The Use of Genetic Markers to Identify Lung Cancer in Fine Needle Aspiration Samples

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Abstract

Purpose: We seek to establish a genetic test to identify lung cancer using cells obtained through computed tomography–guided fine needle aspiration (FNA).

Experimental Design: We selected regions of frequent copy number gains in chromosomes 1q32, 3q26, 5p15, and 8q24 in non–small cell lung cancer and tested their ability to determine the neoplastic state of cells obtained by FNA using fluorescent in situ hybridization. Two sets of samples were included. The pilot set included six paraffin-embedded, noncancerous lung tissues and 33 formalin-fixed FNA specimens. These 39 samples were used to establish the optimal fixation and single scoring criteria for the samples. The test set included 40 FNA samples. The results of the genetic test were compared with the cytology, pathology, and clinical follow-up for each case to assess the sensitivity and specificity of the genetic test.

Results: Nontumor lung tissues had ≤4 signals per nucleus for all tested markers, whereas tumor samples had ≥5 signals per nucleus in five or more cells for at least one marker. Among the 40 testing cases, 36 of 40 (90%) FNA samples were analyzable. Genetic analysis identified 15 cases as tumor and 21 cases as nontumor. Clinical and pathologic diagnoses confirmed the genetic test in 15 of 16 lung cancer cases regardless of tumor subtype, stage, or size and in 20 of 20 cases diagnosed as benign lung diseases.

Conclusions: A set of only four genetic markers can distinguish the neoplastic state of lung lesion using small samples obtained through computed tomography–guided FNA.

Lung cancer is a leading cause of cancer deaths worldwide. In 2008, a total of 215,020 new lung cancer cases and 161,840 deaths are expected in the United States alone (1). The exceptionally high mortality rate of lung cancer is, in part, due to the fact that lung cancer is often diagnosed at a late stage (2) when the prognosis is nearly always poor. The ability to diagnose the disease at an early stage has the potential to save lives.

Indeed, recent progress using computed tomography (CT) to screen high-risk populations has shown highly promising results for detecting lung cancer at an early stage when it is curable (3–6). Several studies have observed that 60% to 85% of lung cancers detected by spiral CT are at stage I (7). However, a major concern in CT screening is the high incidence of finding suspicious nodules that are not cancer. Some studies have shown that 50% of the participants will have at least one noncalcified nodule (8). In CT studies, the number of patients who required further evaluation but did not have cancer ranged from 5% to 50% in prevalence screening and 3% to 12% in incidence screening (9).

Outside of the screening scenario, patients with potentially suspicious nodules are often followed up by CT-guided fine needle aspiration (FNA) and pulmonary cytology in clinical practice (10). Diagnosis can be facilitated by clinical history along with laboratory and radiologic findings, and it has recently been shown that cytologic diagnosis is reliable for early lung cancer (11). However, the cytologic differentiation of reactive pulmonary processes from malignant neoplasms can be challenging as reactive type 2 pneumocytes can be difficult to distinguish from malignant cells (12, 13). In a retrospective analysis of FNA with or without core biopsies involving 95 cases that were identified as benign, 21 (22%) had specific benign diagnosis and all were true negative for malignancy based on radiologic (n = 17) or surgical (n = 4) follow-up (14). The remaining 74 were either nonspecific benign (n = 53, 56%) or nondiagnostic (n = 21, 22%). Significantly, seven of the benign nonspecific (13%) and six of the nondiagnostic cases (29%) exhibited malignancy at excisional biopsy or radiologic follow-up. While this may be related to sampling error, it could also in part be related to difficulties in interpretation based on...
As paraffin cell blocks. Nontumor lung samples (n = 6) were obtained as paraffin sections from the Armed Forces Institute of Pathology. The genetic tests were done on FNA cytology samples obtained at the time of the clinical evaluation when cytologic diagnosis was made. Genetic test was done without knowledge or regard to the clinical diagnosis and was compared with clinical outcome after completion of the analysis. For suspicious nodules that underwent surgery, pathology reports and clinical follow-up was available on all cases. When nodules were benign, cytology and clinical follow-up was used. Outcome of genetic tests were compared with pathology/cytology and clinical follow-up to determine the reliability of the test. A total of 73 FNA cytology samples were used in this study and they included 33 small tumor FNA cytology samples preserved in formalin for the pilot tests, and 40 formalin-fixed (20 tumor and 20 benign) paraffin-embedded cell block sections for the genetic analysis. The final pathologic diagnosis of the testing NSCLC samples included 12 adenocarcinoma, 3 squamous cell carcinoma, 4 large cell carcinoma, 3 neuroendocrine tumors, 1 small-cell lung carcinoma, and 1 poorly differentiated NSCLC. The nontumor samples were diagnosed either as benign nonspecific (8) or benign specific (12) based on cytology findings. In general, one to three sections of 10- to 30-μm-thick sections were used for the 40 paraffin-embedded test samples and one 10- to 50-μm section was used for each of the six formalin-fixed, paraffin-embedded noncancerous lung samples. The patient information and tumor-related information on the 40 test cases are shown in Results.

