RESEARCH ARTICLE

Distribution of EGFR Mutations Commonly Observed in Primary Lung Adenocarcinomas in Pakistan as Predictors for Targeted Therapy

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Abstract

Background: Acquired genetic alterations and presence of sensitizing mutations in the tyrosine kinase domain of EGFR and other signaling molecules have been found in different subsets of primary lung adenocarcinoma. The commonest EGFR mutations are small in frame deletions of exon 19 and a point mutation (L858R) in exon 21, having a combined occurrence of around 90%. The objective of this study was to determine the frequency and types of EGFR mutations in primary lung adenocarcinomas in Pakistan. Materials and Methods: EGFR mutations in tumor samples were screened by multiplex real time PCR. Briefly, DNA from formalin fixed paraffin-embedded tissue was amplified with primers and probes specific to 43 different EGFR mutations in a Cobas z 480 instrument. The assay detects mutations in four exons (18-21) of the EGFR gene. Results: Out of 94 patients, 65 were males and 29 females with a M:F ratio of 2.2: 1. The median age was 62 years (range, 28 - 85 years). In our biopsy samples 70 (74%) cases were of primary lung adenocarcinoma, whereas 24 (26%) were confirmed metastatic adenocarcinoma of primary lung origin. EGFR mutation was positive in 29% of the patients. The highest frequency of L858R was observed in 48% of these, followed by deletion in exon 19 (44%). In addition, other rare mutations such as compound G718X:S768I and insertions in exon 20 insertion were detected in approximately 4% of the patients. Conclusions: This study showed that Del 19 and L858R are the most frequent mutations in Pakistani lung adenocarcinoma patients and around 29% of the patients were found eligible for erlotinib therapy.

Keywords: EGFR - NSCLC - adenocarcinoma - tyrosine kinase - sensitizing mutations

Asian Pac J Cancer Prev, **15 (17)**, 7125-7128

Introduction

Lung cancer is a leading cause of malignancy associated human deaths, which is evident from its high mortality rate of 1.6 million (19.4% of total) cancer deaths worldwide (Ferlay et al., 2013). It is more common in male and elderly group (Makay et al., 2006) risk factors include smoking, pollution, certain metals (chromium, cadmium), some organic chemicals and radiation. The risk of genetic susceptibility can contribute especially in young (Jemal et al., 2008; Ren et al., 2013).

Adenocarcinoma of lung is the commonest subset of lung cancer which is usually seen in nonsmokers and equally involves both males and females (Lindeman et al., 2013). It can present itself in early age as well. Fortunately, in the last decade due to major advances in our understanding of the pathogenesis and management of primary lung adenocarcinoma, new protocols of targeted treatment have emerged. Acquired genetic alterations and presence of sensitizing mutations in the tyrosine kinase domain of EGFR and other signaling molecules were found in different subsets of primary lung adenocarcinoma. Pharmacological agents targeting tyrosine kinase involved in growth factor receptor signaling like EGFR and ALK were developed and their discovery has changed the way, these malignancies are diagnosed and treated today (Rossi et al., 2013).

EGFR is a 170 KD protein, which belongs to subclass 1 of transmembrane receptor tyrosine kinase superfamily. Its gene is located on chromosome 7p11.2 and has 28 exons (Salto-Tellez et al., 2011). It is normally expressed in various tissues of epithelial, mesenchymal and neural origin. Dysregulation of EGFR gene can lead to tumorogenesis. The over- expression of EGFR gene is seen in a variety of tumors including lung, head and neck, colon, pancreas, breast, bladder, kidney and gliomas (Wang et al., 2010; Xu et al., 2010). Around 60% of the primary lung adenocarcinomas are reported to have association with EGFR mutations; these sensitizing mutations are also found in non-smoker women of Asian origin. However, the frequency and types of mutations varied with the population examined (Liu et al., 2009; Wang et al., 2010;

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Xu et al., 2010).

The commonest EGFR mutations are small in frame deletion of exon 19 and point mutation (L858R) in exon 21, having combined occurrence of around 90%, whereas mutations in exon 18 and exon 20 accounted for 5% respectively. The exon 19 and 21 mutations are found to be highly sensitive to reversible TK inhibitors. In contrast, exon 18 and 20 are less sensitive or insensitive to reversible TK inhibitors (Hanif et al., 2009; Ma et al., 2010). In Pakistan like rest of the world lung cancer prevalence is high (15%) and primary lung adenocarcinoma constitute about 40-45% of primary lung cancer (Hanif et al., 2009).

The aim of the present study was to identify spectrum of EGFR mutations in Pakistani lung cancer (adenocarcinoma) patients, and associate them with clinical and histopathological parameters.

Materials and Methods

In this cross sectional study, a total of 94 patients, who approached for EGFR mutation analysis to the Molecular Pathology Section, Department of Pathology and Microbiology, Aga Khan University Hospital were enrolled consecutively from June 2013 to January 2014. The patients were confirmed for the presence of adenocarcinoma of lung on the basis of morphological and immunohistochemical workup. Typical immunohischemical profile of these showed CK 7 positive, TTF1 positive and CK5/6, CK20 and p63 negative. The source of biopsy specimen included primary lung lesions, metastatic lymph node, mediastinal, pleural and peritoneal tissue representing distant or direct metastases. (Figure 1 and 2).

