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CASE REPORT

Molecular biological analysis in a patient with multiple lung adenocarcinomas

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Abstract

The utility of molecular biological analysis in lung adenocarcinoma has been demonstrated. Herein we report a rare case presenting as multiple lung adenocarcinomas with four different *EGFR* gene mutations detected in three lung tumors. After opacification was detected by routine chest X-ray, the patient, a 64-year-old woman, underwent chest computed tomography which revealed a right lung segment S4 ground-glass nodule (GGN). Follow-up computed tomography revealed a 42 mm GGN nodule with a 26 mm nodule (S6) and a 20 mm GGN (S10). Histopathology of resected specimens from the right middle and lower lobes revealed all three nodules were adenocarcinomas. Four *EGFR* mutations were detected; no three tumors had the same mutations. Molecular biological analysis is a promising tool for the diagnosis of primary tumors in patients with multiple lung carcinomas of the same histotype, enabling appropriate treatment.

Introduction

Adenocarcinoma is the most prevalent histotype of primary lung cancer (PLC), accounting for approximately 60% of all lung cancer cases.[1] Genetic abnormalities in pulmonary adenocarcinoma include *ALK* fusion genes, proto-oncogene tyrosine-protein kinase 1 fusion genes, and *EGFR* mutations.[2] *EGFR* mutations are common in East Asians, women, and non-smokers.[3] A 2015 large-scale meta-analysis conducted in Japan reported that 45% (range 21–68%) of Japanese patients with lung adenocarcinoma harbored *EGFR* mutations.[4]

In cases of multiple lung cancer of the same histotype, it can be difficult to determine the primary tumor. This report describes a rare case of multiple primary lung adenocarcinomas where different *EGFR* gene mutations were detected from three tumors in the same right lung field. This case highlights the utility of molecular biological analysis in multiple lung cancers of the same histotype.

Case report

A 64-year-old woman underwent chest computed tomography (CT) after opacification was detected on chest X-ray during a routine health check. CT revealed a 22 mm ground-glass nodule (GGN) in segment S4 of the right lung, but the nodule was monitored without intervention.

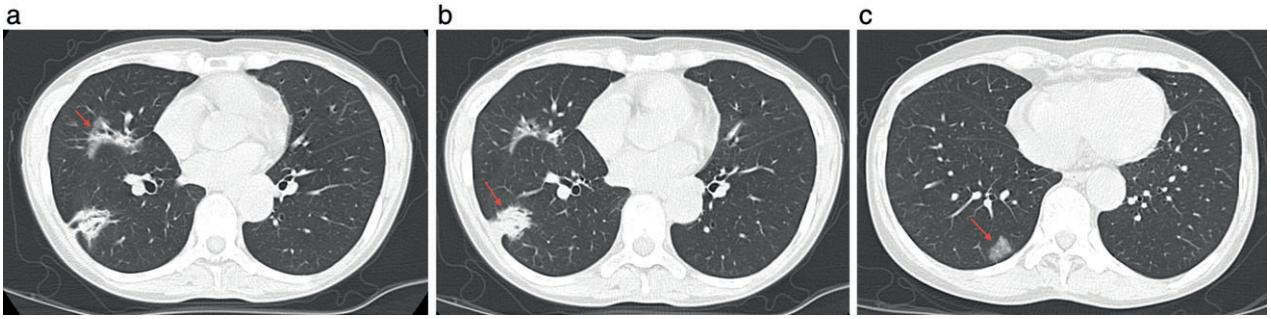

Figure 1 Plain chest computed tomography scan at the patient's first visit to our department. (**a**) The right lung shows a 42 mm irregular part-solid ground glass nodule with well-defined margins adjacent to the interlobar region in the area where a smaller mass was detected two years earlier. (**b**) A 26 mm solid nodule with pleural indentation in S6; and (**c**) a 20 mm ground glass nodule in S10 with no hilar or mediastinal lymphadenopathy. Red arrows indicate the tumors.

She was referred to our department when another routine chest X-ray in 2016 showed enlargement of the GGN. Chest CT revealed a 42 mm irregular part-solid GGN with well-defined margins adjacent to the interlobar region in the area where the right S4 GGN was detected two years earlier (Fig 1a). The right lung showed a new 26 mm solid nodule with pleural indentation in S6 (Fig 1b) and a new 20 mm GGN in S10 (Fig 1c) with no hilar or mediastinal lymphadenopathy.