Identification of genetic markers for lung cancer detection. We selected candidate regions of genomic amplification based on two independent CGH studies involving lung adenocarcinomas and squamous cell carcinomas (19, 20). In total, we evaluated eight different chromosome arms frequently amplified in adenocarcinoma samples (1q, 3q, 5p, 7q, 8p, 12p, and X) and nine chromosome arms often amplified in squamous cell carcinoma tumors (1q, 3q, 5p, 7p, 8q, 12q, 19q, 20p, and X). An in silico test was then designed to determine the maximum number of lung cancers that could be identified based on the minimal number of genetic changes observed in these regions either alone or in combination in a set of 25 squamous cell carcinoma and 59 adenocarcinoma cases. The frequency of amplification for each marker was ranked based on the number of samples that could be detected for both adenocarcinoma and squamous cell carcinoma. Highest ranking markers for adenocarcinoma samples were cross-tested with different high-ranking markers for squamous cell carcinoma in different combinations to derive at a set of markers that jointly detected most cancers (Table 1).

Identification and labeling of FISH probes. For two overlapping sets of bacterial artificial chromosome (BAC) containing human chromosome regions identified via genetic analysis were selected for each region around the target genes (CENPF for 1q31, TERC for 3q25-27, TERT for 5p13-14, and c-myc for 8q23-24) and obtained from public or commercial resources (BACPAC Resources, Children's Hospital Oakland, Oakland, CA, and Invitrogen). DNA was isolated from each BAC, labeled with biotin-dUTP, and hybridized on normal blood lymphocytes metaphase-spread slides. Each BAC was evaluated for intensity and specificity of hybridization at the target region, and a BAC contig composed of two to four overlapping BAC clones was assembled for each region. These contigs served as probes for our genetic test in interphase nuclei by FISH. The probe for chromosome 8q was specific for the c-myc gene and was obtained from Vysis. The resulting chromosome regions are shown in Table 1.

For the probe, 2 μg BAC DNA were labeled with biotin-dUTP or digoxigenin-dUTP by nick translation in the presence of 4 mmol/L labeled nucleotide. Approximately 100 to 200 μg of labeled BAC probe were ethanol precipitated in the presence of 20 μg each of salmon sperm DNA and human Cot1 DNA. The dry pellet was dissolved in 5 μL of hybridization buffer. The hybridization buffer was composed of

**Table 1. Detecting lung cancer based on chromosome copy number changes**

<table>
<thead>
<tr>
<th>Chromosomes with alteration</th>
<th>Detectables based on any alteration (n = 84)</th>
<th>Detectable (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1q, 3q, 5q, 8q</td>
<td>83</td>
<td>98.8</td>
</tr>
<tr>
<td>1q, 5q, 8q</td>
<td>80</td>
<td>95.2</td>
</tr>
<tr>
<td>1q, 3q, 5q, 8q</td>
<td>70</td>
<td>92.3</td>
</tr>
<tr>
<td>3q, 5q, 8q</td>
<td>75</td>
<td>89.5</td>
</tr>
</tbody>
</table>

NOTE: The lung tumor set included 25 squamous cell carcinomas and 59 adenocarcinomas and can be obtained from http://amba.charite.de/~ksch/cghdatabase/index.htm. Chromosome markers, 1q32, 3q26, 5p15, and 8q24 were used in combination or alone to determine the minimum number of markers that could detect the most tumors. Values show the number and the percentage of cancers detected for the indicated marker combinations.

