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Epidermal growth factor receptor mutation subtypes and geographical distribution among Indian non–small cell lung cancer patients

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Abstract

Background—The Medical Oncology Department at Tata Memorial Hospital, the single largest tertiary cancer care center in Asia, receives in-house registered and referral patient samples from all parts of the country. Our recent studies establish 23% EGFR mutation frequency among Indian population. Here, we extend our study and report further analysis of distribution of different types of *EGFR* mutations in 1018 non small cell lung cancer patient, and its co-relation with clinical parameters and geographical variations across the country.

Material and Methods—This study is a retrospective analysis on all the patients who were referred for *EFGR* testing as a routine service over a 1.5 year period. This was part of standard care. EGFR kinase domain mutations in exon 18-21 were probed by TaqMan probe-based assays in 1018 NSCLC patients.

Results and Discussion—While EGFR exon 19 mutations, the most frequent EGFR mutation, were found be higher among non smokers females, we find surprisingly higher incidence of exon 21 mutations among EGFR mutation positive male smokers of Indian ethnicity. Furthermore, as Indian population is known to be composed of a gradient admixture of Ancestral North Indian (with genetic influence from Middle Easterners, Central Asians, and Europeans harboring variant EGFR mutation frequency) and Ancestral South Indians, as a paradox our study indicates comparable EGFR mutation frequency across different geographical locations within India

Conclusion—Geographically there is uniform distribution in the EGFR mutation frequency within India. Further more, while exon 19 mutations are predominant among non smokers, higher incidence of exon 21 mutations exists among EGFR mutation positive male smokers of Indian ethnicity.

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Keywords

Non-small cell lung cancer; epidermal growth factor receptor; adenocarcinoma; real-time PCR

Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer deaths worldwide in both men and women with less than 15% chance of surviving beyond 5 years. Chemotherapy for NSCLC, which accounts for approximately 85% of lung cancer cases, remains marginally effective.[1] Although the majority of lung cancers are linked to environmental carcinogens such as tobacco smoke and environmental pollutants, it is lung cancer in never-smokers, especially adenocarcinomas (ADCs), which are most likely to respond to tyrosine kinase inhibitors (TKI) targeting the epidermal growth factor receptor (EGFR) gene.[2] Recent clinical trials have also suggested that for advanced NSCLC patients with *EGFR* mutant tumors, initial therapy with a TKI instead of chemotherapy may be the best choice of treatment.[1,2]

Of the four major subtypes of *EGFR* mutation present in exon 18-21, the exon 19 and 21 mutations are most frequent.[3–6] They are either in-frame deletions or amino acid substitutions clustered around the ATP-binding pocket of the kinase domain mutations, which confer ligand-independent activation and increased activation duration compared with wildtype receptors.[3] These sensitizing mutations of exon 19 and 21 are both believed to be associated with carcinogenesis, sensitivity to tyrosine kinase drugs and with the prognosis of NSCLCs, but their exact clinical significance remains disputable.[3,7] Significantly, there is a plethora of studies demonstrating that patients with the exon 19 deletions and L858R mutations respond very well to EGFR inhibitors in NSCLC.[5,7]

EGFR mutation subtypes display distinct biological features concerning kinase activity, transforming potential, and sensitivity to EGFR inhibitors. In vitro studies have shown that exon 19 deleted mutants and L858R mutant receptors appear to be more sensitive to gefitinib inhibition as compared with wildtype receptors.[8] It has also been documented that patients with *exon 20* harboring deletions were found to have longer survival following treatment with gefitinib or erlotinib compared with those having L858R mutations in NSCLC.[3,7] However, Mark *et al.*, reported no difference in survival between exon 19 deletions and L858R mutations in the absence of EGFR-targeted therapy.[8,9] Most EGFR exon 19 insertions occur in between amino acids 767–774 of exon 20, within the loop that follows the C-helix of the kinase domain of EGFR.[10–12] It is thus imperative to determine the distribution of *EGFR* mutation subtype in a given population to determine the overall implication for establishing relevance for routine *EGFR* mutation diagnostics for NSCLC patients in clinics and emphasizes effectiveness for adoption of EGFR inhibitors as the firstline treatment in a given population.

