection relates to cancer prevention in terms of detecting microscopic cancer in patients who could be prevented from developing clinical consequences or of identifying very high-risk (noncancer) individuals most in need of chemopreventive approaches.

The notion that it may be possible to detect lung cancer by examining exfoliated cells in the sputum is not new. Over four decades ago, Saccomanno and colleagues developed the current standard techniques for sputum collection, processing, and assessment (4), and by the early 1970s, they had described the cytologic changes that occur during lung cancer development (5). Large randomized studies of sputum cytology and chest X-ray screening in the past, however, could not show that these approaches reduced lung cancer mortality (6). Advances in technology over the intervening decades and an improved understanding of the molecular and genetic basis of lung carcinogene-

sis (7) hold promise for developing molecular diagnostic testing in sputum samples that can improve early detection and diagnostic precision. One important such advance is the detection of chromosomal aberrations by interphase cytogenetics in epithelial cells from sputum samples (8).

Interphase cytogenetics refers to the visualization of chromosomal aberrations in intact cell nuclei based on *in situ* hybridization with labeled nucleic acid probes. Cremer and colleagues introduced the concept of interphase cytogenetics in 1986, when they succeeded in using a centromere-specific DNA probe to score copy numbers of chromosome 18 in nuclei from normal cells and in nuclei from a patient with Edward syndrome carrying a trisomy of this chromosome (9). Shortly thereafter, investigators accomplished the visualization of structural chromosomal aberrations via either whole-chromosome painting probes (10) or probes that target specific chromosomal rearrangements (11, 12). Interphase fluorescence *in situ* hybridization (FISH) uses fluorescently labeled DNA probes to detect chromosomal alterations and can be combined with the simultaneous assessment of the immunophenotype of cells (13). An advantage of interphase FISH is that it can be applied to cytologic specimens such as sputum as well as to paraffin-embedded, formalin-fixed tissue, thus allowing analyses of stored biospecimens and correlation of the results with clinical end points. Another critical advantage is that the results are generated from individual cells rather than from DNA extracted from a pooled sample. Assessment of copy number changes in individual cells is particularly important for analyzing early lesions, for which pooled samples might contain enough “contaminating” cells, including normal surrounding tissue or lymphocytes and connective stroma, to dilute the population of, and thus prevent the detection of copy number changes in abnormal cells.

In this issue of the journal, Varella-Garcia and colleagues report on the prediction of lung cancer by the detection of gene copy number changes via the LAVysion (Abbott Molecular) FISH probe set in sputum (14). This commercially available probe set targets the *MYC* oncogene (8q24),

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epidermal growth factor receptor (*EGFR*) gene (7p12), a band on chromosome 5 (5p15), and the centromere of chromosome 6 (CEP6). These investigators previously evaluated the feasibility of this probe set for lung cancer detection, showing that chromosomal abnormalities could be detected in sputum samples and tumor touch preps from patients with cancer but not in cytologically normal sputum samples from patients without cancer (15). The current report details a case-control study of the association between copy number changes detected by FISH and incident lung cancer; this study was nested in a prospective cohort of individuals with a history of heavy smoking and airflow obstruction. When two or more of these FISH probes showed abnormal copy numbers in sputum samples collected within 18 months before cancer diagnosis, the FISH assay predicted the diagnosis of lung cancer overall with a sensitivity of 76% and specificity of 88%. (This 18-month timing issue is further discussed later.) Lung cancer cases were 29.9 times more likely (95% C.I., 9.5-94.1) to have an abnormal sputum FISH assay than controls. Although abnormalities detected by the FISH assay correlated with conventional cytologic examination of the sputum samples, diagnostic performance did not significantly improve by combining the FISH assay with cytology versus the FISH assay alone, as assessed by receiver operating characteristic curve analyses. In addition, the sensitivity for squamous cell carcinoma was much better (94%) than for other histologies (69%). This finding is not unexpected given that most squamous cell cancers arise in the central airways.