Materials and Methods

**Tissue samples.** All samples were CT-guided FNAs obtained using a 22-gauge needle, at the Weill Medical College of Cornell University, following institutional review board approval (M.V.). Cell aspirates were fixed directly in either formalin until analysis or prepared as paraffin cell blocks. Nontumor lung samples (n = 6) were obtained as paraffin sections from the Armed Forces Institute of Pathology. The genetic tests were done on FNA cytology samples obtained at the time of the clinical evaluation when cytologic diagnosis was made. Genetic test was done without knowledge or regard to the clinical diagnosis and was compared with clinical outcome after completion of the analysis. For suspicious nodules that underwent surgery, pathology reports and clinical follow-up was available on all cases. When nodules were benign, cytology and clinical follow-up was used. Outcome of genetic tests were compared with pathology/cytology and clinical follow-up to determine the reliability of the test. A total of 73 FNA cytology samples were used in this study and they included 33 small tumor FNA cytology samples preserved in formalin for the pilot tests, and 40 formalin-fixed (20 tumor and 20 benign) paraffin-embedded cell block sections for the genetic analysis. The final pathologic diagnosis of the testing NSCLC samples included 12 adenocarcinoma, 3 squamous cell carcinoma, 4 large cell carcinoma, 3 neuroendocrine tumors, 1 small-cell lung carcinoma, and 1 poorly differentiated NSCLC. The nontumor samples were diagnosed either as benign nonspecific (8) or benign specific (12) based on cytology findings. In general, one to three sections of 10- to 30-μm-thick sections were used for the 40 paraffin-embedded test samples and one 10- to 50-μm section was used for each of the six formalin-fixed, paraffin-embedded noncancerous lung samples. The patient information and tumor-related information on the 40 test cases are shown in Results.

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http://amba.charite.de/~ksch/cghdatabase/index.htm
50% deionized formamide, 10% dextran sulfate, and 1× SSC. The probe was denatured for 5 min at 80°C and then preannealed for 1 h at 37°C before adding to the slides.

Sample preparation and FISH hybridization. To enable unambiguous signal evaluation, we prepared single nuclei suspensions from formalin-fixed, paraffin-embedded cell block or tissue sections or from formalin-fixed tissues following the Hedley technique (23). Briefly, for paraffin-embedded cells or tissues, the sections were deparaffinized in xylene, hydrated through the ethanol series, and then incubated overnight in water at 4°C. For formalin-fixed FNA cell aggregates, the formalin solution was removed and the cells were extensively washed with PBS to remove residual formalin and then incubated overnight in water at 4°C. The cells were digested with 0.1% protease Type XXIV (Sigma P-8038) in PBS at 45°C for 45 to 60 min and filtered through serum columns to remove undigested tissue. The purified single-cell suspensions were cytopspin onto the slides. After air drying, slides were baked for 2 h at 60°C, dehydrated, and stored at 4°C in descicators.

For hybridization, slides were incubated with sodium thiocyanate overnight at room temperature in a concentration that varied from 0.1% to 0.5% for paraffin-embedded cells or tissues and 0.5% to 1.0% for samples that were formalin fixed. After overnight incubation, slides were incubated in the same solution at 80°C for 1 h and further treated with Zymed pretreatment (Tissue Pretreatment kit; Zymed) for 30 min at 95°C to 98°C. The nuclei were digested with enzyme solution (Tissue Pretreatment kit; Zymed) for 5–5 min. After washing and dehydration, slides were denatured in 70% formamide and 2× SSC for 10 min at 80°C. The denatured slides were hybridized with preannealed probes, either individually or with another probe for 36 h at 37°C.