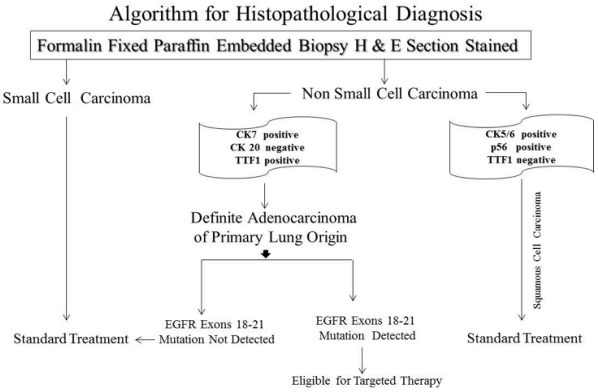

Figure 1. Algorithm for Histopathological Diagnosis

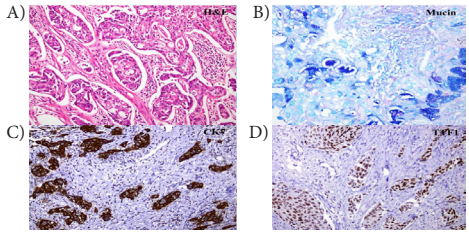

Figure 2. Photomicrographs of Primary Lung Adenocarcinoma. A) H & E showing morphology of typical primary lung adenocarcinoma with glandular differentiation; **B)** Mucin is demonstrated; **C)** Cytokeratin 7 stained tumor cells strongly and diffusely; **D)** TTF1 nuclear positivity in tumor cells

DNA extraction and realtime PCR assay

For the identification of EGFR mutations, depending upon the size of the tissue, 2-5 sections of 5 μM thickness of FFPE specimen were cut and placed in 1.5 ml microfuge tubes. DNA extraction and multiplex PCR was performed using Cobas EGFR mutation test kit and the Cobas z480 Realtime PCR instrument (Roche Molecular Systems, Branchburg, NJ, USA), following the standard package insert protocol. The extracted DNA was quantified by NanoDrop Analyzer (Wilmington, DE, USA) and a total of 150ng DNA was used for amplification. Cobas EGFR Mutation Test uses allele specific polymerase chain reaction (AS-PCR) and can detect 43 different mutations in the four exons (18-21) of EGFR gene, including several point mutations, deletions and insertions. For the assessment of quality parameters and validity of the run a mutant positive and negative controls were included in every batch of the PCR assay. Analysis was performed with the EGFR Analysis Package Software V.1.0 (Roche Molecular Systems).

Statistical analysis

Data were summarized and presented using appropriate descriptive statistics. Chi square test was performed to assess differences in the proportion of EGFR mutations across age, gender and disease severity as categories. Statistical analysis were performed using SPSS version 19.0 for Windows (IBM Inc., Chicago, IL, USA) and p value of <0.05 was set for level of significance.

Results

In this study, Formalin fixed paraffin embedded specimens of primary lung adenocarcinoma were analyzed for mutations in the exon 18-21 of EGFR gene. Out of 94 patients, 65 were males and 29 females with M: F ratio of 2.2: 1. The median age of the patients was 62 years (range, 28-85 years). In our biopsy samples 70 (74%) cases were primary lung adenocarcinoma, whereas 24 (26%) were metastatic adenocarcinoma samples, confirmed to be metastases from lung.

EGFR mutations were positive in 29% of the patients. As shown in Table 2, the highest frequency of L858R was observed in 48% of the patients, followed by deletion in exon 19 (44%). In addition, other rare mutations such as compound G718X:S768I and insetions in exon 20 insertion were detected in approximately 4% of the patients.

Adenocarcinoma of lung in our patients was further categorized into two; primary lung adenocarcinoma

Table 1. Description of Cases Used for EGFR Mutational Analysis

Histology of lung carcinoma	Total cases	No. of EGFR mutation	Exon mutation
Primary adenocarcinoma	70	20	18, 20, 21
Well differentiated	40	10	_____
Moderately differentiated	20	7	_____
Poorly differentiated	10	3	_____
Metastatic adenocarcinoma of primary lung origin	24	7	18, 19, 20, 21

Table 2. Patient Characteristics and Mutational Analyses of 94 Pakistani Lung Adenocarcinoma Cases

		Total cases	EGFR mutation		Mutation types number	
			Positive	Negative		
Cases		N=94	N=27(29%)	N=67(71%)	Del (19exon)	12
					Exon 20	1
					L858R	13
					S768I/G719X	1
Gender	Male	65	14 (60%)			
	Female	29	13 (40%)			
	Age (years) (Median/Range)	62±12/28-85				
Histological type	Primary lung Adenocarcinoma	N=70	N=20 (28%)		del(19exon)	9
	Metastatic Adenocarcinoma primary lung origin	N=24	N= 7 (29%)		Exon 20 ins	1
					Exon21L858R	10
Total No. of cases		N=94			del(19exon)	3
					Exon21L858R	3
					Compound mutation {Exon20S768I, Exon20G719X}	1

samples and metastatic adenocarcinoma samples of primary lung origin. The primary adenocarcinoma form was further classified into well, moderate and poorly differentiated subtypes. The frequency of EGFR mutations in these subsets of the adenocarcinoma was 35% and 25% respectively. EGFR mutations were more frequently found in well differentiated subtypes as presented in Table 1. In addition, no preponderance of gender on mutation status was noted, which may require large data set to avoid selection bias.

