Case Report

Molecular Analysis of Liquid Cytological Samples Collected by Bronchoscopy with Radial Endobronchial Ultrasonography and Guide Sheath

Naomi Iwabu, MD,1 Takehiro Izumo, MD, PhD,1 Yukiko Nakamura, MD,1,2 Christine Chavez, MD,1 Takaaki Tsuchida, MD, PhD,1 and Shinji Sasada, MD, PhD1

A 64-year-old man who underwent sigmoid resection for Stage 4 colon cancer had a growing nodule on the left upper lobe during follow-up. Surgical resection revealed primary pulmonary adenocarcinoma. Subsequently, a new nodule appeared in the contralateral S1b, for which endobronchial ultrasonography with a guide sheath (EBUS-GS) was performed for diagnosis. However, histopathologic examinations were inconclusive. Gene analysis of the liquid samples from this lesion revealed KRAS mutation, which on hindsight was not detected in the metachronous left upper lobe cancer but was detected in the resected sigmoid colon. Hence, the right upper lobe nodule was diagnosed by bronchoscopy as colon cancer metastasis, confirmed after wedge resection. For specimen obtained by EBUS-GS, search for gene mutation in the liquid specimen is useful as an ancillary test especially when histological diagnosis is equivocal. Thus, developments on diagnostic tools using liquid samples are highly expected in the future.

Keywords: bronchoscopy, endobronchial ultrasonography with a guide sheath (EBUS-GS), KRAS mutation, molecular analysis

Introduction

In recent years, molecular targeted drugs have been widely used for many malignant tumors. For advanced lung cancer, histologic-type genetic mutation on specimens obtained by transbronchial biopsy (TBB) has become important targets for treatment as well. The specimen obtained by conventional TBB is usually small in size thus sampling several pieces of tissue is ideal but not always feasible. To help solve the issue, endobronchial ultrasonography with a guide sheath (EBUS-GS), a lung biopsy method that combines a radial probe ultrasound with a guide sheath, was introduced and had been performed in recent years. In addition, the usefulness of EBUS-GS in combination with chest tomosynthesis for bronchoscopic diagnosis of a ground glass nodule or a small nodule has been reported.

However, when the specimen obtained by EBUS-GS is small, diagnosis based on histological examination is often difficult. Probably, a search for gene mutation in these tissues could lead to an accurate diagnosis and consequently, initiation of appropriate treatment.

Case Report

The patient is a 66-year-old male who consulted because of an abnormal chest shadow on chest radiograph.
In 2011, a growing nodular shadow was detected on the left S\(^{1+2}\) by chest X-ray for which wedge resection was done, revealing primary lung adenocarcinoma. On follow-up chest computed tomography (CT) scan after 19 months, a nodular shadow was again apparent, this time on the right upper lung field. He was referred to our department for diagnostic examination.

Past history revealed laryngeal cancer status/post (S/P) radiation therapy in 2006, sigmoid colon cancer S/P resection and adjuvant chemotherapy in 2008 with liver metastasis S/P resection in 2011. He is a tobacco smoker for 58.5 pack years. There is no known history of cancer in the family. Physical examination was likewise unremarkable. Biochemical and coagulation tests were normal. Tumor markers were normal: CEA (2.7 ng/ml), CYFRA (0.6 ng/ml), CA19-9 (22.0 U/ml), NSE (11.3 ng/ml) SCC (1.0 ng/ml), Pro-GRP (33.8 pg/ml). Chest X-ray revealed a nodular shadow on the right upper lung field (Fig. 1A).

Chest CT scan showed a 10 × 9 mm nodule with an irregular border at S\(^{1}\) b of the right lung (Fig. 1B). Fluorodeoxy glucose-positron emission tomography (FDG-PET)/CT showed slight uptake of the same lesion (Fig. 1C).

On January 2013, EBUS-GS was performed in combination with virtual bronchoscopic navigation (VBN) (LungPoint\textsuperscript{®}; Broncus Technologies, Inc., Mountain View, California, USA, Fig. 2A). We used the P-260F scope, K-201 guide sheath kit, and radial ultrasound probe (Olympus Inc., Tokyo, Japan). The radial ultrasound probe with the guide sheath was inserted through the right B\(^{1}\) b. When the target lesion was examined on ultrasound, it was confirmed that the probe reached the lesion adjacently (Fig. 2B). Biopsy and brushing were performed at the same site (Fig. 2C). To ensure that we collected as much cellular components as we could, the biopsy forceps and brush were rinsed in saline after every specimen collection and the GS was flushed with additional 2 ml of saline; all liquid collections were combined in a tube. The samples submitted for examination were: transbronchial biopsy specimen, brush specimen on glass slides, and liquid samples.

Incidentally, our team, with the approval of the Institutional Review Board (IRB) of the hospital, is prospectively working on exploratory researches on gene mutation and expression profile using specimen obtained by bronchoscopic examination. This case was included in the study at that time. So in addition, gene mutation analysis was included in the examination.