At the end of hybridization, the slides were washed in 50% formamide and 2× SSC at 45°C three times for 5 min each, 0.5× SSC, and 0.1% SDS at 65°C four times for 5 min each and 2× SSC at room temperature. After washing, they were incubated with blocking buffer (4× SSC/0.1% Tween 20, 3% bovine serum albumin) containing sheep or goat IgG (1:100 dilution) for 30 min at 37°C to block nonspecific binding. For biotin-labeled probes, the slides were incubated with 1:1,000 dilution avidin-FITC (Vector Laboratories) and then incubated overnight in water at 4°C before adding to the slides. For experiments in which biotin- and digoxigenin-labeled probes were used together, the slides were first incubated with 1:5,000 dilution mouse antidigoxigenin (Sigma) in a developing buffer containing IgG for 1 h at 37°C. For digoxigenin-labeled probe, the slides were first incubated with 1:5,000 dilution mouse antidigoxigenin (Sigma) in a developing buffer containing IgG for 1 h at 37°C. For experiments in which biotin- and digoxigenin-labeled probes were used together, the slides were first incubated with mouse antidigoxigenin and then with a combination of antimouse TRITC and Avidin-FITC each at 1:1,000 dilution. The slides were washed in 4× SSC-0.1% Tween 20 solution four times at 45°C to remove unbound label (FITC and TRITC). The detergent was removed by washing twice with 2× SSC at room temperature, and the slides were air dried and embedded in antifade solution containing 1.4% diaminodiphenylamine-2-pheny lindole.

Fluorescence microscopy and FISH scoring. All samples were analyzed using a Nikon E800 microscope under a ×60 objective. The images were acquired using a Nikon E800 (Nikon, Inc.) equipped with appropriate filters (Chroma Technologies) and acquired using a Retiga Exi digital camera (BioVision Technologies) at five to seven focal planes using the IPLab software.

The signals were evaluated by examining the entire slide without knowledge of the cytology, pathology, or clinical follow-up of the cases. Nuclei were examined individually to determine the number of signals per cell for each marker. Nuclei that could not be evaluated due to insufficient hybridization or cell clusters were excluded from scoring. The number of signals in the nuclei for each marker was counted until five or more signals were observed in three or more cells for at least one probe. When no amplification was observed in a particular sample, all four markers were evaluated and 130 or more nuclei per probe were counted. When amplification of five or more signals was detected for any one probe in more than five cells, the sample was considered a tumor regardless of the status of the other markers. When samples were hybridized with two probes, the chromosome probes for 1q and 3q and the probes for 5p and 8q (c-myc) were combined. In general, 200 nuclei were counted when enough cells were available. However, when cell numbers were limited in a particular sample, a minimum of 130 nuclei were counted. An independent observer (M.H.) also scored samples without amplification.

Results

Detecting lung tumors based on chromosomal amplification. We surveyed chromosome regions frequently amplified in adenocarcinoma and squamous cell carcinoma tumors based on CGH findings (19, 20). A database containing 25 squamous cell carcinoma and 59 adenocarcinoma cases was then used to assess the ability of using genetic markers to identify lung cancers in an in silico test. As shown in Table 1, the number of lung cancers detectable varied from 75 to 80 samples (89.3-95.2%) when three different markers were used. When four markers (1q, 3q, 5p, and 8q) were used, 83 of 84 (98.8%) samples were detected (Table 1). The remaining adenocarcinoma sample did not have amplifications for any of the markers tested, and, therefore, could not be detected by the test. Candidate genes that are frequently amplified in lung cancers in these four different chromosome regions were used as the primary target of BAC probe selection.

To assess the specificity of the assay, we first tested six nontumor lung tissues using the four selected probes. As shown in Fig. 1, 91% to 97% of all nuclei counted had ≤2 specific hybridization signals for each tested marker and 100% of the normal cells have ≤4 signals for all markers tested. The total number of nuclei counted ranged between 537 and 897 among the normal lung samples, and 100% of the nuclei had ≤4 signals for each marker (Figs. 1 and 2A, B). In contrast, of the 25 formalin-fixed FNA tumor samples that were analyzable, 22 of them displayed ≥5 signals in at least two of the tested markers and three had ≥6 signals in at least one marker. The number of cells having more than five signals per nucleus varied from 6 to 178 for at least one marker in the tested
samples. The number of cells with ≤2 signals varied from 3% to 87% depending on tumor content, with an average of 51.8% in the samples. Results for the 25 fully analyzed samples are summarized in Fig. 3 and detailed in Supplementary Table S1.

Based on this result, we considered a sample as nontumor when all four tested markers had ≤4 signals per nucleus for each probe and a sample as tumor when there were five or more hybridization signals per nucleus for any probe in more than five cells.

