

Epidermal Growth Factor Receptor Mutations in Lung Adenocarcinomas: A Single Center Study from Iran

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Abstract

Introduction: Lung cancer is the fifth leading tumor in Iran, and while its incidence remains relatively low, it has been increasing steadily. Targeted therapies have brought new hope to patients with non small cell lung cancer (NSCLC). The epidermal growth factor receptor (EGFR) gene is the prototype member of the type I receptor tyrosine kinase (TK) family and plays a pivotal role in cell proliferation and differentiation. Studies from Asian countries have revealed a higher frequency of EGFR mutations than in the West. The aim of this study was to measure the frequency and type of EGFR mutations in a group of Iranian patients with lung adenocarcinomas. **Methods:** Formalin fixed paraffin embedded (FFPE) lung adenocarcinoma tissues from 103 Iranian patients were sequentially tested for EGFR mutations by the polymerase chain reaction (PCR) followed by direct nucleotide sequencing of exons 18, 19, 20, and 21. Patient's demographics and other clinical details were obtained from the medical records of hospitals affiliated to Iran University of Medical Sciences, Tehran, Iran. Statistical analyses were performed with SPSS v.20. **Results:** EGFR mutations were detected in 25/103 (24.3%) patients. The most frequent was an exon 21 point mutation (L858R) (15 patients; 60%), followed by one in exon 19 (10 patients; 40%). The frequency of EGFR mutations in never-smoker patients was significantly higher than in smokers (68% versus 32%; $p < 0.01$). **Conclusion:** EGFR mutation frequency is higher than in the West but lower than in East Asian and almost equal to reported rates for Indian and North African populations. Smoking is negatively associated with EGFR mutations in Iranian lung adenocarcinomas.

Keywords: Lung adenocarcinomas- epidermal growth factor receptor- mutations- receptor tyrosine kinase

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Introduction

According to WHO, Cancer is a major leading cause of death worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths. Lung cancer is one of the leading causes of death and responsible of 1.59 million deaths around the world and is predicted to be the 7th cause of death and responsible for 3% of mortalities by 2030 (Karbalaie Niya et al., 2017; Moradi et al., 2017; Siegel et al., 2016). In Iran, lung cancer ranks second and third as the cancer-causing death in men and women, respectively and has been increasing steadily in both men and women during the recent years (Hajmanoochehri et al., 2014; Rezaei et al., 2016). Non-small cell lung cancer (NSCLC) is the most common type of lung cancer and primarily occurs in elderly patients. As life expectancy will be better gradually, NSCLC and related social burden will increase, indiscriminately. In patients that have recurrent or metastatic tumors, chemotherapy remains the initial treatment of choice (Codony-Servat et al., 2016).

Traditional treatment with empirically chosen cytotoxic chemotherapeutic agents, have given small, but real survival benefits. Recent advances and insights into molecular pathogenesis of lung cancers have provided some novel molecular targets. This so-called somatic driver mutation can serve as Achilles heels for tumors (Chirieac and Kobzik, 2017). Patients with non-small-cell lung cancer sometimes show a dramatic clinical response to Gefitinib or Erlotinib that are reversible tyrosine kinase inhibitors (TKI) specific for the epidermal growth factor receptor (EGFR). However, until April 2004, it was unclear how to identify patients who would benefit from these drugs. Then, two groups from Boston reported that EGFR gene mutations in the kinase domain are strongly associated with tyrosine kinase inhibitor sensitivity (Kashima et al., 2017; Yang et al., 2017).

Mutations in epidermal growth factor receptor (EGFR), KRAS, and anaplastic lymphoma kinase (ALK) are mutually exclusive in patients with NSCLC, and the

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presence of one mutation in lieu of another can influence response to targeted therapy. Therefore, testing for these mutations and tailoring therapy accordingly is widely accepted as standard practice (Genestreti et al., 2017; Karbalaie Niya et al., 2016). The frequency of EGFR mutations varies from 27 to 60% in East Asians, from 8 to 13% in Europeans, and from 12 to 16% in African and white Americans. Among Asian patients the incidence of EGFR mutations is approximately 30% compared with 7% among Caucasians. Even higher mutation rates (ranging from 47% to 64% among various East Asian countries) were observed in the initial study. It is worthwhile to keep in mind differences between mutation frequencies and clinical response among different ethnic populations while preparing local guidelines for treatment of EGFR mutations (Daniels et al., 2016).

