Identification of Epidermal Growth Factor Receptor Mutations and Anaplastic Lymphoma Kinase Gene Rearrangement in Lung Adenocarcinomas

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ABSTRACT

Objective: The identification of molecular alterations has an important therapeutic implication in lung adenocarcinoma patients. Herein, we presented our experience with the identification of epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) mutations in primary and metastatic lung adenocarcinomas.

Methods: Epidermal growth factor receptor mutations in exons 18, 19, 20 and 21 were evaluated by pyrosequencing. A total of 101 cases of lung adenocarcinomas, including 64 primary and 37 metastatic tumours were studied. Mutation analyses were performed on six cell blocks obtained from fine-needle aspiration, 64 small biopsies and 31 resection materials. For ALK gene rearrangement, 23 of 101 cases with no EGFR mutation were evaluated by fluorescence *in situ* hybridization using an ALK break-apart probe.

Results: The median age of the 101 patients was 61 years (range, 33–85 years). Among the patient population, 19 patients were female. Epidermal growth factor receptor mutations were found in 12 of 101 patients (11.8%), with five primary tumours (41.7%) and seven metastatic tumours (lymph node, pleura, brain, liver; 58.3%), and four of these 12 patients (33.3%) were female. A total of six patients with delE746-A750 (exon 19), three patients with delE747-A750insP (exon 19), two patients with L858R (exon 21), and 1 patient with Gly719Ser; L861Q (exon 18; 21) were noted. ALK gene rearrangement was evident in 4 of 23 patients (17.4%): one (25%) was female and had primary tumour and three (75%) were male and had metastatic tumours.

Conclusion: Metastatic adenocarcinomas demonstrated EGFR mutation more than primary tumours; therefore, repeating molecular testing in metastatic lung adenocarcinomas may uncover newly acquired molecular alterations.

Keywords: Anaplastic lymphoma kinase mutation, epidermal growth factor receptor mutation, lung adenocarcinoma

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INTRODUCTION

Non-small cell lung cancer (NSCLC) constitutes about 80% of lung cancers and among them, adenocarcinomas have increasing trend. More than 70% of patients are diagnosed with advanced stage disease; therefore, systemic treatment plays a central role in the clinical management of adenocarcinomas. Besides platinum-based chemotherapy recently, several targeted agents were introduced for the treatment of advanced patients. Gefitinib and erlotinib are selective reversible epidermal growth factor receptor - tyrosine kinase inhibitors.

Mutations in the epidermal growth factor receptor (EGFR) gene are critical determinants of tumour cells in the response to epidermal growth factor receptor - tyrosine kinase inhibitors. Therefore, it is very important to define the status of EGFR mutations in tumours to predict response to epidermal growth factor receptor - tyrosine kinase inhibitors treatment (1, 2).

Anaplastic lymphoma kinase (ALK) was first identified as a fusion partner in t (2;5) chromosomal translocation. Fusion of ALK with a variety of partner genes results in the expression of oncogenic chimeric proteins that lead to constitutive activation of the ALK kinase domain and the downstream signaling pathways. In lung cancer, fusion of ALK and the echinoderm microtubule-associated protein-like 4 (EML4) was identified. Rearrangements of ALK gene in adenocarcinomas define a molecular subgroup of tumours characterized clinically by sensitivity to ALK tyrosine kinase inhibitors such as crizotinib (3–5).

In the current study, we presented our experience on Turkish population (Mediterranean region) with the identification of EGFR and ALK gene rearrangement using tissue specimens of primary and metastatic lung adenocarcinoma.

SUBJECTS AND METHODS

Patients diagnosed with lung adenocarcinoma and the tumour specimens subjected to EGFR mutational analysis in Akdeniz University Hospital between 2011 and 2013 were included in this study. A total of 101 consecutive cases of lung adenocarcinoma were retrieved from molecular diagnostic unit, Department of Pathology, Akdeniz University School of Medicine, Antalya. Consultation cases (diagnosed elsewhere) which are subjected to EGFR mutational analysis were excluded.

Epidermal growth factor receptor mutations were detected from genomic DNAs of tumour cells were isolated from formalin - fixed paraffin embedded tumour tissues / cell blocks using a QIAamp DNA FFPE Tissue Kit (Qiagen). PCR was performed with 15 ng of genomic DNA. Mutations in exons 18–21 of the EGFR gene were detected by pyrosequencing. The pyrosequencing was performed on a PyroMark Q24 system (Qiagen) according to manufacturer's instructions and analysed in AQ mode of the PyroMark software. Among studied 101 – adenocarcinoma tumour tissues / cells, 64 were obtained from primary side of tumour and 37 were obtained from several metastatic organs and regions including: 14 – brain, ten – lymph node, five – liver, four – pleura, three – bone and one – thyroid. Epidermal growth factor receptor mutation analysis was performed on six cell blocks gathering from fine needle aspiration, 64 – small biopsies and 31– resection materials.

