Comparison of EGFR mutation of surgically resected primary lung cancer and metastatic lymph node obtained by Endobronchial ultrasound-guided transbronchial needle aspiration

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Introduction

The presence of a mutation of the EGFR gene is a strong predictor of a better outcome with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs). In advanced lung cancer with mediastinal and/or hilar lymph node metastasis, EBUS-TBNA can be performable for multiple passages for a target lymph node to obtain samples for both cytological diagnosis and DNA mutation analysis. We retrospectively investigated EGFR mutation status of both EBUS-TBNA samples and surgically resected primary tumors with the use of peptide nucleic acid-locked nucleic acid polymerase chain reaction (PNA-LNA PCR) Clamp methods, which was reported to be more

sensitive than conventional direct sequencing in order to validated the usefulness of EBUS-TBNA sample for EGFR mutation analysis.

Methods

Patients and EBUS-TBNA tissue sampling

From April 2006 to October 2009, the consecutive 14 cases of lung cancer patients who were diagnosed with mediastinal or hilar lymph node metastasis proven by EBUS-TBNA were enrolled in the study. The main tumor and metastatic lymph node were surgically resected. The EGFR genetic homologies were evaluated between the surgically resected primary tumor and the metastatic lymph node obtained by EBUS-TBNA.

DNA extraction and EGFR mutation analysis

DNA was extracted using QIAamp DNA Blood Kit from all fresh samples. The pinpoint DNA extraction was performed with DEXPAT.

PNA-LNA PCR Clamp assay

EGFR mutation was examined for EGFR mutations by means of the PNA-LNA PCR clamp method. This method results in preferential amplification of the mutant sequence, which is then detected by a fluorescent primer that incorporates locked nucleic acids to increase the specificity.

PCR-based Direct Sequencing for exons 19 and 21

EGFR mutations were examined using PCR-based direct sequencing for exons 19 and 21.

Immunohistochemistry for surgically resected tissue samples

The primary antibodies used were mouse monoclonal antibodies against thyroid transcription factor-1 (TTF-1), surfactant-associated protein A (PE-10), and anti-cytokeratin 7 (CK7).

Results

EGFR mutation analysis of all 14 EBUS-TBNA samples showed wild-type EGFR in 11 cases, exon21: L858R mutation in 3 cases. EGFR status obtained from EBUS-TBNA specimen was identical to that of primary tumor in 13 cases among the 14 surgically treated cases (92.9%). One case of large cell carcinoma exhibited the contradictory results. EGFR mutation was negative in the

specimen obtained from the metastatic lymph node by EBUS-TBNA, both the surgically removed primary tumor and the surgically removed metastatic subcarinal lymph node exhibited the positive EGFR mutations.

In this case, further investigation of the surgically resected metastatic lymph node sample clarified that the metastatic lymph node includes the small number of cancer cells with immunologically epithelial differentiations with positive expression for PE-10 and TTF-1 protein. Pin-point DNA extraction from the surgically removed metastatic lymph node which had been targeted by EBUS-TBNA revealed that area A exhibited positive for EGFR mutation including two overlapping peaks of guanine and adenine which means both wild type and L858R co-existed in the area, whereas only wild type EGFR gene was detected in DNA extracted from area B; the part of tumor which were negative for neither PE-10 or TTF-1.

Discussion

For one case, EBUS-TBNA lymph node sample exhibited wild-type EGFR, the surgically removed primary tumor exhibited L858R mutation. The result suggested that the population of EGFR mutation might be different depending on the sampling part of the tumor.

We confirmed that the result supports that more sensitive methods for detecting EGFR mutations than direct sequencing method would be required for EGFR mutation analysis of small amount of specimen with few number of EGFR mutation positive cancer cells. PNA-LNA clamp method is considered as one of such highly sensitive method that is capable of detecting EGFR mutation of one cancer cell in 100 cells of wild type DNA.

Since the assessment of EGFR mutation has been widely used for guidance in the clinical management of lung cancer, extreme care must be taken when working with small amounts of tissue sample. The occurrence of artifacts may be prevented with the use of larger amounts of template DNA, so it would be desirable to collect the multiple passage of samples as possible in EBUS-TBNA.

Conclusion

The EGFR mutations in non-small cell lung cancer can be accurately detected by the combination of EBUS-TBNA tissue sampling from the metastatic lymph node and PNA-LNA clamp EGFR mutation analysis method. Heterogeneity of EGFR mutation status in the metastatic lesion should be taken into consideration in EGFR mutation diagnosis.