The patient had no remarkable past medical or occupational history and no history of smoking. Adenocarcinoma was diagnosed based on transbronchial biopsy of the S4 GGN and S6 nodule. Positron emission tomography and head magnetic resonance imaging were performed to detect systemic metastases, and revealed no findings suggestive of extrapulmonary metastasis. These results could indicate triple lung cancer (cT2aN0M0) or ipsilateral pulmonary metastasis (cT4N0M0, with the T4 nodule being an ipsilateral metastasis in a different lobe). Blood counts and biochemical tests showed no abnormal findings. Preoperative tumor marker levels were: carcinoembryonic antigen 8.1 ng/mL, stage-specific embryonic antigen-1 37 U/mL, squamous cell carcinoma antigen < 0.5 mg/mL, pro-gastrin-releasing peptide 55.4 pg./mL, and neuron-specific enolase 17.1 ng/mL.

Right middle and lower lobectomy with hilar and mediastinal lymph node dissection was performed at the Department of Thoracic and Cardiovascular Surgery at our hospital. In accordance with World Health Organization (WHO) Classification, the S4 tumor (Fig 2a) was subclassified as invasive adenocarcinoma, the S6 tumor (Fig 2b) as minimally invasive adenocarcinoma of the acinar and papillary type, and the S10 tumor (Fig 2c) as minimally invasive adenocarcinoma.[5] We considered the possibility of using this classification (a modification of the WHO classification), as our diagnostic criteria for primary lung adenocarcinoma, so we screened for *EGFR* using specimens from each of the three tumors. Four *EGFR* mutations were detected, and no three tumors had the same mutations (Table 1). *ALK* fusion genes were not detected in any of the tumors by immunohistochemical (IHC) analysis. Each tumor had different *EGFR* mutations and each was

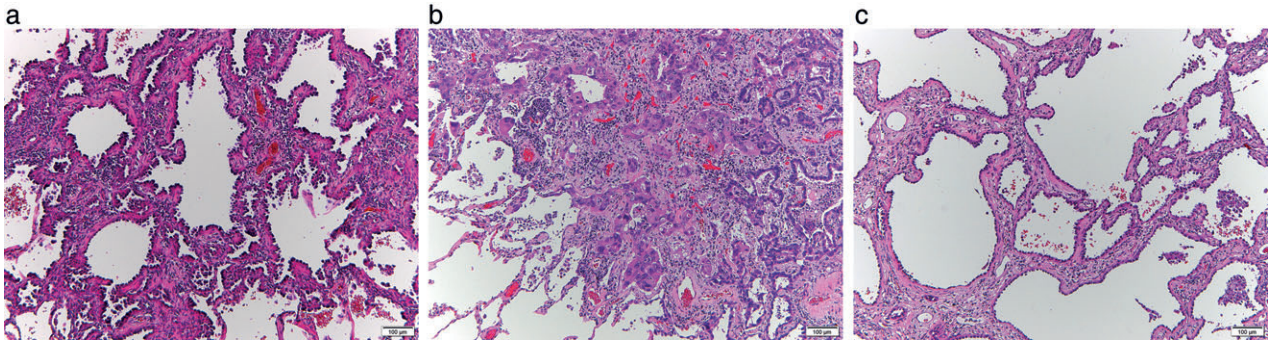

Figure 2 Hematoxylin and eosin stains from histopathological analysis of lung tissue obtained from the right S4, S6, and S10 tumors. (**a**) The S4 tumor was subclassified as invasive adenocarcinoma (36 × 15 mm, papillary pattern predominant: papillary 80% > lepidic 20%); (**b**) the S6 tumor as minimally invasive adenocarcinoma (23 × 25 mm, acinar and papillary pattern predominant) of the acinar and papillary type because both were mixed and had poorly defined margins (80% > lepidic 15%, solid 15%); and (**c**) the S10 tumor as minimally invasive adenocarcinoma (18 × 11 mm, lepidic pattern predominant: lepidic 80% > papillary 20%). Scale bar indicates 100 μm.