Besides variation of individual subtypes, overall incidence of *EGFR* mutation (including all subtypes) varies across different ethnicity; 10%–15% in Caucasians and 20%–30% in various East Asians, we recently showed an intermediate *EGFR* mutation in Indian population to be of 23%. We argue that this intermediate frequency is reminiscent of an

ancestral admixture of genetic influence from Middle Easterners, Central Asians, and Europeans on modern Indian population. A recent study has as well showed that Indian population is composed of a mixture of original Ancestral South India and Ancestral North Indians with genetic influence from Middle Easterners and Europeans.[13] As genetic diversity is known to influence incidence of *EGFR* mutations, we asked if *EGFR* mutation frequency varies across four different geographical locations within India. In this extensive study, our main objective was to determine the distribution of *EGFR* mutation subtype in NSCLC patients registered in TMH by using rapid and sensitive technique of RQ-PCR with *TaqMan*® primer probes, as well as to evaluate the actual prevalence of mutation types in NSCLC.

Material and Methods

This study has been done on 1018 cases that were referred for *EGFR* testing from medical oncology as a routine service over a 1.5-year period. This was part of standard care; therefore, no Institutional Review Board approval was obtained. The patient characteristics including the age, gender, smoking/tobacco use, and histopathology were recorded.

Collection of patient samples

The FFPE blocks of the patients were collected from the Pathology Department, TMH. The hematoxylin and eosin-stained sections of the blocks were viewed under the microscope to confirm that the tumor region constitutes more than 75% of the tissue mass.

DNA extraction

For DNA isolation, three FFPE tissue sections of 14 μ m each were taken using microtome (Leica). After deparaffinizing the sections with xylene,[14] the DNA was extracted as per the kit insert (QiaAmp FFPE tissue kit, Cat no 56404).

Mutation analysis by PCR and sequencing

Extracted DNA was amplified for the exons 18, 19, 20, and 21 using a nested-PCR method, [15–17] with a 100 ng DNA as the template as shown recently. With the above PCR products as template, second round of nested PCR amplification was carried out using a different set of primers specific for these regions. After the amplification of the regions of interest, the amplicons were then purified using the Qiaquick PCR purification kit (Qiagen). About 2.5 ng of the PCR product, along with 1.6 pmols of the forward or reverse primer was used for sequencing in the Applied Biosystems DNA Analyzer.[5] The resulting sequence was compared with the available normal sequence in the NCBI database using the NCBI BLAST and the variations observed thereupon were recorded.

Mutation analysis by *TaqMan*®-based real-time PCR technique

The reaction mixture was carried out in 10 μ L volumes, which contains 5 μ L of the *TaqMan* probe,[15,16] master mix (Roche), primers at a final concentration of 9 μ M and probes at a final concentration of 2 μ M and the remaining volume was made up to 5 μ L using PCR grade water. The instrument used for the assay was LC 480 II. The program used was, 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

The detectors used were FAM/VIC at 5' *and TAMRA/MGB at 3' fluorescence for the wildtype and mutant, respectively. The assay sensitivity has a limit of 1% detection and each run consisted of a positive and a negative control to check the integrity of the assay.

Statistical analysis: The Chi-square test was performed to reveal any significant correlation between the mutation status and age, gender, and habits of the patient and tumor histology.

Results

Total 1018 patients diagnosed with lung cancer in TMH from February 2011 to December 2012 were tested for common *EGFR* mutation subtypes by RQ-PCR using *TaqMan* primer probes in exon 18 point mutation, exon 19 in frame deletions, and exon 21 L858R point mutation. Exon 20 assay was incorporated from August 2012 onward in 215 cases. After standardization and validation with direct sequencing, the RQ-PCR test, which was found to be robust, rapid, and highly sensitive, was implemented as the routine diagnostic test.

Out of 1018, the maximum number of patients were from the west of India and lowest from the south of India, but the percentage positivity almost remained between 20% and 28% with highest in south and the west of India [Table 1]. Similar to our recent report (under communication), we observe an overall frequency of 25 % incidence of *EGFR* mutation in this extended population study. Of note, approximately 53% mutations were in-frame deletions in exon 19, whereas 38% are missense mutations in L858R in exon 21, 6% of the mutations were found in exon 18 and 3% in exon 20, which is similar to that described in Spanish and Japanese populations.[18–21] Exon 20 mutation analysis was performed in 215 patients, of whom 3% were positive. This is similar to that reported in the literature.[22] *EGFR* exon 20 insertions comprise approximately 4% of all *EGFR* mutant lung tumors [Table 2].

Our finding suggests exon 19 and 21 in ADC group of 53% and 38%, respectively, and 7% in exon 18, were similar to East Asian population with a mutation rate. In our earlier study, exon 19 mutation was present in 67% cases and exon 21 in 29% of cases, this difference could be because of the smaller sample size of 111 patients[17,23] [Table 3].