The current study highlights the value of the University of Colorado Lung Cancer Specialized Program of Research Excellence cohort that was established in 1993 for testing promising biomarkers identified in the laboratory (16). Conceptual frameworks for the validation of biomarkers that have been described emphasize the importance of establishing cohort studies with repositories of biospecimens collected and stored from participants who are followed over time for disease development (17, 18). The Colorado Specialized Program of Research Excellence cohort includes approximately 2,500 current or former adult smokers at a high risk for lung cancer based on a heavy tobacco smoking history and the presence of chronic obstructive pulmonary disease indicated by airflow obstruction on spirometry (19). Limiting enrollment in this modest-sized cohort to these high-risk adults has ensured the development of a sufficient number of lung cancer cases over a relatively short period of follow-up. Relevant biological specimens including serum, DNA, and sputum were collected at baseline. Previous studies have shown high rates of moderate atypia in sputum samples from this cohort and that moderate atypia or worse was a weak predictor for incident lung cancer (16, 20). More recently, Belinsky and colleagues used this resource to show that aberrant gene promoter methylation in sputum samples was predictive of lung cancer (21). In the present study, Varella-Garcia and colleagues have expanded on their previous work (15, 22) to show that the FISH assay applied to sputum samples was a much stronger predictor of lung

cancer than was either cytology or aberrant gene promoter methylation in this cohort. Although this new work provides a significant step toward the identification and validation of an early-detection molecular marker, there are important points that should be considered in interpreting the results and setting future research directions.

It is important to note that enrollment in this cohort was limited to high-risk individuals with significant airflow obstruction, a group for whom it is relatively easy to obtain spontaneous expectorated sputum (23). Spontaneous sputum samples from high-risk individuals without airflow obstruction may not have sufficient cellularity for FISH analysis. Even in the present study, approximately 14% of specimens were considered inadequate for FISH analysis because they contained fewer than 100 nucleated epithelial cells with good hybridization signals. Sputum also can be collected by induction after saline inhalation with a nebulizer, although this procedure needs to be performed in a clinic and is therefore less attractive for population screening. Pooling the first-morning cough specimen over 3 days has been shown to be as efficacious as induced sputum for diagnosing lung cancer in individuals with airflow obstruction (24). It remains to be determined whether high-risk individuals without airflow obstruction can produce adequate spontaneous sputum for FISH analysis. Future studies are needed to evaluate the FISH assay characteristics when applied to sputum specimens collected in high-risk cohorts without airflow obstruction to ensure that the methods and findings are reproducible in these populations.

Another important point to consider is that the FISH assay was more sensitive for squamous cell carcinoma than for other subtypes, raising the question of whether the test is sufficiently sensitive for the detection of other histologic subtypes of lung cancer. Lung cancer is a complex and heterogeneous neoplasm probably having multiple preneoplastic pathways of development (25). The sequence of changes that occur in bronchial epithelium leading to the development of squamous cell carcinoma are well described, but events leading to other types of lung cancer are less clear. Furthermore, the specific genetic changes found in small-cell lung cancers differ from those found in non-small-cell lung cancers (26). The cytogenetic literature suggests that it might be possible to improve sensitivity for other tumor histologies by including additional probes targeting common genomic imbalances. For example, the inclusion of a probe for the long arm of chromosome 3 should increase sensitivity, especially for small-cell lung cancers, whereas adenocarcinomas frequently show copy number increases of chromosomes 1 and 20 (27, 28); including probes for these latter two chromosomes likely would increase sensitivity for detecting adenocarcinoma. It is also possible to target genes that are lost in lung cancer, although this can be problematic because reduced or nonexistent signal numbers may be the result of hybridization failure. Nevertheless, recent studies have shown that deletions that map to chromosome bands 3p22.1 and 10q22 can be reliably detected by FISH

applied to bronchial brushings in noncancerous epithelium adjacent to tumors (29) and in sputum samples (30).

It is intriguing to hypothesize why the FISH assay used in the study by Varella-Garcia and colleagues is more sensitive within rather than more than 18 months before diagnosis. As suggested by the investigators, this finding likely reflects either that this assay is detecting late epithelial-cell field effects that predict a very high risk of developing lung cancer or that lung cancer is already present and the tumor cells are being exfoliated. Cancers diagnosed within a very short window of the initial sputum collection in this study may best be considered prevalent lung cancer cases rather than incident cases. This issue might be clarified by comparing the signal pattern in cells in sputum with that in cells of the primary tumor. If the aneuploidy pattern matches closely, it is reasonable to assume that exfoliated cells were indeed detected. Alternatively, general chromosomal instability in the sputum samples versus a clonal pattern in the primary cancers could suggest that the process of field cancerization resulted in random chromosomal gains and losses in premalignant bronchial epithelium and that the clones with a "lung cancer pattern" were selected for progression to cancer.

