EGFR mutation testing in non-small cell lung cancer (NSCLC)

Fouad Al Dayel

Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

Received 22 January 2012; received in revised form 22 January 2012; accepted 20 September 2012

KEYWORDS
Adenocarcinoma of lung; EGFR mutation; Targeted therapy

Summary Lung carcinoma is subdivided into small cell carcinoma and non-small cell carcinoma (NSCLC). NSCLC is a heterogeneous group of carcinomas and accounts for 70–80% of lung cancer. NSCLC is further divided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.

Activating somatic mutations of the tyrosine kinase domain of epidermal growth factor receptor (EGFR) have recently been characterized in a subset of patients with non-small cell lung cancer (NSCLC). These mutations involve exons 18, 19, 20 and 21. Patients harboring these mutations in their tumors show good response to EGFR tyrosine kinase inhibitors (EGFR-TKIs). The aim of this manuscript is to provide an overview of EGFR mutations in NSCLC as well as to briefly discuss sample requirements and testing guidelines for EGFR mutation.

© 2012 Published by Elsevier Limited on behalf of King Saud Bin Abdulaziz University for Health Sciences. All rights reserved.

Introduction

As per current World Health Organization (WHO) [1], lung carcinoma is subdivided into small cell and non-small cell carcinoma (NSCLC). The latter compromise 70–80% of lung carcinoma. Although NSCLC consists of squamous cell carcinoma, adenocarcinoma and large cell carcinoma, it was considered as one group mainly because of lack of specific therapy for various histologic subtypes. Nowadays the distinction between adenocarcinoma and squamous cell carcinoma is extremely important due to availability of therapy.

There is increasing interest in adenocarcinoma of lung for various reasons. One reason is adenocarcinoma incidence is increasing (now considered to be the most predominant histologic subtype). Other reason is the potential uses of targeted therapy in cases showing EGFR mutations.

Since 1980s, many studies showed EGFR over-expression in lung carcinoma particularly squamous cell carcinoma using various techniques including immunohistochemistry. However, the significance of these over-expressions as prognostic marker...
continued to be controversial. Clinical trials revealed variability in response to tyrosine kinase inhibitor, with higher response seen in Japanese patients than European patients (27.5% vs. 10.4%). In USA, partial response was noticed in women, in non-smoker and patient with adenocarcinoma. The breakthrough took place in 2004, Lynch et al. [2] reported that activating mutations of EGFR gene kinase domain resulted in responsiveness to tyrosine kinase inhibitors (TKIs) in patients with lung adenocarcinoma. Simultaneously two independent groups reported similar results [3,4]. Up to 20% of NSCLC shows EGFR mutation and up to 80% of these patients respond to TKIs (only 10% of EGFR mutation negative cases respond to TKIs). However, most of these patients will develop resistance to treatment within one year [5]. Secondary resistance is either due to second EGFR mutation T790M, or MET amplification.

The most frequent mutations in EGFR are exon 19 deletions and exon 21 point mutation: L858R (replacement of leucine at position 858 in the protein with arginine). Mutations detection start with extracting good quality DNA followed by amplifications of exon 18–21 of EGFR tyrosine kinase domain then bidirectional sequencing.

The recommendation from International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS) and European Respiratory Society (ERS) [6] is to test for EGFR mutation in all cases of lung adenocarcinoma, possible adenocarcinoma and NSCLC—not otherwise specified. If EGFR testing is negative, Alkfusion Test should be performed. It is optional to proceed to KRAS mutation testing (codon 12 and 13). Activating mutations in KRAS gene were shown to be of negative predictive value to TKIs. Also, KRAS mutations correlate with smoking history and poor prognosis.

