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Case report

Analysis of significantly mutated genes as a clinical tool for the diagnosis in a case of lung cancer

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ABSTRACT

Bronchoendoscopic examination is not necessarily comfortable procedure and limited by its sensitivity, depending on the location and size of the tumor lesion. Patients with a non-diagnostic bronchoendoscopic examination often undergo further invasive examinations. Non-invasive diagnostic tool of lung cancer is desired. A 72-year-old man had a 3.0 cm × 2.5 cm mass lesion in the segment B1 of right lung. Cytological examination of sputum, bronchial washing and curetted samples were all "negative". We could confirm a diagnosis of lung cancer after right upper lung lobe resection pathologically, and also obtained concordant results by genomic analysis using cytological negative samples from airways collected before operation. Genetic analysis showed mutational profiles of both resected specimens and samples from airways were identical. These data clearly indicated the next generation sequencing (NGS) may yield a diagnostic tool to conduct "precision medicine".

1. Introduction

Patients with a non-diagnostic bronchoendoscopic examination often undergo further invasive examinations. Bronchoendoscopy is relatively safe, with less than 1% of procedures complicated by pneumothorax [1]. However bronchoendoscopic examination is not necessarily comfortable procedure and limited by its sensitivity, which ranges from 34 to 88%, depending on the location and size of the lesion [2]. In this study, we report a case of lung cancer diagnosed by genomic analysis, but not by usual bronchoendoscopic examinations including cytological and pathological measures.

2. Case presentation

A 72-year-old man was pointed out to have an abnormal mass lesion by computed tomography of the chest by a routine health check-up. He was referred to our hospital and underwent the clinical examinations and surgery. During these procedures, we collected several specimens for diagnosis and genomic analyses (Table 1).

The abnormal mass lesion was located in the upper lung field and the size of mass was 3.0 × 2.5 cm in diameter (Figs. 1 and 2). He had a smoking history of 50 pack-year. Interstitial lung fibrosis was also pointed out but he had no respiratory symptom. The result of laboratory examination of hematology and blood chemistry was within normal ranges including several tumor markers. Bronchoendoscopy was performed to confirm a diagnosis of the mass lesion. Because the introducible bronchus to mass lesion was not identified, we could not use endobronchial ultrasonography (EBUS) using a guided sheath (EBUS-GS) [3]. Instead, by an angulated curette forceps, we obtained a small amount of liquid samples from bronchus adjacent to the mass lesion. We also obtained bronchial washing, bronchial brushing samples and sputum. A report from cytological examination of bronchial washing and curetted samples indicated there were no apparent tumor cells in these specimens (all "negative").

Imaging findings including local invasion to adjacent vessels

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Table 1
The timeline of clinical examinations, collection of samples and genomic analyses was exhibited.

Date	Events	Results
5/26/2015	First visit	Symptom free
5/26/2015	Chest X-P	A mass of right upper lung field was found.
6/12/2015	Chest CT	Local invasion to innominate vein was found.
6/25/2015	Sputum Collection	No apparent tumor cells were detected.
	Bronchial brushing/washing	No apparent tumor cells were detected.
	Bronchial curetting	No apparent tumor cells were detected.
	Blood sample collection	Collection for genomic analysis
7/7/2015	Bone Scintigraphy	No metastasis was found.
7/8/2015	Brain MRI	No metastasis was found.
8/10/2015	Surgical Resection	No invasive findings were revealed.
9/25/2015	Genomic analysis	

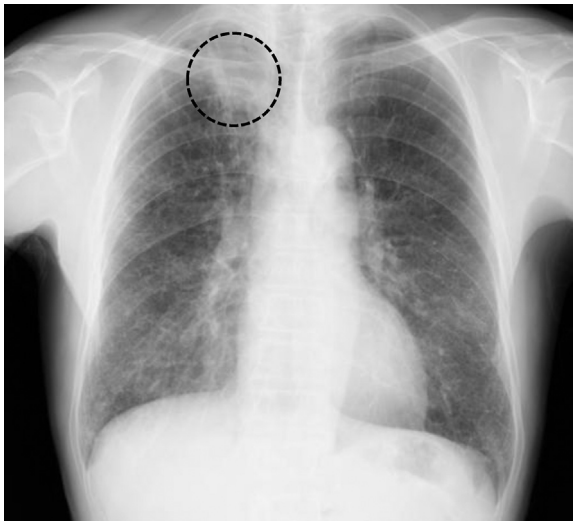

Fig. 1. Chest radiography revealed a mass lesion in mediastinal right upper lung field. Tumor lesion was indicated by dotted circle.

