Multiplexed Molecular Profiling of Lung Cancer Using Pleural Effusion

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Introduction: Pleural effusion is frequently observed in patients with advanced lung cancer. Although effusion can be obtained less invasively and repeatedly, its use in multiplexed molecular profiling has not been fully investigated.
Methods: Between July 2011 and April 2013, pleural effusion samples were obtained from patients with lung cancer at Shizuoka Cancer Center. They were analyzed for *EGFR, KRAS, BRAF, PIK3CA, NRAS, MEK1, AKT1, PTEN,* and *HER2* mutations, *EGFR, MET, FGFR1, FGFR2,* and *PIK3CA* amplifications, and *ALK, ROS1,* and *RET* fusion genes using pyrosequensing and/or capillary electrophoresis, quantitative reverse-transcriptase polymerase chain reaction, and reverse-transcriptase polymerase chain reaction, respectively.
Results: One hundred and two samples from 84 patients were analyzed. Adenocarcinoma was the most common histological subtype (82%). Genetic abnormalities were detected in 42% of patients. The most common abnormality was *EGFR* mutation (29%), followed by *EML4-ALK* rearrangement (5%), *KRAS* mutation, *and EGFR* amplification (4%, each). Concordance rates between pleural effusion and matched formalin-fixed, paraffin-embedded samples were 88%. Among 11 patients who provided samples at multiple time points, changes in molecular profile over the course of treatment were observed in five patients.
Conclusions: The use of pleural effusion for multiplexed molecular testing and real-time monitoring in lung cancer was demonstrated.

Key Words: Multiplexed molecular testing, Driver mutation, Pleural effusion, Lung cancer

(*J Thorac Oncol.* 2014;9: 1048–1052)

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This work was supported by JSPS KAKENHI Grant Numbers 24591186 (N.Y.) and 24501363 (Y.K.).
Disclosure: The authors declare no conflicts of interest.
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ISSN: 1556-0864/14/0907-1048

Development of molecular cancer therapeutics has brought numerous benefits to patients with driver mutations. Non–small-cell lung cancer (NSCLC) is one of such malignancies with targetable genetic alterations. In NSCLC patients who harbor epidermal growth factor receptor (*EGFR*) mutation, EGFR-tyrosine kinase inhibitors double progression-free survival compared with platinum containing chemotherapy.[1] In 2007, *anaplastic lymphoma kinase (ALK)* rearrangement was discovered in approximately 5% of NSCLC,[2] and ALK inhibitor (crizotinib) was rapidly approved in 5 years, both in the United States and Japan. Today, druggable oncogenes other than *EGFR* and *ALK* have been detected,[3,4] and development of specific inhibitors is underway.

In the era of multiplexed molecular profiling, tumors should be tagged with some genetic abnormalities before treatment. To detect such abnormalities, an ample yield of tumor cells is necessary. Among lung cancer patients, pleural effusion is observed in 7–15%,[5] and it can be obtained less invasively and repeatedly compared with primary lesion. Although pleural effusion is a potential candidate for molecular testing, its use in multiplexed molecular profiling has not been fully investigated.

In July 2011, we started the "Shizuoka lung cancer mutation study," a prospective tumor genotyping study of patients with thoracic malignancies. Using these samples, we conducted a multiplexed molecular profiling of advanced lung cancer with pleural effusion.

MATERIALS AND METHODS

Patient Selection and Samples

Between July 2011 and April 2013, consecutive patients with pathologically confirmed lung cancer at Shizuoka Cancer Center were enrolled in the "Shizuoka lung cancer mutation study". Written informed consent was obtained from all participants, and this study was approved by the institutional review board of our hospital. The diagnosis of the tumor was done by institutional pathologists in accordance with the 2004 World Health Organization classification. Pleural effusion samples of up to 250 ml were obtained at the time of diagnosis or therapeutic drainage. To analyze concordance rate of molecular profile between pleural effusion and tissue samples, formalin-fixed, paraffin-embedded (FFPE) samples at the time of diagnosis were provided. Results of mutational testing were communicated to clinicians.