Table 1 Results of screening for EGFR mutations with PCR using surgical specimens from each of the three tumors

	Lung Sections		
EGFR mutations	S4	S6	S10
Exon 18 6719X	ND	ND	Detected
Exon 19 deletions	ND	Detected	ND
Exon 20 57 681	ND	ND	ND
Exon 20 insertions	ND	ND	ND
Exon 20 T790M	ND	ND	ND
Exon 21 L858R	Detected	ND	ND
Exon 21 LtitiliQ	ND	ND	Detected

ND, not detected.

classified as a primary tumor. Tumors were staged individually according to the WHO Classification as pT2aN0M0 (stage IB S4 tumor), pT1bN0M0 (stage IA S6 tumor), and pT1aN0M0 (stage IA S10 tumor). Adjuvant chemotherapy was deemed necessary for the stage IB tumor according to postoperative treatment guidelines;[5] oral tegafur/uracil was initiated. The cancer has not recurred 21 months after resection. Written informed consent for this publication was obtained from the patient.

Discussion

The most common type of *EGFR* mutation is exon 19 deletion, followed by codon 858 of exon 21 (L858R) point mutation. These two mutations account for 90% of all *EGFR* mutations observed.[3] We detected these mutations in the S4 (exon 21 L858R) and S6 (exon 19 deletion) tumors in our case. Other rare mutations include codon 719 of exon 18 (G719X) point mutation, and exon 20 (S768I, insertion, T790M) and exon 21 L861Q insertions.[6] We detected a rare compound *EGFR* mutation comprising exons 18 G719X and 21 L861Q in the S10 tumor in our case. Kobayashi *et al.* reported that 13.75% of 79 patients with non-small cell lung cancer with *EGFR* mutations had two *EGFR* mutations, and 1.25% had a compound mutation comprising exons 18 G719X and 21 L861Q.[7] Few reports have described three lung tumors with four different *EGFR* mutations, as observed in the present case. Most recently, Rafael *et al.* detected seven bilateral adenocarcinomas, with genetic analysis of three right lung tumors revealing two different *KRAS* mutations in two of the tumors and *EGFR* mutation in the third.[8] Yamazaki *et al.* reported the association of synchronous multiple lung cancer diagnosed as adenocarcinoma with *EGFR* mutation in 72.2% of cases,[9] consistent with the present case. Important characteristics of driver mutations in lung adenocarcinoma have been reported.[8] The majority of driver mutations described in lung adenocarcinoma are mutually exclusive clonal events. In our case, we think that the driver mutations were present at three places in the lung tissue almost simultaneously. Independently arising adenocarcinomas are expected to have different mutational profiles, whereas metastatic tumors are expected to share the same driver mutation.[8] Many lung adenocarcinomas exhibit varying admixtures of histological features and are thus subclassified into histotypes.

The 2015 WHO Classification broadly classifies lung adenocarcinomas into preinvasive lesions, minimally invasive adenocarcinoma, invasive adenocarcinoma, and invasive adenocarcinoma variant.[5] In our case, two tumors were minimally invasive adenocarcinomas (lepidic pattern predominant and acinar/papillary pattern predominant) and one was invasive adenocarcinoma (papillary pattern predominant). *EGFR* mutations occur more frequently in tumors with papillary and lepidic growth patterns, *KRAS* mutations in mucinous carcinomas, and *ALK* fusion genes in tumors with solid and acinar growth patterns with signet ring cell features.[10] Lung adenocarcinoma is a highly heterogeneous disease, and comprehensive histologic subtyping cannot be used to reliably predict the underlying genetic differences among tumors.[10] Thus, Stella *et al.* recommend extensive molecular profiling in cases with multiple lung cancers, as it might clarify the association between the lung lesions found.[11] Several investigators have reported the utility of molecular biological analysis for diagnosis and treatment in multiple primary cancers, not only in lung cancer[12] but also breast,[13] gastric,[14] hepatocellular,[15] renal,[16] and colorectal cancers.[17] Thus, molecular biological analysis is necessary when multiple cancers of the same histotype are identified in the same tissue where there are multiple primary tumors.

In conclusion, when different genetic mutations are found in multiple lung tumors, molecular biological analysis enables diagnosis of the primary lung cancer and can also help to distinguish primary from metastatic lung cancer. In addition, analysis of a larger number of various genetic mutations will facilitate improved diagnosis and treatment. Molecular biological analysis appears to be a useful tool for the diagnosis of primary tumors in patients with multiple lung adenocarcinomas, enabling appropriate treatment.

Disclosure

No authors report any conflict of interest.

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