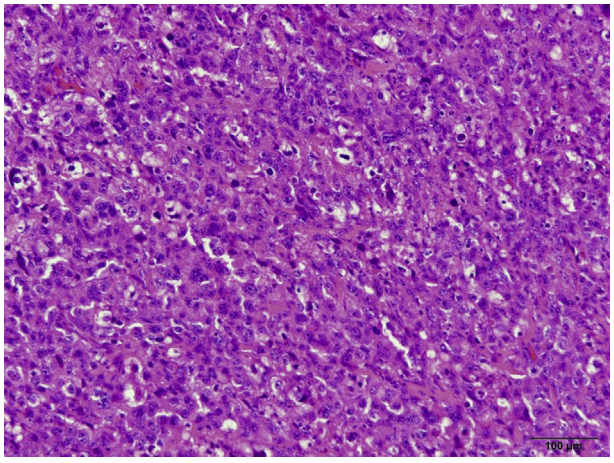

Fig. 3. A pathological diagnosis of a resected tumor was solid type of adenocarcinoma by new WHO classification.

and mediastinal nodal swelling was compatible with lung cancer as the stage of cT3N2M0 (stage IIIA) clinically. Because an innominate vessel was located over the tumor, we could not find the route of percutaneous CT guided needle biopsy (Fig. 2). Upper lung lobe resection was performed to confirm the diagnosis and the stage of the tumor. A pathological diagnosis of a resected tumor (3.0 × 2.5 cm) was solid type of adenocarcinoma by new WHO classification (Fig. 3) [4]. After surgery, we could confirm the non-invasiveness of the tumor to adjacent innominate vessel and no mediastinal lymph node metastasis. (pT2aN0M0, stage IB). However, because of local recurrence of mediastinal lymph nodes metastases with malignant pleural effusion, radiation therapy and chemotherapy with carboplatin and nab-paclitaxel was administered as advanced stage of lung cancer.

Recent international collaborative studies from The Cancer Genome Atlas (TCGA) identified a set of 53 significantly mutated genes (SMGs) by studying the whole exons of 230 cases of lung adenocarcinoma and 178 of squamous cell carcinoma [5,6]. Instead of analyzing approximately 20,000 genes of whole exons, these SMGs will be able to disclose principal mutations and signaling

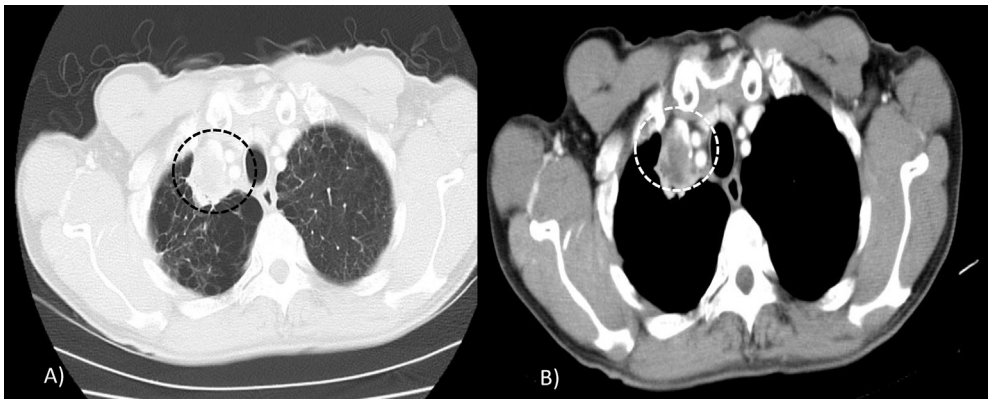

Fig. 2. Computed tomography of the chest revealed a mass lesion adjacent to vessels in mediastinum. Tumor lesion was indicated by dotted circle. A) The introducible bronchus to mass lesion was not identified. B) Because an innominate vessel was located over the tumor, we could not find the route of percutaneous CT guided needle biopsy.

pathways in tumor. These 53 SMGs may be significant, and sufficient for the diagnosis and basic understanding of lung cancer-related oncogenes [7]. In order to elucidate the utility of this genomic analysis for definite diagnosis of lung cancer, we performed targeted sequencing of the 53 lung cancer associated SMGs using an *in house* panel, which covers 205,684 base pairs and 95% of targeted regions (see Table 2) [7].