For ALK gene rearrangement, 23 of 101 cases with no EGFR mutation were evaluated by fluorescence *in situ* hybridization (FISH). Fluorescence *in situ* hybridization analysis was performed on the formalin-fixed paraffin-embedded tumour tissues using a break-apart probe specific to the ALK locus (Vysis LSI ALK Dual Color, break-apart rearrangement probe; Abbott Molecular, Abbott Park, IL) according to the manufacturer's instruction. Anaplastic lymphoma kinase FISH was considered positive when more than 15 % of 100 or more analysed cells showed splitting of the fluorescent probes flanking the ALK locus. Among 23 tumour

tissue, 15 were gathered from primary side of tumour and eight were obtained from several metastatic organs and regions including four – brain, two – lymph node, one – liver and one – soft-tissue.

Statistical analyses

The patient demographic data and clinicopathologic characteristics were obtained from pathology reports. Statistical analyses were performed using the Statistical Software Package for the Social Sciences, version 15.0 for Windows (SPSS Inc, Chicago, IL). Differences in proportions for categorical variables were compared using X^2 tests or Fisher's exact test as appropriate, while the *t*-test was used for difference in means. A result was considered significant if the *p*-value was < 0.05.

RESULTS

The median age of 101 patients was 61 years (range, 33–85 years.) Among them, 19 patients were female and 82 were male (F/M=1/4).

Twelve of 101 cases (11.8 %) had EGFR mutations in tumour DNA samples by pyrosequencing. The median age of 12 EGFR mutated patients was 62.5 years (range, 33–82 years.) Four of mutated patients were female (F/M: 1/3). Among them, five mutations (41.7 %) were detected in tumours obtained from primary site. The rest of seven mutations (58.3 %) were detected in tumours obtained from metastatic sites including lymph node, pleura, brain and liver.

Nine patients had exon 19 mutation, six case with delE746 - A750 and three cases with delE747 - A750insP. Two patients had L858R mutation in exon 21. One patient had two different mutations localized in exon 18, Gly719Ser and in exon 21, L861Q (Fig. 1).

