Quantitative Detection of Lung Cancer Cells by Fluorescence *In Situ* Hybridization*

Comparison With Conventional Cytology

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Study objective: **The aim of this study was to clarify whether fluorescence *in situ* hybridization (FISH) can diagnose lung cancer in various clinical specimens in comparison with conventional cytology.**
Design: **Prospective study.**
Setting: **University hospital in a metropolitan area.**
Patients: **Fifty consecutive patients with abnormal chest radiography or CT scan findings were enrolled. The patients included 32 men and 18 women, with an average age of 64 years. The final definitive diagnosis was made by histologic examination, as follows: 38 primary lung cancers (24 adenocarcinomas, 8 squamous cell carcinomas, 2 large cell carcinomas, and 4 small cell carcinomas); 1 metastatic renal cell carcinoma; and 11 benign lesions.**
Methods: **Four types of clinical specimens were analyzed. Cells obtained by transbronchial brushing and transbronchial fine-needle aspiration using a fiberoptic bronchoscope under fluoroscopy, CT scan-guided percutaneous needle biopsy, and bronchial washings. On every examination, duplicate slides were made for analyses of conventional cytology and FISH.**
Results: **Classifications according to conventional cytology were as follows: class I, 4 patients; class II, 15 patients; class IIIa, 3 patients; class IIIb, 5 patients; and class V, 23 patients. A classification higher than class IIIb was considered to be positive for cancer. For cytology, we found no false-positive cases and 11 false-negative cases. The specificity was 100%, and the sensitivity was 71.8%. By FISH, 34 cases showed aberrant copy numbers in either chromosome 3 or 17. We found no false-positive cases and five false-negative cases. The specificity was 100%, and the sensitivity was 87.1%.**
Conclusion: **The ability of FISH to detect aneusomic lung cancer cells is superior to conventional cytology for the diagnosis of lung cancer.** ***(CHEST 2005; 128:906–911)***

Key words: aneuploidy; aneusomy; cytology; fluorescence *in situ* hybridization; lung cancer

Abbreviations: BW = bronchial washing; FISH = fluorescence *in situ* hybridization; PN = percutaneous needle biopsy; SSC = standard saline citrate; TB = transbronchial brushing

Conventional cytology plays an important role for the diagnosis of lung cancer, especially in the examination of sputum and pleural effusions. In addition, cell specimens obtained by transbronchial brushing (TB)[1] and needle aspiration under fluoroscopy,[2] percutaneous needle biopsy (PN) under CT scanning,[3] and bronchial washings (BWs)[4] provide important information for the differential diagnosis between benign and malignant disease. Cytologic diagnoses are made by experienced cytologists who can properly evaluate the morphologic features of malignant cells. However, this judgment is some-

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times difficult when the morphologic changes associated with malignancy are mild. Such cells are usually classified as class III, using the classification of Papanicolaou,[5] which is suggestive of, but not conclusive for, malignancy. This is an ambiguous judgment for clinical decision making. In addition, when one obtains a small number of cells from the lesion, the definitive diagnosis is even more difficult. These limitations of morphology-based conventional cytology have stimulated the search for more objective and quantitative methods for an accurate cytologic diagnosis of cancer.

Aneuploidy is the most common feature of many solid tumors, including lung cancer.[6] Solid tumors are characterized by complicated karyotypes by classic cytogenetics.[7,8] Chromosomal instability[9,10] may cause the uneven distribution of chromosomes during cell division.[11,12] Thus, malignant tumors can be diagnosed by detecting aneuploid, usually hyperdiploid, cells. A rapid and sensitive method for detecting aneusomy of a specific chromosome in an individual cell is fluorescence *in situ* hybridization (FISH). For this purpose, specific centromeric DNA probes enumerated the chromosomes. FISH was originally developed as a method to detect chromosomal aberrations,[13] and is now widely used for gene mapping,[14] the diagnosis of congenital diseases,[15] and detecting specific gene copy number changes in malignant cells.[16–18]

One advantage of FISH in detecting malignant cells is its objective and quantitative evaluation. However, the specificity and sensitivity of FISH in the diagnosis of lung cancer is unclear. We report the results of a prospective study comparing FISH with conventional cytology to detect lung cancer cells.