We analyzed resected surgical specimens, bronchial washing, sputa and plasma using next generation sequencer (Ion Proton platform, Thermo Fisher Scientific, Waltham, MA, USA). Sequencing data was obtained by 4,623,928 mapping reads (range: 3,482,251–7,431,995) with 94.1% of reads aligned onto the targeted regions (range: 83%–97%) and with an average of 1669-fold coverage (range: 1271–2731) (Table 3). The analysis of resected primary tumor revealed two somatic mutations in *TP53* and *COBL*, namely, the former Gln317Ter, and the latter Pro828Thr, respectively (Table 4). The mutations found in supernatant of bronchial washing fluid, sputum and plasma were identical to those of tumor (Table 4). Of interest, we observed the concentration

Table 3
Summary of mapping sequencing reads and coverage data.

Sample name	Mapped reads	On target	Coverage depth	Uniformity
Tumor	4,542,280	96.72%	1644	84.64%
BrW/ppt	3,482,251	97.14%	1271	70.13%
BrW/Sup	3,647,609	95.95%	1357	82.41%
Buffy Coat	4,512,224	97.44%	1722	84.85%
Plasma	4,127,207	83.04%	1286	74.97%
Sputum	7,431,995	94.60%	2731	85.07%

Abbreviations: BrW/ppt = bronchial washing precipitates.
BrW/Sup = bronchial washing supernatant.

Table 2
Lung cancer-associated 53 significantly mutated genes in targeted sequence panel.

Number of gene	Gene symbol	Chromosome	Number of amplicons	Total bases	Covered bases	Missed bases	Overall coverage
1	*AKT1*	chr14	26	1573	1497	76	95%
2	*AKT2*	chr19	27	1576	1543	33	98%
3	*AKT3*	chr1	30	1624	1624	0	100%
4	*ARID1A*	chr1	76	7058	6023	1035	85%
5	*ARID1B*	chr6	75	6950	5965	985	86%
6	*ARID2*	chr12	71	5718	5643	75	99%
7	*ASCL4*	chr12	5	532	382	150	72%
8	*ATM*	chr11	147	9791	9439	352	96%
9	*BRAF*	chr7	37	2481	2224	257	90%
10	*CDKN2A*	chr9	9	962	612	350	64%
11	*COBL*	chr7	48	4151	3977	174	96%
12	*CREBBP*	chr16	96	7639	7071	568	93%
13	*CTNNB1*	chr3	32	2486	2486	0	100%
14	*CUL3*	chr2	42	2561	2495	66	97%
15	*EGFR*	chr7	60	4189	4135	54	99%
16	*EP300*	chr22	90	7555	7182	373	95%
17	*EPHA7*	chr6	44	3175	3154	21	99%
18	*ERBB2*	chr17	57	4080	3808	272	93%
19	*ERBB3*	chr12	59	4440	4374	66	99%
20	*FGFR1*	chr8	41	2825	2816	9	100%
21	*FGFR2*	chr10	43	2910	2842	68	98%
22	*FGFR3*	chr4	34	2752	2215	537	81%
23	*FOXP2*	chr7	36	2487	2469	18	99%
24	*HRAS*	chr11	11	683	683	0	100%
25	*KEAP1*	chr19	24	1925	1845	80	96%
26	*KMT2D*	chr12	192	17,154	15,854	1300	92%
27	*KRAS*	chr12	10	737	681	56	92%
28	*MAP2K1*	chr15	18	1292	1239	53	96%
29	*MET*	chr7	59	4427	4396	31	99%
30	*MGA*	chr15	110	9428	9345	83	99%
31	*MLL*	chr11	144	12,279	11,875	404	97%
32	*NF1*	chr17	136	9161	9023	138	99%
33	*NFE2L2*	chr2	23	1868	1826	42	98%
34	*NOTCH1*	chr9	99	8008	7078	930	88%
35	*NOTCH2*	chr1	101	7809	7539	270	97%
36	*NRAS*	chr1	9	610	610	0	100%
37	*PIK3CA*	chr3	50	3407	3282	125	96%
38	*PTEN*	chr10	18	1302	1223	79	94%
39	*RASA1*	chr5	55	3412	3216	196	94%
40	*RB1*	chr13	55	3057	2902	155	95%
41	*RBM10*	chrX	48	3228	3079	149	95%
42	*RIT1*	chr1	13	771	771	0	100%
43	*SETD2*	chr3	91	7905	7663	242	97%
44	*SLIT2*	chr4	76	4972	4854	118	98%
45	*SMAD4*	chr18	24	1769	1715	54	97%
46	*SMARCA4*	chr19	74	5399	5055	344	94%
47	*SOX2*	chr3	9	964	883	81	92%
48	*STK11*	chr19	23	1392	1343	49	97%
49	*TP53*	chr17	22	1383	1351	32	98%
50	*TP63*	chr3	34	2360	2227	133	94%
51	*TSC1*	chr9	49	3705	3603	102	97%
52	*TSC2*	chr16	92	5834	5677	157	97%
53	*U2AF1*	chr21	15	880	870	10	99%

Table 4
Detection of somatic mutations in primary lung tumor, bronchial washing samples, sputum and plasma.

