ORIGINAL ARTICLE

Heterogeneity of EGFR Aberrations and Correlation with Histological Structures: Analyses of Therapy-Naive Isogenic Lung Cancer Lesions with *EGFR* Mutation

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ABSTRACT

Introduction: *EGFR* gene somatic mutation is reportedly homogeneous. However, there are few data regarding the heterogeneity of expression of mutant EGFR protein and *EGFR* gene copy number, especially in extrathoracic lesions. These types of data may enhance our understanding of the biology of *EGFR*-mutated lung cancer and our understanding of the heterogeneous response patterns to EGFR TKIs.

Methods: An 81-year-old never-smoking female with lung adenocarcinoma could not receive any systemic therapy because of her poor performance status. After her death, 15 tumor specimens from different sites were obtained by autopsy. Expression of mutant EGFR protein and *EGFR* gene copy numbers were assessed by immunohistochemical analysis and by silver in situ hybridization, respectively. Heterogeneity in these EGFR aberrations was compared between metastatic sites (distant versus lymph node) or histological structures (micropapillary versus nonmicropapillary).

Results: All lesions showed positive staining for mutant EGFR protein, except for 40% of the papillary component in one of the pulmonary metastases (weak staining below the 1+ threshold). Expression of mutant-specific EGFR protein, evaluated by H-score, was significantly higher in the micropapillary components than in the nonmicropapillary components (Mann-Whitney *U* test, $p = 0.014$). *EGFR* gene copy number was quite different between lesions but not correlated with histological structure or metastatic form. However, *EGFR* gene copy numbers were similar between histological structures in each lesion.

Conclusion: These data indicate that expression of EGFR mutant protein and EGFR gene copy number do not change as a consequence of tumor progression. This also justifies using the biopsy specimens from metastases as a surrogate for primary tumors.

Keywords: Mutant-specific antibody; Silver in situ hybridization; Autopsy; Micropapillary; Lung adenocarcinoma

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Introduction

Lung adenocarcinoma with the *EGFR* gene mutation is a clinically and therapeutically distinct molecular subtype in NSCLCs.[1] To date, several groups have analyzed the heterogeneity of the *EGFR* mutation status, as summarized in a recent review.[2] Although a certain number of studies have reported the heterogeneous distribution of the *EGFR* mutations, at the present time, most of the researchers and clinicians believe that *EGFR* mutation status is usually homogeneous and the low *EGFR* gene copy number and the high rate of contaminated noncancerous cells sometimes cause failure to detect the *EGFR* mutation.[3]

A smaller number of studies, on the other hand, have reported that *EGFR* gene copy number[4–6] is heterogeneous between lesions in a patient, although these studies did not focus on the *EGFR*-mutated lung cancers. In a recent literature search, no data were found regarding the intertumor heterogeneity in expression of mutant EGFR protein. These data might contribute to our understanding of *EGFR*-mutated lung cancer biology and enhance our understanding of the heterogeneous response patterns to EGFR tyrosine kinase inhibitors (TKIs).

Currently, EGFR TKI treatment is considered the first choice for patients with advanced *EGFR*-mutated NSCLCs, and treatment is thought to potentially alter *EGFR* gene copy number status and/or mutant EGFR protein expression status.[7–11] Therefore, it is very challenging to ascertain the natural status of intertumor heterogeneity of these EGFR aberrations. In this study, we examined specimens from the autopsy of a patient with *EGFR*-mutated lung adenocarcinoma who did not receive systemic therapy because of her poor performance status. Each metastatic lesion was analyzed for EGFR aberrations and compared. All the lesions were independently examined for both micropapillary and nonmicropapillary histological components.

Materials and Methods

Clinical Course of the Patient

An 81-year-old never-smoking female with clinical stage IIIA lung adenocarcinoma (*EGFR* exon 19 deletion [del]) was treated by thoracic radiotherapy (objective response: stable disease). Eighteen months later, brain and pulmonary metastases developed in the patient, but she did not receive systemic therapy (including EGFR TKIs) because of her poor performance status. After her death, tumor specimens were obtained by autopsy in accordance with ethical guidelines and written informed consent from her legal guardians. Approval for the use of tumor specimens for the current analyses was obtained from the institutional review boards of Kinki University Faculty of Medicine and Higashihiroshima Medical Center.

Immunohistochemical Analysis

Paraffin-embedded tumor tissue was sectioned at a thickness of 4 μm and mounted on glass slides. All staining was performed on the Benchmark XT automated stainer (Ventana Medical Systems, Inc., AZ). For detection, a mutation-specific antibody to EGFR that recognizes the del E746_A750 mutation in exon 19 (XP Rabbit mAb #2085 [Cell Signaling Technology, Danvers, MA]) was used. The staining platform utilized Ultraview development reagents (Ventana Medical Systems). Specimens were evaluated using the H-score assessment, which combines staining intensity (0–3) and the percentage of positive cells (0%–100%) as previously described.[12] For each specimen, the individual intensity level was multiplied by the percentage of cells and all values were added to obtain the final IHC score, which ranged from 0 to 300. The micropapillary component and nonmicropapillary component were assessed independently in each lesion.

DNA Extraction and Mutation Analysis

Genomic DNA was extracted from a specimen using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Mutational status of the *EGFR* exon 19 was analyzed by a polymerase chain reaction–based method as described previously.[11]

Silver In Situ Hybridization Analysis

The *EGFR* gene copy number was analyzed using brightfield microscopy and silver in situ hybridization (SISH) technology. Probing was carried out with both EGFR-specific (#760-1216) and centromere 7–specific (#760-1219) probes according to protocols from the manufacturer by using the Benchmark XT automated stainer (Ventana Medical Systems). The assessment of gene copy number was performed independently and blinded from IHC analysis. *EGFR* gene status results were grouped according to the Colorado scoring system,[13] and classified into six main categories, including disomy, low trisomy, high trisomy, low polysomy, high polysomy, and gene amplification. The micropapillary component and nonmicropapillary component were assessed independently within each lesion. For further comparison between the micropapillary component and nonmicropapillary component within each metastatic lesion, the percentage of cells with four or more copies of *EGFR* gene was used.

Statistical Analyses

The Mann-Whitney *U* test was used to compare H-scores between histological structures. The X^2 test was used to evaluate the significance of the relationship