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LETTER TO THE EDITOR

Next-generation sequencing analyses using biopsy forceps and cytology brush rinse fluids for lung cancer genotyping: Report of five cases

Genotype-directed cancer therapy is assisting the management of non-small cell lung cancer (NSCLC) in patients with actionable molecular alterations. Epidermal growth factor receptor (*EGFR*) mutation analysis has played an important role in the treatment of NSCLC. However, in 7.5% of patients *EGFR* mutation was impossible to detect with a transbronchial lung biopsy (TBLB) because of low-tumor cell content. In addition, repeated TBLBs should be avoided when collecting specimens for genotyping, given the higher risk of developing complications.[1] Recently, the targeted application of next-generation sequencing (NGS) technology has enabled the detection of specific mutations that can provide treatment opportunities for lung cancer patients, whereas rejection rates resulting from the use of inappropriate TBLB specimens are presumed to be similar to those experienced with *EGFR* mutation analysis. Taking these factors into consideration, we have developed a method that employs cryopreserved samples from biopsy forceps and cytology brush rinse fluids for NGS analyses, when the tumor cells were not histologically detected in the TBLB specimens. In this study, we report our experience with NGS analyses in five cases where we used cryopreserved samples obtained from biopsy forceps and cytology brush rinse fluids.

At Dokkyo Medical University Hospital between August 2019 and March 2020 there were 66 instances of NGS analysis using endobronchial ultrasound with guide-sheath guided TBLB specimens for NSCLC patients. There were also five cases of NGS using cryopreserved samples from biopsy forceps and cytology brush rinse fluids due to inappropriate biopsy specimens. Whenever TBLB was undertaken at our hospital, cytological examinations, namely stamp cytology, brush cytology, and bronchoalveolar lavage cytology, were also performed at the same time. We obtained TBLB specimens that were originally prepared from 10% buffered formalin-fixed, paraffin-embedded tissue according to our routine hospital procedure. By contrast, the biopsy forceps and cytology brushes were thoroughly washed with 20 ml of physiological saline solution. All the biopsy forceps and cytology brushes that we used were washed in the same physiological saline solution each time to increase the cell yield of the rinse fluid. Cell pellets were obtained by centrifugation for 5 min at 3000*g* and 4°C and washed twice with physiological saline solution. The cell pellets were stored as cryopreserved samples in a −80°C freezer. In cases where the diagnosis could not be confirmed histologically with TBLB specimens and cytologically detected tumor cells, the cryopreserved samples were subjected to NGS analysis.

For the NGS analysis we used the Oncomine Dx Target Test (Ion Torrent PGM Dx Sequencer, Thermo Fisher Scientific) performed by MUQS Laboratory (SRL Inc. SRL Hachioji, Japan). DNA was extracted using the Quant-iT dsDNA HS Assay Kit (Invitrogen). The extracted DNA was quantified using fluorometric quantification with a Qubit 3.0 Fluorometer (Thermo Fisher Scientific). RNA was extracted using a Quant-iT RNA Assay Kit (Invitrogen). The quantity of extracted RNA was measured by fluorometric quantification with a Qubit 3.0 Fluorometer (Thermo Fisher Scientific). The tests were conducted using the Ion Torrent PGM Dx platform, and the panel included 46 genes.[2] All procedures conducted with human participants were performed in accordance with the ethical standards of the appropriate institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards. Approved written informed consent was obtained from the patients and their families. This study was approved by the Ethics Committee of Dokkyo Medical University Hospital (approval no. R-41-2).

Table 1 summarizes the results of the NGS analysis of five cases using a cryopreserved sample obtained from the rinse fluid of biopsy forceps and cytology brushes. All five cases were successfully sequenced by NGS. Cytologic diagnoses revealed NSCLC in three cases and adenocarcinoma in two cases, although histologic diagnoses revealed atypical cells in four cases and there was insufficient material in one case. The DNA concentration could be measured in all five cases, and the median concentration was 16.4 ng/ul (3.3–16.9). The RNA concentration could be measured in three cases, and the median concentration was 1.0 ng/ul (0.0–2.8). In one case a genetic alteration was detected, and *MET* exon 14 skipping mutation was observed.

TBLB provides a definitive histological diagnosis for most lung cancer patients, although it is an invasive diagnostic technique whose repetition is not recommended.[1] NGS analysis is also very expensive. It is well known that combining TBLB with cytological procedures such as stamp cytology, brush cytology, and bronchoalveolar lavage cytology increase the probability of a pathological diagnosis. In

TABLE 1 Results of next-generation sequencing analysis for lung cancer using biopsy forceps and cytology brush rinse fluids

Case	Age (years)	Sex	Histologic diagnosis	Cytological diagnosis	DNA concentration (ng/ul)	RNA concentration (ng/ul)	Genotype
1	82	Male	Atypical cells	NSCLC[a]	10.3	2.8	*MET* exon 14 skip mutation
2	82	Male	Atypical cells	NSCLC	16.9	0.0	Not found
3	70	Male	Insufficient material	Ad[b]	17.6	1.9	Not found
4	84	Male	Atypical cells	Ad	3.3	0.0	Not found
5	82	Female	Atypical cells	NSCLC	16.4	1.0	Not found

[a]NSCLC, non-small cell lung cancer.
[b]Ad, adenocarcinoma.

addition, Rosell et al. reported that the cytology of bronchial biopsy rinse fluid enhances the bronchoscopy diagnostic yield for malignant endobronchial tumors.[3] Accordingly, we considered the NGS analysis of the rinse fluid of biopsy forceps and cytology brush to be possible without prolonging procedures and increasing costs.

The DNA quantity is an important factor as regards the success of NGS analysis. It has also been reported that NGS with cytology samples can be performed successfully in solid tumor samples even if the quantity of DNA is less than the manufacturer's recommended threshold.[4] Furthermore, the success of NGS analysis is affected not only by the DNA quantity but also by the DNA quality. Cryopreserved samples have been shown to provide high quality DNA for NGS analysis.[5] Our results suggested that cryopreserved samples prepared from the rinse fluid of biopsy forceps and cytology brushes could ensure sufficient DNA quantity and quality for NGS analysis.

In summary, we performed NGS analyses using biopsy forceps and cytology brush rinse fluid in five cases where TBLB specimens failed to diagnose NSCLC histologically and cytology was used to confirm the presence of tumor cells. As a result, sufficient DNA concentration was obtained, and in one case, a specific genetic alteration was found. TBLB is an invasive procedure that whose repetition is not recommended. NGS analyses using biopsy forceps and cytology brush rinse fluid may aid in the detection of specific mutations indicating lung cancer, when TBLB is unable to provide a definitive histological diagnosis.

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CONFLICT OF INTEREST

There are no conflicts of interest regarding the publication of this article.

AUTHOR CONTRIBUTIONS

Yoshimasa Nakazato and Kazuyuki Ishida were responsible for the acquisition and interpretation of patient data and manuscript preparation. Ryo Arai and Seiji Niho participated in interpretation of patient clinical data. Hiromi Machida and Yukimi Horii performed pathological examination. Masato Onozaki, Kensuke Ohikata, and Yuko Kaneko performed pathological diagnoses and critically revised the manuscript. All the authors approved the final manuscript.

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