Mutation analysis was performed in an institutional genetic laboratory. DNA was extracted using a QIAmp DNA micro Kit (Qiagen, Valencia, California, USA). After polymerase chain reaction, we evaluated for epithelial growth factor receptor (EGFR) gene mutations (EGFR exon 19 deletion and exon 21 point mutation), v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations, and v–raf murine sarcoma viral oncogene homolog B1 (BRAF) mutations by high resolution melting (HRM). We extracted RNA by using QIAamp RNA Blood Mini Kit (Qiagen, USA). With extracted RNA, we performed RT-PCR by using LAMP-1 (housekeeping gene) primers and evaluated the qualities of RNA on LightCycler (Idaho Technology, Salt Lake City, Utah, USA) platform. Then, anaplastic lymphoma kinase (ALK)-rearrangements were analyzed by multiplex PCR. The concentrations of DNA and RNA were assessed by UV Spectrophotometry (260 nm absorption) BioSpec-nano (Shimadzu, Kyoto, Japan).
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The anamnestic sigmoid colon cancer (Fig. 3D). In addition, immunohistochemical (IHC) examination revealed that the tumor cells were positive for CDX2 and negative for TTF-1. Thereafter, the nodule in the right S1b was officially signed out as lung metastasis from sigmoid colon cancer.

Discussion

to our knowledge, this is the first report demonstrating the suitability of liquid samples obtained by bronchoscopy for gene mutation analysis for the diagnosis of a pulmonary nodule. In this case wherein malignancy was suspected, we performed EBUS-GS with the hope of making definite diagnosis. However, the biopsy and cytology samples that we obtained were not diagnostic. Although we performed EBUS-GS in combination with VBN, the closest we could reach was only adjacent to the target lesion.

Kurimoto, et al. reported only a 42% diagnostic yield of EBUS-GS when the probe was adjacent to the lesion.
In such cases, it is therefore difficult to make a diagnosis by histological examination alone.

KRAS has an important role of transmitting tyrosine kinase receptor -activated signals such as EGFR, and is involved in the growth, differentiation, and survival of cells. KRAS gene mutation is observed in various cancers, occurring in 10%–15% of lung adenocarcinoma cases and in 40% of colorectal cancer cases. It is commonly found in smokers and in those with mucinous adenocarcinoma and adenocarcinoma associated with goblet cells. It is also reported that the status of KRAS gene mutation in the primary site and the metastatic site correspond with each other in more than 95% of cases.

In this case, pathological diagnosis of malignancy using the samples obtained by bronchoscopy was difficult. However, lung metastasis from colorectal cancer was suspected and lung resection was performed on account of the following reasons: (1) KRAS gene mutation was also positive in the anamnestic colorectal cancer cells; (2) the tissue obtained by bronchoscopy was necrotic and necrosis due to the tumor was undeniable.

The lung is the most frequent site of cancer metastasis, and common primary sites are digestive organs, urogenital system and breast cancer, majority present as multiple and bilateral pulmonary lesions but 10% present as isolated metastasis, 30% to 40% of which are accounted for by colorectal cancer.

From the histopathological point of view, colorectal cancer has a strong tendency to develop necrosis in the tumor cell nest because high pillar-shaped atypical epithelium with spindle-shaped nucleus grows in a duct-like, cribriform manner. On the other hand, adenocarcinoma is less likely to develop major necrosis unless it is large in size or poorly differentiated. Analyzing now our case, atypical cells in the surgical specimen obtained from the nodule in the right S1b were growing, represented by the irregularly fusing glandular tubular structure and papillary structure, as a result of which necrotic materials were accumulated.
Furthermore, IHC patterns were discordant between the two contralateral pulmonary nodules. The tumor cells in the resected right S1b nodule were positive for CDX2 and negative for TTF-1 while the surgical specimen previously obtained from the left S1+2b was negative for CDX-2 and positive for TTF-1.

Our experience enlightened us that for cases suspected to be malignant but with equivocal results on histology and cytology of bronchoscopy samples, gene mutation analysis might be of help in clinching the diagnosis. In advanced cancer, it is common to perform gene mutation analysis on tissues obtained by transbronchial biopsy for targeted therapy. However, histological diagnosis may be dependent on the size of the tissue obtained as well as the presence of a direct bronchus leading to the lesion. This case further suggests that gene mutation analysis is feasible even for samples with scant amount of malignant cells, be it a tissue specimen or a liquid specimen obtained by bronchoscopy. Therefore it can be considered as a useful ancillary diagnostic tool in determining the presence of malignancy.

Conclusion

Even when histological diagnosis of a small specimen obtained by TBB was difficult, search for gene mutation, such as KRAS, in the liquid specimen was useful for diagnosis. Thus, improvements in diagnostic tests using liquid specimen are highly expected in the future.

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Disclosure Statement

The authors have no conflicts of interest.

References