The authors also note that the FISH assay they used is labor-intensive and not ready for immediate clinical application. Although this is true, several automated imaging systems are being developed, or are already available, that achieve certain levels of automation for image acquisition and analysis (visit <http://www.riedlab.nci.nih.gov/links.asp> for a listing of systems). Quantitative measurements of the nuclear DNA content, which is fast, would be an additional parameter to consider for at least preselecting cells most likely to contain chromosomal aneuploidies. Although the application of interphase FISH to solid tumors has lagged behind its utilization in hematologic malignancies, its usefulness for detecting tumor cells in cytologic specimens seems to be more and more appreciated. For example, a probe cocktail for the visualization of aneuploidies in urine samples has shown great potential for the diagnosis of recurrent bladder cancer (31). Likewise, the detection of gains of chromosome 3q (i.e., genomic amplification of the human telomerase gene *TERC*) in low-grade cervical dysplasia can discern lesions with a low or high risk of progression (32). Therefore, it is fair to conclude that interphase FISH for the early detection of solid tumors has not yet entered prime time and that once its usefulness is fully shown, the present throughput problems in terms of image acquisition and analysis likely will be solved.

The last important point with regard to the interphase FISH study to be addressed here is that technologic advances in radiologic imaging methods, which have occurred in parallel with advances in molecular biology and genetics, point to an emerging role for noninvasive biomarkers in sputum and to potential resources for validating these markers. Early nonrandomized studies of helical computed tomographic (CT) imaging yielded promising results, particularly for detecting cancers arising

in the peripheral airways; helical CT, however, may be less sensitive for centrally located tumors (33). Furthermore, distinguishing benign from malignant nodules, particularly very small ones, can be difficult, and false-positive CT tests may result in unnecessary invasive procedures. The lung cancer community is currently awaiting results of the National Lung Cancer Screening Trial launched in 2002 by the National Cancer Institute. This randomized, controlled trial is comparing the effect of helical CT with that of standard chest X-ray on lung cancer mortality, the gold standard end point for screening studies, in approximately 53,000 current or former smokers across the United States. The National Lung Cancer Screening Trial has measured spirometry to assess airflow and collected biospecimens including sputum, blood, and urine from a subset of participants. The Dutch-Belgian Lung Cancer Screening Trial [also known as the Nederlands-Leuvens Longkanker Screenings Onderzoek (NELSON) trial] is another large randomized, controlled CT screening trial that will produce informative results (34). Even if the findings of these randomized trials show a favorable benefit-to-risk ratio for CT, there will likely still be a role for the development of noninvasive biomarkers in refining the high-risk population to optimize the clinical benefits and harms of screening, enhancing the sensitivity of CT for centrally located lesions and aiding in the clinical management of CT-detected nodules of uncertain clinical significance.

Biomarkers such as the FISH assay applied to sputum samples described by Varella-Garcia and colleagues (14) could complement CT scanning. Supporting this concept, a recent small cross-sectional study from another group using a different set of FISH probes showed that testing for *HYAL2* (3p21.3), *FHIT* (3p14.2), *p16* (9p21), and *SP-A* (10q22) in sputum samples by FISH increased the diagnosis of stage I central tumors compared with CT alone (35). Interphase FISH techniques also may aid in the diagnosis of CT-detected nodules in the peripheral airways. For example, a recent study showed the ability of interphase FISH to distinguish benign from malignant CT-detected nodules when applied to cells obtained by fine-needle aspiration, including the identification of adenocarcinomas, which typically present in peripheral locations (36). In conclusion, the detection of chromosomal copy number changes by FISH has the potential to improve early lung cancer detection, particularly as a complement to CT imaging. Further evaluation and validation of this approach in other cohorts will help fully define the role of interphase FISH for lung cancer early detection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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