Molecular Analyses of Pleural Effusion and Tissue Samples

Cell isolation from pleural effusion was performed and sored at −80°C until use. Genomic DNAs were extracted using QIAamp DNA mini kit (QIAGEN, Hilden, Germany). From FFPE samples, DNAs were extracted using QIAamp DNA FFPE tissue kit (QAIGEN). DNA concentration was measured using Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA). Total RNAs were extracted from pleural effusion with RNeasy Mini kit (QIAGEN) and measured by spectrophotometer (NanoDrop 2000C; Thermo Scientific, Wilmington, DE). They were analyzed for *EGFR, KRAS, BRAF, PIK3CA, NRAS, MEK1, AKT1, PTEN,* and *HER2* mutations, *EGFR, MET, FGFR1, FGFR2,* and *PIK3CA* amplifications, and *ALK, ROS1,* and *RET* fusion genes using pyrosequensing and/or capillary electrophoresis, quantitative reverse-transcriptase polymerase chain reaction, (qRT-PCR) and RT-PCR, respectively (Tables 1 and 2). Each analyzing method is described in the Supplemental Digital Content 1 (http://links.lww.com/JTO/A601).

Statistical Analysis

The primary purpose of this study was to explore the use of pleural effusion in multiplexed molecular profiling of advanced lung cancer. Detection rate was defined as the proportion of samples with genetic abnormalities. If multiple samples were obtained from the same patient, results for the first sample were adopted. Detection rate between the two groups (pathologically positive or negative) were analyzed using Fisher's exact test. Concordance rate of molecular profile between pleural effusion samples and matched FFPE samples were also analyzed. Probability values of < 0.05 indicated a statistically significant difference. All the analyses were performed using JMP ver.7 (SAS Institute Inc., Cary, NC).

TABLE 1. Multiple Tumor Genotyping Panel

Mutations Gene	Position	AA Mutant	Nucleotide Mutant
EGFR	G719	G719C/S	2155G>T/A
		G719A	2156G>C
	exon 19	Deletion	
	T790	T790M	2369C>T
	exon 20	Insertion	
	L858	L858R	2573T>G
	L861	L861Q	2582T>A
KRAS	G12	G12C/S/R	34G>T/A/C
		G12V/A/D	35G>T/C/A
	G13	G13C/S/R	37G>T/A/C
		G13D/A	38G>A/C
	Q61	Q61K	181C>A
		Q61R/L	182A>G/T
		Q61H	183A>T/C
BRAF	G466	G466V	1397G>T
	G469	G469A	1406G>C
	L597	L597V	1789C>G
	V600	V600E	1799T>A
PIK3CA	E542	E542K	1624G>A
	E545	E545K/Q	1633G>A/C
	H1047	H1047R	3140A>G
NRAS	Q61	Q61K	181C>A
		Q61L/R	182A>T/G
MEK1 (MAP2K1)	Q56	Q56P	167A>C
	K57	K57N	171G>T
	D67	D67N	199G>A
AKT1	E17	E17K	49G>A
PTEN	R233	R233*	697C>T
HER2	Exon 20	Insertion	

TABLE 2. Amplifications and Fusion Genes

Gene Amplifications	Fusion Genes
EGFR	EML4-ALK
MET	CD74-ROS1
PIK3CA	SLC34A2-ROS1
FGFR1	KIF5B-RET
FGFR2	CCDC6-RET

RESULTS

Patient Characteristics

Among 845 consecutive patients enrolled in the "Shizuoka lung cancer mutation study" during the study period, pleural effusion samples were obtained from 92 patients. Eight patients were ineligible because further investigations indicated that they did not have lung cancer (three non-thoracic malignancies, one incidence each of malignant pleural mesothelioma, invasive thymoma, thymic carcinoma, tracheal carcinoma, and spindle cell sarcoma). Then, 102 samples from 84 patients were analyzed (Fig. 1).

Patient characteristics are summarized in Table 3. Median age was 69 (range 29–85), 69% were male, 63% were current smokers, and the most common histology was adenocarcinoma (82%). Forty-three samples (42%) were obtained at the time of initial diagnosis. In 11 patients (13%), samples were obtained at multiple time points.

Detection of Genetic Abnormalities

Genetic abnormalities were detected in 35 patients (42%; 95% confidence interval: 31–52%). In 80 patients,

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