**EGFR mutations**

EGFR is a member of receptor tyrosine kinase family and a major factor in regulating cellular proliferation, invasion, metastasis, angiogenesis and inhibition of apoptosis. EGFR signals activate at least two parallel intracellular pathways [7]. One of these pathways, is the MAP kinase pathway (MAPK) that regulates G1 checkpoint in the cell cycle and control cellular proliferation [8]. Once EGFR is activated, the MAPK pathway transmits the signal to the nucleus via the active forms of RAS, RAF and MEK genes [7,9]. The RAS proto-oncogene consists of H-ras, K-ras and N-ras [10], located in the inner plasma membrane layer and present in either the active form (GTP bound) or the inactive form (GDP bound) [11].

Mutant EGFR binds ATP less tightly and binds TKIs more tightly than wild type EGFR.

**EGFR testing**

The sample available is usually paraffin embedded tissue. Preferably primary tumor tissue is used, when this is not available one may consider sample from metastatic tissue. Ideally, the tissue sample should contain at least 50% of viable tumor cells. Methods with higher detection sensitivity can detect mutation with lower tumor content levels. 4–10 μm sections of non-baked unstained slides prepared from paraffin block and one H&E reference slide to mark the area of interest. The tumor area of interest selected by the pathologist should be a minimum of 2 mm × 2 mm.

**Mutation detection**

Detection of mutation can be performed using a variety of mutation platforms, direct sequencing is widely used (amplify and sequence EGFR exons 18–21). Other methods includes real-time-PCR (amplification refractory mutation system), high resolution melting analysis, and denaturing high performance liquid chromatography (DHPLS).

**Testing facility**

Mutation analysis testing should be performed in accredited, quality assured facility participating in external proficiency testing schemes. EGFR testing should be validated before reporting the test results. Requirements for validation for molecular testing are both analytical and clinical. There are published guidelines for validating and reporting molecular testing [12]. The College of American Pathologists developed recommendations for testing, validating and reporting molecular testing [13].

**Summary**

Adenocarcinoma is the most common histologic type of NSCLC. Treatment decisions of NSCLC are dependent on two important factors. The first one
is accurate histologic classification using H&E stain as well as several immunohistochemical stains particularly in poorly differentiated carcinoma. The other factor is testing the tumor tissue for the presence or absence of specific mutations for targeted therapy. Since most of the tissue specimens are biopsy specimen, the pathologists play important role in managing the tissue carefully for immunohistochemical studies, molecular testing and for possible research.

Utilizing the 2011 IASLC/ATS/ERS proposal for classification of lung adenocarcinoma is highly recommended. In this classification, histologic subtypes are correlated well with EGFR mutations [14].

Funding: No funding sources.

Competing interests: None declared.

Ethical approval: Not required.

Appendix A. Lung Cancer Guidelines Committee Members

Dr. Abdul Rahman Jazieh, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

Dr. Abdulrahman Al Hadab, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

Dr. Adnan Hebschi, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Dr. Ahmed Abdulwarith, King Fahad Specialist Hospital, Dammam, Saudi Arabia.

Dr. Ahmed Bamoussa, Riyadh Military Hospital, Riyadh, Saudi Arabia.

Dr. Ahmed Saadeddin, Riyadh Military Hospital, Riyadh, Saudi Arabia.

Dr. Ashwaq Al Olayan, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

Dr. Azzam Khankan, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

Dr. Foad Al Dayel, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Dr. Hamed Al Husaini, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Dr. Hamdan Al Jahdali, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

Dr. Hana Bameifieh, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

Dr. Khalid Al Kattan, Al Faisal University, Riyadh, Saudi Arabia.

Dr. Loutfi, Shukri, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

Dr. M. Hasan Rajab, Al Faisal University, Riyadh, Saudi Arabia.

Dr. Sara Al Ghani, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

Dr. Turki Al Fayae, King Abdulaziz Medical City, Princess Noorah Oncology Center, Jeddah, Saudi Arabia.

Dr. Yasir Bahadur, King Faisal Specialist Hospital & Research Center, Jeddah, Saudi Arabia.

References