The purpose of this investigation was to examine the frequency and characteristics of EGFR mutations in Iranian patients with NSCLC and to assess the association between this mutation and clinicopathological characteristics.

Materials and Methods

Patients and Methods

One hundred-twenty six patients of NSCLC were sequentially tested for EGFR mutation. Patient's demographics, history and treatment details were obtained from the medical records from hospitals affiliated to Iran University of medical sciences, Tehran, Iran. The data and information were collected retrospectively from a period of December 2010 to April 2014.

All samples were formalin fixed paraffin embedded (FFPE) tissues (biopsies or surgically resected specimens), from primary tumors and metastatic sites, were used for the mutation analysis. Genomic deoxyribonucleic acid (DNA) was extracted by previous method (Karbalaie Niya et al., 2016; Karbalaie Niya et al., 2017; Safarnezhad Tameshkel et al., 2016) and exons 18, 19, 20, and 21 of EGFR gene were amplified by nested polymerase chain reaction (PCR). The amplified PCR product was subjected to the direct nucleotide sequencing for the detection of mutations. PCR condition and protocol was used same as previous works (Kelly et al., 2017; Rossi et al., 2016).

Our research ethic committee approved the study as the mutation analysis of EGFR is the standard practice that helps to optimize treatment planning. Chi-square analysis was used to determine the relationship between the levels of two categorical variables and the linear relationship between continuous variables. The Student's t-test was used to compare the two independent groups. SPSS Statistics version 21 (SPSS Inc., Chicago, IL, USA) was used for data analysis. All tests were two-sided, and p-value < 0.05 was considered statistically significant.

Results

One hundred three cases out of one hundred twenty six cases of NSCLC were adenocarcinoma (81.7%). The study population consisted of 51 (49.5%) men and 52 women (50.5%). The age of patients ranged from 34

Table 1. Demographic Characteristics of the Study Population

Characteristics	All patients (n=103)
Age	
Median	67
Range	34-83
Sex	No. (%)
Male	51(49.5%)
Female	52(50.5%)
Smoking history	No. (%)
Never	66 (64%)
Ever	37(36%)

years to 83 years. Among all patients, 37 (35.9%) were smokers. Patients' demographic characteristics are shown in Table 1. Out of 103 cases with adenocarcinomas, 37 cases were current smokers or ex-smokers and 66 cases were never smoker.

Mutations in the EGFR gene were found in 25 (24.3%) of 103 patients distributed between male patients (14 positives, 56%) and female patients (11 positives, 44%). Of 25 cases with EGFR mutations, 8 patients were smokers (4 female patients, 4 male patients) and 17 were non smokers (7 female patients, 10 male patients). Association between EGFR mutation and sex of the patients was not statistically significant (P =0.45). The most frequent EGFR mutation was detected in exon 21 as point mutations (L858R) (15 patients; 60%), followed by exon 19 (10patients; 40%).

No mutations were seen in any of the tested cases of squamous cell carcinoma (11 patients), large cell carcinoma (6 patients) and small cell carcinoma (6 patients). The frequency of never smokers in patients with tumors having EGFR mutations was significantly higher than that observed in patients without mutations (68% versus 32%; p < 0. 01).

Discussion

Most patients with lung cancer have distant metastases or pleural effusion at the time of initial diagnosis and are not candidates for surgical treatment. Systemic chemotherapy is the preferred method of treatment in these patients but the efficacy of anticancer agents is limited and patients with advanced diseases rarely live long (Tomoda et al., 2016). Epidermal growth factor receptor (EGFR), a cell membrane receptor with tyrosine kinase activity, is expressed in most patients with NSCLC and plays a role in cellular proliferation, inhibition of apoptosis, angiogenesis, metastatic potential, and chemoresistance (Higgins et al., 2016). EGFR gene mutation can lead to amplification and overexpression of EGFR protein as well as to other carcinogenic mechanisms of EGFR tyrosine kinase activity disorder. In appropriate activation of EGFR tyrosine kinase can promote tumor angiogenesis, tumor cell proliferation, adhesion, invasion and metastasis. In theory, specific blocking of the receptors can inhibit tumor formation and angiogenesis, and this theory has been confirmed in clinical studies (Songet al., 2016; Wang

et al., 2016).