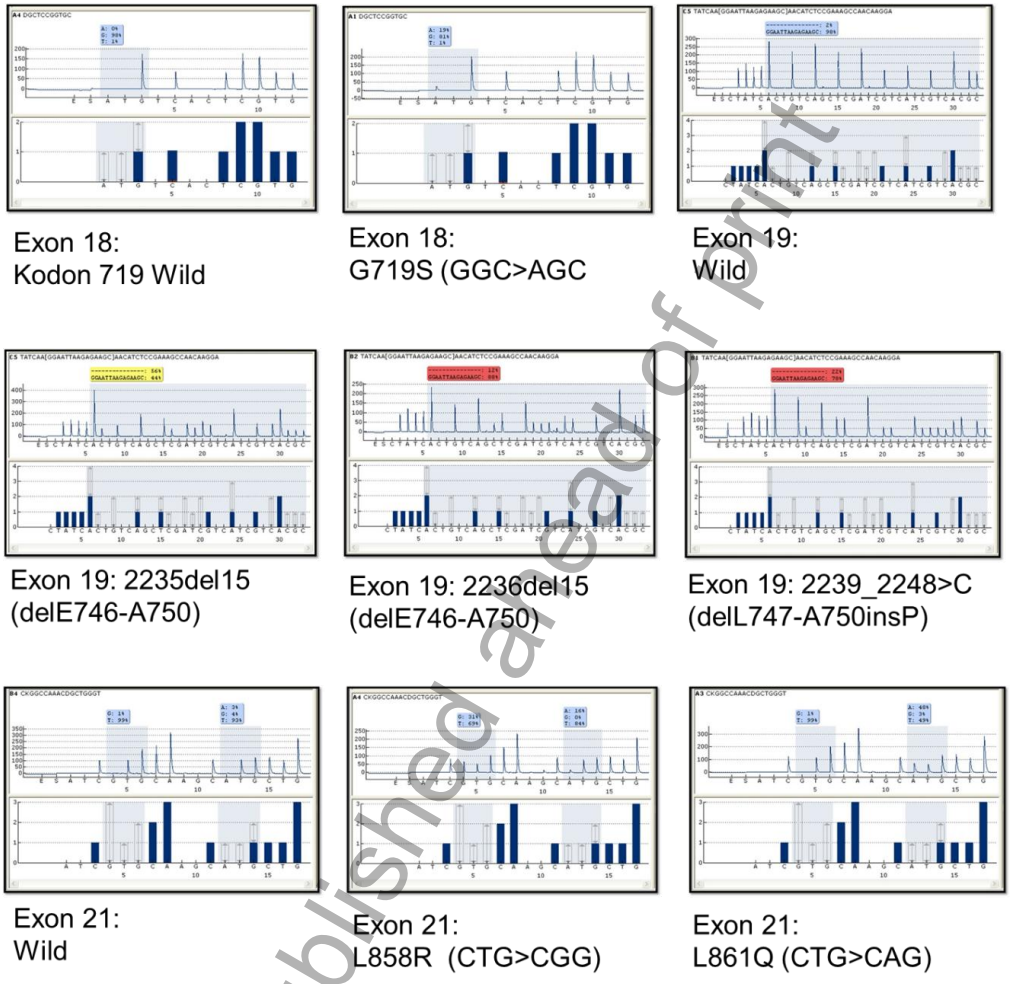

Fig. 1: Results of pyrosequencing for EGFR mutations in tumour DNA samples.

Anaplastic lymphoma kinase gene rearrangement was found in 4 of 23 cases (17.4%) without EGFR mutations. The median age of ALK mutated patients was 61 years (range, 33–84 years.) One of them was female (F/M=1/4). Among ALK mutated patients, one mutation (25%) was detected in primary tumour tissue. The rest of three mutations (75%) were detected in tumours obtained from metastatic sites including lymph node (2 cases) and brain [1 case] (Fig. 2).