**Optimal sampling for FISH analysis using FNA cytology samples.** To determine the optimal method for biopsy sample processing, we examined the robustness of FISH signals among biopsy samples preserved in a formalin fixative for a long period (3-18 months) in the test set of 33 FNA cytology samples. Overall, the hybridization signal was observed in 27 samples. Two cases were analyzable only for two of the four markers and these markers had ≤4 signals (data not shown). Optimal results were routinely obtained for samples that were fixed for ≤45 days. The hybridization results were much more variable for those samples stored in formalin for >45 days. In these cases, although sufficient nuclei were isolated from the remaining six samples, the hybridization signals were too weak for evaluation despite repeated effort. These six cases together with the two partially analyzable samples were excluded from analysis. In contrast, all six paraffin-embedded nontumorous lung samples generated robust signals by FISH hybridization (100%). Therefore, we used routine formalin-fixed, paraffin-embedded sections for the genetic analysis by FISH.

**Specificity of cancer detection using genetic markers by FISH.** We next used the criteria established above to examine 40 routine paraffin sections of FNA cell blocks (Table 2) to determine if FISH alone could identify the neoplastic status of the samples. After the completion of the FISH analysis, genetic assessments on the FNA cytology samples were compared with the cytology, histopathologic diagnosis, and clinical follow-up of the patients (Table 2). Overall, 36 of 40 paraffin-embedded FNA cytology samples (90%) were analyzable by FISH. Four samples could not be analyzed due to the lack of nuclei after sample processing. Samples were analyzed for all four markers in most cases (24). In some cases, fewer markers were used when a conclusive diagnosis could be made for the case (three markers in one case; two markers in five cases). Examples of tumor and nontumor samples analyzed by FISH are shown in Fig. 2 and summarized in Fig. 3. In total, 15 cases had amplified signals for at least two markers and thus were considered as tumors based on the genetic test. Nineteen samples showed ≤4 signals per nucleus per marker for all tested markers and were considered as normal. Sample 21 was analyzable for only two markers but all cells analyzed had ≤4 signals per nucleus for the two tested markers. Furthermore, >92% of the nuclei (94% for 1q32 and 92% for 3q26) in this sample had ≤2 hybridization signals per cell and this is the same pattern as in nontumors where the hybridization signals of ≤2 for all four markers were observed in >90% of the nuclei. In sample 23, a total of 959 nuclei were counted for all four markers and only one nucleus had five signals for the 3q probe. Therefore, both these cases were considered as nontumor based on the scoring criteria established in the pilot test.

![Fig. 2. In situ hybridization in tumor FNA and normal lung samples. A and B, nontumor lung sample 503. C and D, tumor samples 14 and 18. Hybridization probes and colors are as indicated.](image1)

![Fig. 3. Summary of FISH analysis on pilot and test samples. Samples were classified into three categories (≤4, 5, and ≥6) based on the observed maximum number of signals for each probe. Normal samples have a maximum number of ≤4 signals for each marker and tumors have a minimum number of ≥6 signals per marker or ≥5 per marker in five or more cells. N, normal; nontumor lung samples. N, normal; nontumor lung samples. □, FNA-pilot: tumors used in the pilot test. □, FNA test: samples used in the testing analysis.](image2)
Comparison of genetic diagnosis to pathology and clinical follow-up. When the genetic diagnosis by FISH was compared with available histopathologic and clinical data, genetic markers accurately identified 15 of 16 tumors and all 20 clinically nontumor samples (i.e., 35 of 36 analyzable cases; summarized in Fig. 3). Sample 23 has been followed up for more than 3.5 years and is clinically stable with no further disease complication. This further supports the molecular diagnosis for this sample as nontumor. In sample 40, the genetic analyses for all four markers were within reference range, with 88.9% to 94% cells having ≤2 signals per nucleus for each marker. By using CT-guided FNA, sample 40 was obtained from a complex part-solid mass with large areas of mucus plugging and inflammation in the initial cytology diagnosis. A second FNA analysis was recommended and used for the diagnosis of adenocarcinoma with bronchioloalveolar features. Genetic analysis was done using cell block samples prepared from the first FNA cytology sample. A second test using another tissue section of the same cell block revealed chromosome amplifications in three of four markers examined.