Sample name	Mutations		Histological diagnosis
	TP53 Gln317Ter AF (Var/Total reads)	*COBL* Pro828Thr AF (Var/Total reads)	
Tumor	81.7% (277/339)	14.8% (293/1979)	Adenocarcinoma
BrW/Sup	21.5% (85/414)	4.5% (90/1985)	Negative
Sputum	7.3% (93/1282)	3.6% (72/1985)	Not applicable
Plasma	2.4% (10/415)	1.8% (37/2010)	Not applicable
BrW/ppt	Not detected	Not detected	Negative
Buffy Coat	Not detected	Not detected	Not applicable

Abbreviations: AF = allelic fraction; Var: variant.
BrW/ppt = bronchial washing precipitates.
BrW/Sup = bronchial washing supernatant.

gradient of mutant allelic fraction (AF) (Table 4).

The AFs were highest, as was expected, in the primary tumor e.g., 82% and 15% at *TP53* Gln317Ter and *COBL* Pro828Thr, respectively (Table 4). The AFs of the two mutations were stepwisely decreased from bronchial washing supernatant, sputum and plasma (Table 4). In contrast, we could not confirm a diagnosis of lung cancer by conventional cytological and histological examinations.

3. Discussion

When lung cancer was suspected, the method of EBUS-GS is useful for collecting samples from peripheral pulmonary lesions to yield high diagnostic accuracy (77%) [3]. In this case, a peripheral mass lesion of 3.0 cm in diameter was located in mediastinal area of upper lung lobe, and several attempts to find out an introducible bronchus for EBUS-GS failed. However, we obtained curetted cytological samples and bronchial washing, but conventional cytological examinations were all reported “negative” or “non-diagnostic”.

Genomic analysis was performed at our laboratory of GAC (Genome Analysis Center) established 3 years ago at our hospital [7–14]. We already developed lung cancer panel consisting of 53 genes [7]. We tested whether it will be useful to detect lung SMGs in cytologically negative samples collected by bronchial washing.

Bronchial samples were usually collected from segmental bronchus connecting to peripheral nodules by bronchoendoscopy. If we were able to use EBUS-GS, we might have had specimens which contain more cellular component. That could have made conventional pathological and cytological diagnosis more informative. However, we were not able to find the leading bronchus to the nearest site of the tumor. We were only able to obtain a small amount of liquid samples probably far away the tumor. But still, we were able to obtain almost complete concordant results among the primary tumor, endobronchial washings, sputum and plasma with different concentrations (Table 4).

Evaluation of circulating tumor DNA in plasma/serum is a promising non-invasive diagnostic tool [15]. Of particular interest in this case was that we were able to detect the same mutations in circulating plasma collected beforehand at initial evaluation by bronchoscopy, clearly indicating that we may not need cellular components which are pre-requisite for classical cytological diagnosis. This was also reflected that the mutations were detected in supernatant, but not in the cellular rich buffy coats (Table 4).

Because genomic analysis of sputum before bronchial washing by using an *in house* lung cancer panel was positive in this case, genomic analysis may have a possibility of non-invasive tool for the diagnosis of lung cancer in patients with negative cytological examinations. In a previous report from The Johns Hopkins Lung Cancer Project, *KRAS* or *TP53* mutation identical to primary lesion was also detected in the sputum by using a polymerase chain reaction-based assay [16]. Lung cancer panel consisting of 53 genes used in this case may be more utilizing procedure to diagnose lung cancer. Experience of a large number of patients is needed to reach the final conclusion about the utility of genomic analysis as a non-invasive diagnostic tool.

4. Conclusion

Genomic analysis by lung cancer panel consisting of 53 genes used in this case may have a possibility of a non-invasive diagnostic tool of lung cancer.

Financial disclosure and conflict of interest

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The authors have stated that they have no conflict of interest.

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