Discussion

Clinical trials and published data show that EGFR mutation analysis is highly desirable on primary lung adenocarcinoma as patients significantly benefit from specific tyrosine kinase inhibitors. Therefore to choose best option for these patients the analysis is a prerequisite.

Salto-telkz et al (2011) approximated that 30%-80% of lung adenocarcinoma patients are tested for EGFR mutation in East Asia. In this part of the world EGFR testing in primary lung adenocarcinoma is very limited. Reason includes lack of testing facilities and high cost. Our institute is the first in the 6th most populated countries of the world with an estimated population of 186 million, hence is the first report of mutational analyses from this country.

The worldwide occurrence of lung cancer is approximately 25% in lifelong nonsmokers (Ferlay et al., 2012) in which around 30 to 40% are from Asian countries (Xiao et al., 2011). In both male and female nonsmokers the mortality rates from primary lung adenocarcinoma are 17.1 and 14.7 /1000person/year respectively. The proportion of primary lung adenocarcinoma in nonsmokers is indeed one of the most common causes of cancer-related death (Parkin et al., 2005).

Ma et al (2010) reported high prevalence of EGFR mutation in primary lung adenocarcinoma from northeast (China, Japan, Korea) and part of Southeast Asia (excluding India) nonsmokers, predicting high sensitivity to TKI. Another study from Indian reported around 30% of primary adenocarcinoma of lung belonged to subgroup of NSCLC, their data matched with other global study where EGFR mutation rate was 1.7 times more in female verses 1.5 times more in Indian cohort. Their prevalence of EGFR mutation being matched with East Asian data of 27-60%.

Noronha et al. (2013) recently showed, data shows 96% of patients who primary adenocarcinoma of lung in which majority was nonsmoker with significant proportion of females (48%), 35% of these patients were found to harbor an EGFR mutation.

Regarding the types of EGFR mutation detected in their study, 74% of patients were noted to have deletion (exon 19), 23% patients had L858R point mutation (exon21) and only 2.5% had G719C point mutation exon 18.

Published literatures strongly suggest an association between grade, age, ethnicity and EGFR positivity. Multiple studies have also shown improved response and better quality of life along with high survival in the subset of EGFR positive patients with response to TKIs (Salto-Tellez et al., 2011).

Our study for the first time reveals information regarding EGFR mutational prevalence in Pakistani population, where 94 lung cancer patients of primary lung adenocarcinoma, specimens received from every part of Pakistan was analyzed, using the most sensitive diagnostic technique. As compared to other studies which showed that adenocarcinoma is more common in female (Wang et al., 2011), our study could not comment yet because of small size of the cases tested. Overall prevalence of EGFR mutation slightly matched with reported Asian data which ranges between 27-60% in various studies (Cohen et al., 2010).Another similarity in our data with Western and Asian data is the highest mutation rate of 48% noted for L858R (exon 21) followed by deletions of exon 19 (44%) of EGFR mutant isoform.

Other exons mutations were also identified like compound G718X (exon18) with S768I (exon20) and isolated exon 20 insertions seen in 4% of cases. In published data these are rare mutations and also resistant to targeted therapy but chemotherapy is another optional choice for these rare cases. The current recommendation for testing EGFR mutation is limited to adenocarcinoma of lung or nonsquamous lung cancer with adenocarcinoma component. The significant finding of positive EGRF mutation is high among patients of East Asian origin and female nonsmokers, however it is recognized that the immunohistopathological feature alone cannot be used to select for EGFR mutation analysis (Lindeman et al., 2013).

The benefit of EGFR positive lung adenocarcinoma have better response rates when treated with TKI than with chemotherapy (Maemondo et al., 2010; Zhou et al., 2011; Han et al., 2012; Sequist et al., 2013) For assessment of treatment response and emergence of resistance in our patients we need larger studies with clinical data and follow up would determine impact of TKIs treatment of EGFR sensitizing lung adenocarcinoma.

In conclusion, L858R (exon 21) and deletion 19 exon were most frequent mutations in primary lung adenocarcinoma patients of Pakistani origin. These observations have led to changes in the overall treatment strategies for lung adenocarcinoma. Therefore, a genetic testing before the treatment is considered essential for primary adenocarcinoma of lung in order to select the appropriate treatment option according to the patient's molecular characteristics.

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