Discussion

Chromosomal aberrations occur frequently in different cancers, including lung cancer. Extensive catalogues of recurrent abnormalities in a wide range of solid tumors have been compiled from cytogenetic (Mitelman Database of Chromosome Aberrations in Cancer) and CGH (25) studies and are available online. The data indicate that each tumor type displays

Table 2. Clinical and genetic diagnoses of all 40 test FNA biopsies

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Age/Gender</th>
<th>Cytology diagnosis</th>
<th>Nodule location</th>
<th>Pathology diagnosis</th>
<th>Genetic diagnosis</th>
<th>Agreement*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55/F</td>
<td>Atypical carcinoid</td>
<td>RUL</td>
<td>Carcinoid</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>78/F</td>
<td>Mucinous type adenocarcinoma</td>
<td>LUL</td>
<td>Adenocarcinoma</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>55/M</td>
<td>Well-differentiated adenocarcinoma</td>
<td>RLL</td>
<td>Adenocarcinoma</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>69/M</td>
<td>Adenocarcinoma</td>
<td>LLL</td>
<td>Adenocarcinoma</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>62/M</td>
<td>Adenocarcinoma</td>
<td>LLL</td>
<td>Adenocarcinoma</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>55/M</td>
<td>Hamartoma</td>
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<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
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<td>45/M</td>
<td>High-grade neuroendocrine carcinoma</td>
<td>RUL</td>
<td>Neuroendocrine carcinoma</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>45/M</td>
<td>High-grade neuroendocrine carcinoma</td>
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</tr>
<tr>
<td>9</td>
<td>83/F</td>
<td>Poorly differentiated non–small cell carcinoma</td>
<td>LLL</td>
<td>Non–small cell carcinoma</td>
<td>NA</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>69/F</td>
<td>Non–small cell carcinoma</td>
<td>RLL</td>
<td>Non–small cell carcinoma</td>
<td>Tumor</td>
<td>Yes</td>
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<tr>
<td>11</td>
<td>65/M</td>
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<td>RUL</td>
<td>Squamous cell carcinoma</td>
<td>Tumor</td>
<td>Yes</td>
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<tr>
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<td>LLL</td>
<td>Adenocarcinoma</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>NA/NA</td>
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<td>Normal</td>
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<td>48/M</td>
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<td>Adenocarcinoma</td>
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<td>—</td>
</tr>
<tr>
<td>15</td>
<td>64/M</td>
<td>Bronchioloalveolar carcinoma</td>
<td>RLL</td>
<td>Adenocarcinoma</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>60/M</td>
<td>Poorly differentiated squamous cell carcinoma</td>
<td>LUL</td>
<td>Squamous cell carcinoma</td>
<td>NA</td>
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<td>17</td>
<td>78/F</td>
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<td>LLL</td>
<td>Large cell carcinoma</td>
<td>Tumor</td>
<td>Yes</td>
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<tr>
<td>18</td>
<td>70/M</td>
<td>Non–small cell carcinoma</td>
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<td>Non–small cell carcinoma</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>19</td>
<td>66/M</td>
<td>Small-cell carcinoma</td>
<td>RUL</td>
<td>Small-cell carcinoma</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>20</td>
<td>72/F</td>
<td>Adenocarcinoma</td>
<td>LUL</td>
<td>Adenocarcinoma</td>
<td>Tumor</td>
<td>Yes</td>
</tr>
<tr>
<td>21</td>
<td>85/M</td>
<td>Bronchioloalveolar cell hyperplasia</td>
<td>LLL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
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<tr>
<td>22</td>
<td>67/F</td>
<td>Acute inflammation</td>
<td>RML</td>
<td>Benign</td>
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<tr>
<td>23</td>
<td>36/M</td>
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<td>RUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>24</td>
<td>36/M</td>
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<td>LUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
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<tr>
<td>25</td>
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<td>RUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>26</td>
<td>54/M</td>
<td>Atelectasis/scarring</td>
<td>RUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>27</td>
<td>72/F</td>
<td>Atelectasis/scarring</td>
<td>Lingular mass</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>28</td>
<td>69/F</td>
<td>Inflammatory pseudotumor</td>
<td>Para-arotic mass</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>29</td>
<td>56/F</td>
<td>Pseudomonas aeruginosa</td>
<td>RUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>30</td>
<td>43/M</td>
<td>Chronic inflammation with caseating granulomatous features</td>
<td>RUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>31</td>
<td>55/M</td>
<td>Pulmonary hamartoma</td>
<td>LLL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>32</td>
<td>55/F</td>
<td>Fibromyxoid stroma</td>
<td>RUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>33</td>
<td>75/M</td>
<td>Acute inflammation and necrosis with fungus forms present</td>
<td>RUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>34</td>
<td>51/M</td>
<td>Acute pneumonia with bacilli</td>
<td>RUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>35</td>
<td>89/M</td>
<td>Acute pneumonia with bacilli</td>
<td>RUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>36</td>
<td>77/M</td>
<td>Acute inflammation with few dysplastic squamous cells</td>
<td>LUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>37</td>
<td>75/M</td>
<td>Reactive intrapulmonary lymph node</td>
<td>RUL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>38</td>
<td>34/M</td>
<td>Fibrous scar with elastosis and chronic inflammation</td>
<td>LLL</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>39</td>
<td>58/F</td>
<td>Chronic inflammation</td>
<td>RML</td>
<td>Benign</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>40</td>
<td>84/M</td>
<td>Fibrinous pneumonia</td>
<td>LUL</td>
<td>Adenocarcinoma</td>
<td>Tumor</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: RUL, right upper lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; RML, right medium lobe; NA, not applicable.