Materials and Methods

Patients

Fifty consecutive patients who underwent cytologic examination for abnormal chest radiography or CT scan findings at Tokyo Medical University Hospital from July 2003 to January 2004 were enrolled in this prospective study. The patients included 32 men and 18 women, with an average age of 64 years. The final definitive diagnosis was made by histologic examination, as follows: 38 primary lung cancers (24 adenocarcinomas, 8 squamous cell carcinomas, 2 large cell carcinomas, and 4 small cell carcinomas); 1 metastatic renal cell carcinoma; and 11 benign lesions. All patients with lung cancer were staged according to the latest Union Internationale Centre le Cancer criteria.[19] Cases included 10 tumors in stage IA, 5 in stage IB, 1 in stage IIA, 3 in stage IIB, 10 in stage IIIA, 6 in stage IIIB, and 3 in stage IV (Table 1).

Cells gathered from lung lesions were independently analyzed by conventional cytology and FISH. Informed consent for the cytologic examinations and genetic analyses of the specimens were obtained from all patients.

Table 1—*Histology and Stage of Lung Cancer in This Series of Patients

Case	Age	Gender	Specimen	Histology	Stage
1	59	M	TB	Sq	cIIIA
2	42	F	PN	B	NA
3	43	M	TB	Ad	PIIIA
4	70	M	TB	Ad	CIV
5	77	M	TB	Ad	PIA
6	73	M	PN	Ad	PIA
7	58	M	TN	Ad	PIA
8	71	M	BW	Ad	pIIIA
9	71	M	BW	La	pIB
10	66	F	TN	RCC	NA
11	65	F	TB	Sm	cIIIB
12	68	F	PN	Ad	pIA
13	69	F	BW	Ad	pIV
14	52	F	TB	Ad	pIIA
15	75	F	TN	Sq	pIA
16	37	M	PN	B	NA
17	64	M	PN	Ad	pIIB
18	58	F	TB	B	NA
19	73	F	PN	Ad	pIA
20	62	M	PN	B	NA
21	69	M	TB	B	NA
22	76	M	BW	La	cIIIB
23	76	M	PN	Ad	cIIIB
24	75	M	PN	Ad	pIB
25	65	M	BW	Sm	cIIIA
26	23	M	PN	B	NA
27	74	M	BW	Ad	cIV
28	72	F	PN	B	NA
29	80	M	TB	B	NA
30	56	M	BW	Ad	pIB
31	64	M	TB	B	NA
32	58	M	PN	Ad	cIIIA
33	72	F	PN	Ad	pIA
34	72	M	PN	Ad	cIIIA
35	66	F	TB	Sm	cIIIB
36	61	M	TB	Ad	pIIB
37	72	F	TB	Ad	pIB
38	52	M	PN	B	NA
39	64	M	TB	Ad	pIA
40	79	F	TB	Sq	cIB
41	39	M	TB	B	NA
42	78	M	BW	Sq	cIIIB
43	62	M	TB	Sq	cIIIA
44	57	M	TB	Sq	pIIIA
45	70	M	PN	Ad	pIA
46	55	F	PN	Ad	pIA
47	58	F	TN	Sq	cIIIB
48	66	F	TN	Ad	pIIB
49	62	M	BW	Sm	cIIIA
50	72	F	BW	Sq	cIIIA

*M = male; F = female; TN = transbronchial needle biopsy; Ad = adenocarcinoma; Sq = squamous cell carcinoma; La = large cell carcinoma; Sm = small cell carcinoma; RCC = renal cell carcinoma; B = benign lesion; c = clinical stage; p = pathologic stage; NA = not applicable.

Cell Samples

In this study, the following four types of cell specimens were analyzed: cells obtained by TB (n = 18) and transbronchial fine-needle aspiration (n = 5) using a fiberoptic bronchoscope