Case report

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

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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...
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

| 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. 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.

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

| 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            | CTNNB2      | chr9       | 9                   | 962         | 612          | 350          | 64%             |
| 11            | CUL3        | chr1       | 48                  | 4151        | 3977         | 174          | 96%             |
| 12            | CREBBP      | chr1       | 96                  | 7639        | 7071         | 568          | 93%             |
| 13            | CTNNB1      | chr3       | 5                   | 2487        | 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       | 36                  | 2752        | 2215         | 537          | 81%             |
| 23            | FOXP2       | chr7       | 36                  | 2487        | 2469         | 18           | 99%             |
| 24            | Hras        | chr11      | 11                  | 683         | 683          | 0            | 100%            |
| 25            | KEAP1       | chr9       | 24                  | 1925        | 1845         | 80           | 96%             |
| 26            | KMT2D       | chr12      | 192                 | 17,154      | 15,914       | 1300         | 92%             |
| 27            | Kras        | chr10      | 12                  | 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            | Nfl         | chr17      | 136                 | 9161        | 9023         | 138          | 99%             |
| 33            | Nfe2l2      | chr9       | 23                  | 1868        | 1826         | 42           | 98%             |
| 34            | Notc1       | chr9       | 99                  | 8008        | 7078         | 930          | 88%             |
| 35            | Notc2       | chr1       | 101                 | 7809        | 7539         | 270          | 97%             |
| 36            | Nras        | chr1       | 9                   | 610         | 610          | 0            | 100%            |
| 37            | Pkca        | chr3       | 50                  | 3407        | 3282         | 125          | 96%             |
| 38            | Ptene        | chr10      | 18                  | 1302        | 1223         | 79           | 94%             |
| 39            | Ras1        | chr5       | 55                  | 3412        | 3216         | 196          | 94%             |
| 40            | Rb1         | chr13      | 55                  | 3057        | 2902         | 155          | 95%             |
| 41            | Rbmi10      | chrX       | 48                  | 3228        | 3079         | 149          | 99%             |
| 42            | Rbt1        | 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        | 5039         | 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       | 45                  | 3705        | 3691         | 102          | 97%             |
| 52            | Tsc2        | chr12      | 92                  | 5834        | 5677         | 157          | 97%             |
| 53            | U2af1       | chr21      | 15                  | 880         | 870          | 10           | 99%             |

Abbreviations: 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 EBGS-US, we might have had specimens which contain more cellular component. That could have made conventional pathologial 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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Table 4

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

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