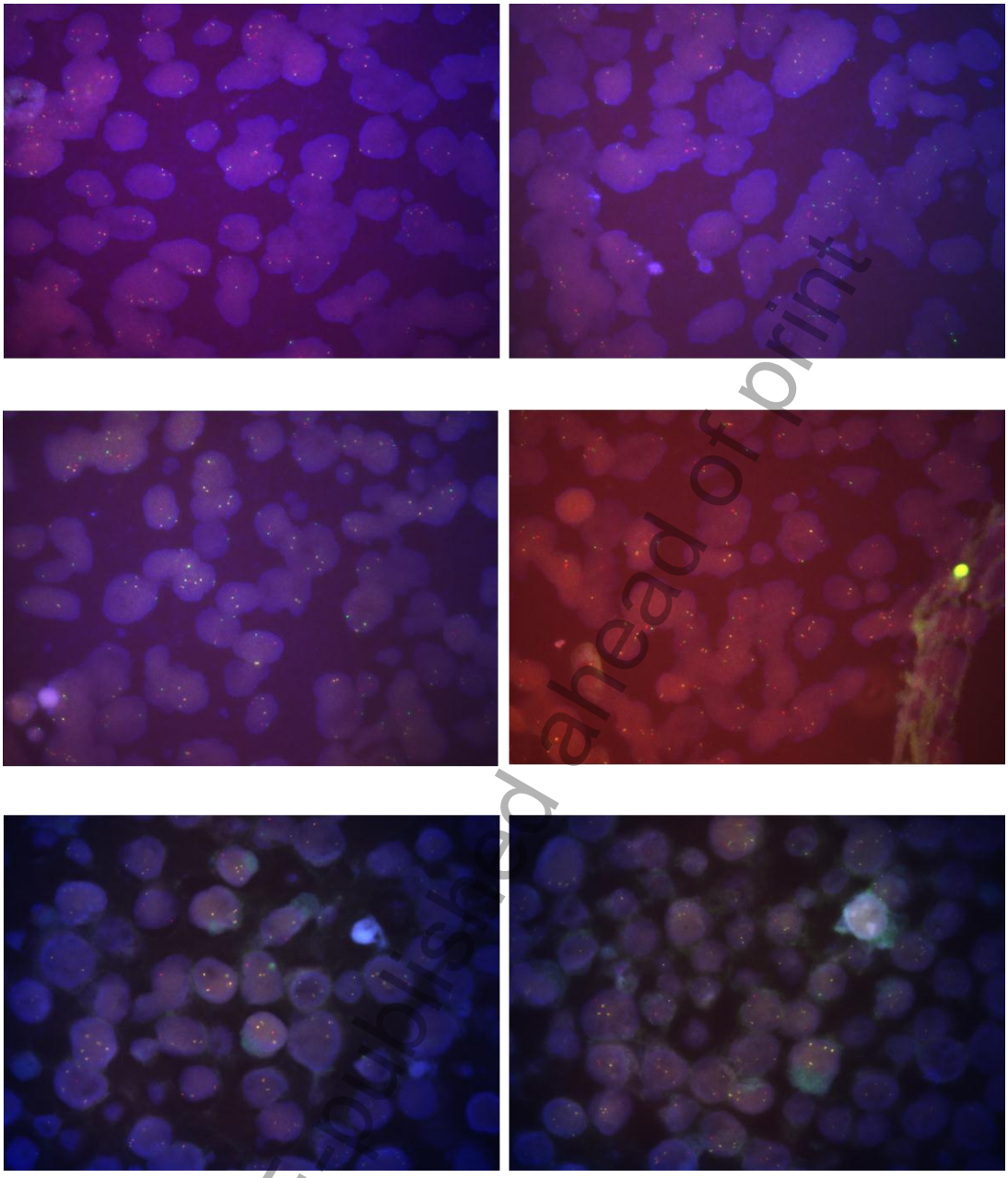

Fig. 2: ALK gene rearrangements were demonstrated (Vysis LSI ALK Dual Color, breakapart rearrangement probe).

DISCUSSION

In routine practice, to identify genotype-specific subsets of patients can lead to successful treatment with appropriate kinase inhibitors and determine survival outcomes differed among genotypes. In the present study, we demonstrated our experience on EGFR and ALK mutation analyses (6–9).

Although it has been shown that smears can be used effectively for DNA extraction for EGFR mutation assays, use of a cell block is recommended for cytology specimens and in our practice, we gained enough tumour DNA from cell blocks. Pyrosequencing is a sensitive method for the detection of mutations in DNA isolated from paraffin embedded tissues.

The previously reported EGFR mutation rates of NSCLC in the Turkish population were given 4% and 7%. The EGFR mutation rate we have found is 11.8%. This difference can be explained by the study cohort which contains only adenocarcinomas and it is known that adenocarcinomas have EGFR mutations more than other histologic subtypes of lung carcinomas. We also used only tumours that contained part of the formalin-fixed paraffin-embedded tumour tissue slides. In previous studies on the Turkish population, the slide examination and preparation was not clear. Another reason could be the detection technique we used, because in previous studies dideoxy sequencing was used for EGFR mutation analyses and it is known that pyrosequencing is superior to dideoxy sequencing in the detection of EGFR mutations (10–12).

Soda *et al* reported an inversion on chromosome arm 2p resulted in the creation of an EML4 - ALK fusion gene in lung cancer in 2007 (13). The EML4-ALK fusion gene was identified in 5 of 75 (7%) NSCLC patients examined. Subsequent studies have indicated that the prevalence of EML4 - ALK fusion gene is about 2 % to 7% of all NSCLCs with enrichment in adenocarcinomas in never or light smokers (3, 4). In this study, we found ALK gene rearrangement as 17.4%. This result is quite different from the existing data. It could be

explained by the number of patients that we were studied are low and we choose the adenocarcinoma patients without EGFR mutations.

Male predominance is evident in our study cohort (F/M=1/4). It could be the explanation of male predominance in EGFR and ALK mutated cases. In the current study, metastatic lung adenocarcinoma tissues demonstrated EGFR and ALK mutations more than the tumours obtained from primary site. We detected EGFR mutations in exons 18, 19 and 21 which are associated with TKI sensitivity. Although, we did not know the EGFR and ALK status of primary tumour tissues that we found EFGR and ALK mutations on metastatic counterparts, it seems reasonable to repeat molecular testing in metastatic lung adenocarcinomas (4, 9).

CONCLUSION

Metastatic adenocarcinomas demonstrated EGFR mutation more than primary tumours; therefore, repeating molecular testing in metastatic lung adenocarcinomas may uncover newly acquired molecular alterations.

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Author Contributions

IH Ozbudak conceived paper, oversaw data collection, conducted data analysis, wrote manuscript and approved final version. M Ozcan participated in study design, data analysis and interpretation, revised manuscript and approved final version. G Ozbilim participated in study design, data analysis, revision of manuscript and approved final version. The authors declare that they have not received any financial support and that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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