The genomic discoveries in EGFR and the resultant targeted treatment opened up a new horizon of treatment in lung cancer biology and therapy. Current available drugs that target EGFR can be divided into 2 categories: small-molecule EGFR tyrosine kinase inhibitor (TKI)-gefitinib and erlotinib and monoclonal anti-EGFR antibody cetuximab that have become the standard first-line therapy for patients with advanced NSCLC that harbor EGFR mutation (El Guerrab et al., 2016; Husain et al., 2017). EGFR mutations are currently used as predictive markers of clinical response to EGFR-TKIs, with 70% to 80% of patients deriving substantial benefit from these targeted therapies. This shows the clinical relevance of EGFR mutational status for therapeutic decision making in patients with advanced NSCLC (Gainor et al., 2016; Samuels et al., 2016).

The nature and frequencies of lung cancer driver mutations have been shown to be different among racial and ethnic groups. The rates of EGFR mutations in lung adenocarcinoma is also recognized to vary across ethnic groups with higher prevalence observed in East-Asian trials than in European and North American studies, and the response rate to TKI in patients previously treated with traditional chemotherapy regimens showed better response rate in Asian patients than that in patients of other ethnicities as well (Cheema et al., 2016).

In the present study exon 21 mutations accounted for 60% while exon 19 mutations accounted for 40% of the mutations. Interestingly, no exon 18 and exon 20 mutations were seen. As far as we know our study has been the first of its kind in Iran and the results showed that overall frequency of EGFR mutation was higher than western ethnics and lower than East Asian countries and almost equal the frequency reported by Errihani in North Africans (Errihani et al., 2013).

The frequency of never smokers in patients with tumors harboring EGFR mutations was like many other studies (Tissot et al., 2016; Zenke et al., 2016). We know that failure to enroll the appropriate number of patients in a study of a new diagnostic technology can lead to overestimates of both the sensitivity and specificity of the new technology and similarly in a study of a new therapy, it can lead to an overestimate of the effectiveness of that therapy, so we are conducting another study involving a larger number of subjects are to confirm these findings.

In conclusion, in this first study of EGFR mutation frequency in Iranian population of patients with NSCLC we showed that the frequency of this mutation is higher than that western continent but lower than in East Asian and almost equal to Indian population and North Africans. Exact definition of type of mutation will add significant clinical value to the management of NSCLC.

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References

- Cheema PK, Menjak IB, Winterton-Perks Z, et al (2016). Impact of reflex EGFR/ALK testing on time to treatment of patients with advanced nonsquamous non-small-cell lung cancer. *J Oncol Pract*, **13**, 130-8.
- Chirieac LR, Kobzik L. (2017). Pathology and molecular pathology of lung cancer. Pathology and epidemiology of cancer Springer, pp 367-90.
- Codony-Servat J, Molina-Vila MA, Bertran-Alamillo J, Rosell R, D'Hondt E (2016). Inhibition of epidermal growth factor receptor pathway by epidermal growth factor antibodies in non-small cell lung cancer. *AACR*, DOI: 10.1158/1538-7445.AM2016-2368.
- Daniels AR, Attanoos RL, Butler R, Davies H (2016). The frequency of Egfr mutations in lung adenocarcinoma: The Cardiff experience. *J Pathol*, **238**, 18.
- El Guerrab A, Bamdad M, Kwiatkowski F, et al. (2016). Anti-EGFR monoclonal antibodies and EGFR tyrosine kinase inhibitors as combination therapy for triple-negative breast cancer. *Oncotarget*, **7**, 73618-37.
- Errihani H, Inrhaoun H, Boukir A, et al (2013). Frequency and type of epidermal growth factor receptor mutations in Moroccan patients with lung adenocarcinoma. *J Thorac Oncol*, **8**, 1212-14.
- Gainor JF, Shaw AT, Sequist LV, et al (2016). EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung cancer: A retrospective analysis. *Clin Cancer Res*, **22**, 4585-93.
- Genestreti G, de Biase D, Di Battista M, et al (2017). Epidermal Growth factor receptor (EGFR) mutation in exon 19 (p. E749Q) confers resistance to gefitinib in one patient with Lung Adenocarcinoma. *Clin Lung Cancer*, **18**, 215-17.
- Hajmanoochehri F, Mohammadi N, Zohal MA, Sodagar A, Ebtehaj M (2014). Epidemiological and clinicopathological characteristics of lung cancer in a teaching hospital in Iran. *Asian Pac J Cancer Prev*, **15**, 2495-2500.
- Higgins GS, Krause M, McKenna WG, Baumann M (2016). Personalized radiation oncology: epidermal growth factor receptor and other receptor tyrosine kinase inhibitors Molecular radio-oncology, Springer, pp 107-22.
- Husain H, Scur M, Murtuza A, et al (2017). Strategies to overcome bypass mechanisms mediating clinical resistance to EGFR tyrosine kinase inhibition in lung cancer. *Mol Cancer Ther*, **16**, 265-72.
- Karbalaie Niya MH, Basi A, Koochak A, et al (2016). Sensitive high-resolution melting analysis for screening of KRAS and BRAF mutations in Iranian human metastatic colorectal cancers. *Asian Pac J Cancer Prev*, **17**, 6047-52.
- Karbalaie Niya MH, Safarnezhad Tameshkel F, Panahi M, et al (2017). Human papillomavirus investigation in head and neck squamous cell carcinoma: Initial report from the low risk HPV types associations. *Asian Pac J Cancer Prev*, **18**, 2573-9.
- Kashima J, Okuma Y, Miwa M, Hosomi Y (2017). Retrospective analysis of survival in patients with leptomeningeal carcinomatosis from lung adenocarcinoma treated with erlotinib and gefitinib. *Jpn J Clin Oncol*, **47**, 357-62.
- Kelly D, Mc Sorley L, O'Shea E, et al (2017). A regional analysis of epidermal growth factor receptor (EGFR) mutated lung cancer for HSE South. *Ir J Med Sci*, **186**, 855-7.
- Moradi P, Keyvani H, Mousavi SJ, et al (2017). Investigation of viral infection in idiopathic pulmonary fibrosis among Iranian patients in Tehran. *Microb pathog*, **104**, 171-4.
- Rezaei S, Akbari Sari A, Woldemichael A, et al (2016). Estimating the economic burden of lung cancer in Iran. *Asian*