*Samples were scored for concordance (yes) or discordance (no) between genetic and clinical diagnosis in the Agreement column.

1 Confirmed to be tumor in repeat test.
a nonrandom recurrent pattern of chromosomal aberrations that can be used to distinguish between normal tissues and tumors (26, 27). In contrast to DNA-based tests such as CGH and loss of heterozygosity analysis, which require the use of samples highly enriched for tumor cell content, FISH visually identifies the chromosomal aberrations on the metaphase chromosome or interphase nuclei of the individual cells. This approach has been used to identify colorectal carcinogenesis (28, 29), bladder cancer (30), head and neck cancer (24), lung carcinoma (31), cervical cancer (32–34), and germ cell tumors (35).

The value of FISH analysis in the diagnosis of lung cancer has been explored with a specificity of 82% to 100% and sensitivity of 72% to 87% when two chromosome probes were used (36–38). In our study using FNA samples, we accurately identify all nontumor cases (20 of 20, 100% specificity) using just four genetic markers. We were able to identify lung tumors based on the molecular status of the cells obtained by FNA in 15 of 16 testable cases (94%). The work presented here shows the potential for a genetic-based cytologic test performed synergistically to increase the specificity and sensitivity of the clinical diagnoses using small lung lesions. Furthermore, the genetic markers that we used (Table 1) to distinguish tumor from nontumor samples in CT-guided FNA specimens coincide with the high-resolution genomic profiles of human NSCLC identifying chromosome regions 1q 31, 3q25-27, 5p13-14, and 8q23-24 as the minimum common regions most often amplified in lung cancer (39). Our results support the notion that although chromosomal aberrations are associated with various cancers, they are absent or are very rare in nontumor cells (40). In contrast, tumors almost always contain genetic amplifications of chromosomes regardless of size, stage, and pathologic subtype (37). These specific changes can be used as specific molecular markers.

Although highly specific, the genetic method described here is imperfect because it is limited by the cellular content of the tumor, the sampling precision of the FNA procedure, the presence of the genetic changes in the tumor sample, and the added cost as well as time for the analysis. In our study, 4 of 40 (10%) of the FNA cytology samples could not be tested genetically due to the lack of sufficient cells. Furthermore, 1 of the 16 tumor samples (sample 40) required a second test by both cytologic as well as genetic analysis for accurate diagnosis possibly due to the fact that the tumor contained large areas of mucus plugging and inflammation. Finally, although all testable tumor samples of this study had genetic changes in one or more makers, our initial survey using a larger set of 83 samples include one that would have been missed by the genetic test. Nonetheless, our experience using routine formalin-fixed, paraffin-embedded cell block of FNA cytology samples showed that ~90% of the samples could be successfully analyzed by genetic testing using FISH. Of these analyzable samples, our genetic test identified all 20 (100%) benign cases and 15 of 16 (93.8%) tumors in the testing samples.