Pac J Cancer Prev, **17**, 4729.

- Rossi S, D'Argento E, Basso M, et al (2016). Different EGFR gene mutations in Exon 18, 19 and 21 as prognostic and predictive markers in NSCLC: A single institution analysis. *Mol Diagn Ther*, **20**, 55-63.
- Safarnezhad Tameshkel F, Sohrabi MR, Babae MR, et al (2016). Mutation analysis of KRAS and BRAF genes in metastatic colorectal cancer: a first large scale study from Iran. *Asian Pac J Cancer Prev*, **17**, 603-8.
- Samuels ML, Read C, Jackson J, et al (2016). Multiplex digital PCR analysis of EGFR mutations for lung cancer characterization and monitoring. [abstract]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research; 2016 Apr 16-20; New Orleans, LA. Philadelphia (PA): AACR; Cancer Res 2016;76(14 Suppl):Abstract nr 3647.
- Siegel RL, Miller KD, Jemal A (2016). Cancer statistics, 2016. *CA Cancer J Clin*, **66**, 7-30.
- Song Z, Wang X, Zheng Y, Su H, Zhang Y (2016). MET gene amplification and overexpression in Chinese non-small-cell lung cancer patients without EGFR mutations. *Clin Lung Cancer*, **18**, 213-19.
- Tissot C, Couraud S, Tanguy R, et al (2016). Clinical characteristics and outcome of patients with lung cancer harboring BRAF mutations. *Lung Cancer*, **91**, 23-8.
- Tomoda C, Ogimi Y, Saito F, et al (2016). Outcome and characteristics of patients with malignant pleural effusion from differentiated thyroid carcinoma. *Endocrine J*, **63**, 257-61.
- Wang X, Niu H, Fan Q, et al (2016). Predictive value of EGFR overexpression and gene amplification on icotinib efficacy in patients with advanced esophageal squamous cell carcinoma. *Oncotarget*, **7**, 24744.
- Yang JJ, Zhou Q, Yan HH, et al (2017). A phase III randomised controlled trial of erlotinib vs gefitinib in advanced non-small cell lung cancer with EGFR mutations. *Br J Cancer*, **116**, 568-74.
- Zenke Y, Yoh K, Matsumoto S, et al (2016). Clinical impact of gastric acid-suppressing medication use on the efficacy of erlotinib and gefitinib in patients with advanced non-small-cell lung cancer harboring EGFR mutations. *Clin Lung Cancer*, **17**, 412-18.



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