In summary, we show here that genetic markers applied to spiral CT-guided FNA cytology samples are highly sensitive for the diagnosis of lung cancer and highly specific in their ability to exclude cancer within a given specimen. This approach should be particularly useful in complementing cytology diagnosis of benign or nonspecific benign diseases, especially when there is radiological confirmation that the needle has been properly placed within the lesion. The use of the larger core biopsies could allow better sample acquisition and further increase its detection sensitivity in identifying tumor from small lung lesions. A larger prospective study is also needed to further validate the genetic test described here for its potential clinical application.

Disclosure of Potential Conflicts of Interest

Dr. Yankelevitz has the following relationships with PneumRx, Inc., and with General Electric.

PneumRx, Inc.: Dr. Yankelevitz is an inventor on a pending patent related to biopsy needles assigned to PneumRx, Inc., is a paid medical advisor, and holds stock in the company.

General Electric: Dr. Yankelevitz is a co-inventor on a patent and other pending patents owned by Cornell Research Foundation (CRF), which are nonexclusively licensed and related to technology involving computer-aided diagnostic methods, including measurement of nodules. He receives royalties from the CRF pursuant to Cornell policy, which in turn is consistent with the Bayh-Dole Act.

Receives research support in the form of grants and contracts from the American Legacy Foundation, Flight Attendants’ Medical Research Institute, NCI AstraZeneca, Inc., OSI Pharmaceutical, GlaxoSmithKline, Visiongate, Carestream Health, Inc., and the Foundation for Lung Cancer: Early Detection, Prevention and Treatment (primary source of funding was an unrestricted gift by the Vector group, the parent company of Liggett Tobacco).

Dr. Henschke has the following relationship with General Electric and the National Cancer Institute (NCI).

General Electric: Dr. Henschke is a co-inventor on a patent and other pending patents owned by Cornell Research Foundation (CRF), which are nonexclusively licensed and related to General Electric for technology involving computer-aided diagnostic methods, including measurement of nodules. She receives royalties from the CRF pursuant to Cornell policy, which in turn is consistent with the Bayh-Dole Act.

NCI: Dr. Henschke is compensated for serving as a study section member for the NCI.

Dr. Henschke also receives research support in the form of grants and contracts from the American Legacy Foundation, Flight Attendants’ Medical Research Institute, NCI, AstraZeneca, Inc., Carestream Health, Inc., and the Foundation for Lung Cancer: early detection, prevention and treatment (primary source of funding was an unrestricted gift by the Vector group, the parent company of Liggett Tobacco).

The following is a listing of the patent and patent applications in which Claudia Henschke and David Yankelevitz are both listed as a co-inventor.

1. U.S. Patent No. 7,274,810 “System and Method for Three-Dimensional Image Rendering and Analysis” (Patent Issued to CRF September 25, 2007); the CRF has two pending divisional applications related to this Patent.
2. The CRF has two pending patent applications for “System, Method and Apparatus for Small Pulmonary Nodule Computer-Aided Diagnosis from Computed Tomography Scans.”
3. The CRF has a pending patent application for “System and Method for Providing Remote Analysis of Medical Data.”
4. The CRF has a pending patent application for “System and Method for Analyzing Medical Data to Determine Diagnosis and Treatment.”
5. The CRF has a pending patent application for “System and Method for Conducting a Clinical Trial Study.”
6. The CRF has a pending PCT application for “Medical Imaging Visibility Index System and Method for Cancer Lesions.”
7. The CRF has a pending patent application (and foreign pending patent applications) for “Method for Expanding the Domain of Imaging Software in a Diagnostic Workup.”
8. The CRF has a pending patent application for “System and Method for Position Matching of a Patent for Medical Imaging.”
9. Dr. Henschke and Dr. Yankelevitz are inventors on a pending patent application for Medical Imaging System for Accurate Measurement Evaluation of Changes in a Target Lesion (foreign patent applications also pending).

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References

Clinical Cancer Research

The Use of Genetic Markers to Identify Lung Cancer in Fine Needle Aspiration Samples


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