Correlating with the smoking status among *EGFR* mutation-positive patients, exon 19 mutations were found in 62% nonsmoker males and 57% females compared with 39% smoker males. However, exon 21 mutations were found in 46% male smokers and compared with 29% male nonsmokers. These results reveal that exon 19 mutations are predominant among nonsmokers, whereas exon 21 more frequently exists among smokers [Table 4].

Discussion

Despite historical evidence for decreasing admixture content of genetic influence while moving from North to South India, due to amalgamation of major ancestral populations over past thousands of years at different points of time, no significant difference was observed in the incidence of *EGFR* mutation across different geographical locations within India. Further study is needed to confirm these findings due to sparse representation of patients from South and Central India. However, within the cohort of different mutations, we

analyzed the clinical and pathological characteristics of lung ADC patients. Our results suggest that exon 21 mutation was more common in male smokers compared with nonsmokers, whereas exon 19 shows higher mutation rate in never-smokers than smokers, irrespective of gender bias. It has been reported that exon 18 EGFR mutations are detected more frequently in younger patients, which was also noted in our study.[24] However, Kosaka *et al.*, [7] found no association between EGFR mutations and age, whereas Huang *et al.*, [25] found no significant association between the presence of an EGFR mutation and gender or smoking history in Chinese patients.[26]

Conclusion

Geographically there is uniform distribution in the *EGFR* mutation frequency within India. Furthermore, while exon 19 mutations are predominant among nonsmokers, higher incidence of exon 21 mutations exists among *EGFR* mutation-positive male smokers of Indian ethnicity.

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References

1. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004; 304:1497–500. [PubMed: 15118125]
2. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004; 350:2129–39. [PubMed: 15118073]
3. D'Angelo SP, Pietanza MC, Johnson ML, Riely GJ, Miller VA, Sima CS, et al. Incidence of EGFR exon 19 deletions and L858R in tumor specimens from men and cigarette smokers with lung adenocarcinomas. *J Clin Oncol*. 2011; 29:2066–70. [PubMed: 21482987]
4. Jackman DM, Yeap BY, Sequist LV, Lindeman N, Holmes AJ, Joshi VA, et al. Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. *Clin Cancer Res*. 2006; 12:3908–14. [PubMed: 16818686]
5. Riely GJ, Pao W, Pham D, Li AR, Rizvi N, Venkatraman ES, et al. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. *Clin Cancer Res*. 2006; 12:839–44. [PubMed: 16467097]
6. Zhang Y, Sun Y, Pan Y, Li C, Shen L, Li Y, et al. Frequency of driver mutations in lung adenocarcinoma from female never-smokers varies with histologic subtypes and age at diagnosis. *Clin Cancer Res*. 2012; 18:1947–53. [PubMed: 22317764]
7. Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: Biological and clinical implications. *Cancer Res*. 2004; 64:8919–23. [PubMed: 15604253]
8. Gazdar AF. Activating and resistance mutations of EGFR in non-small-cell lung cancer: Role in clinical response to EGFR tyrosine kinase inhibitors. *Oncogene*. 2009; 28(Suppl 1):S24–31. [PubMed: 19680293]

9. Kumar A, Petri ET, Halmos B, Boggon TJ. Structure and clinical relevance of the epidermal growth factor receptor in human cancer. *J Clin Oncol.* 2008; 26:1742–51. [PubMed: 18375904]
10. Arcila ME, Nafa K, Chaft JE, Rekhtman N, Lau C, Reva BA, et al. EGFR exon 20 insertion mutations in lung adenocarcinomas: Prevalence, molecular heterogeneity, and clinicopathologic characteristics. *Mol Cancer Ther.* 2013; 12:220–9. [PubMed: 23371856]
11. Suda K, Onozato R, Yatabe Y, Mitsudomi T. EGFR T790M mutation: A double role in lung cancer cell survival? *J Thorac Oncol.* 2009; 4:1–4. [PubMed: 19096299]
12. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: Preclinical data and clinical implications. *Lancet Oncol.* 2012; 13:e23–31. [PubMed: 21764376]
13. Reich D, Thangaraj K, Patterson N, Price AL, Singh L. Reconstructing Indian population history. *Nature.* 2009; 461:489–94. [PubMed: 19779445]
14. Cronin M, Pho M, Dutta D, Stephans JC, Shak S, Kiefer MC, et al. Measurement of gene expression in archival paraffin-embedded tissues: Development and performance of a 92-gene reverse transcriptase-polymerase chain reaction assay. *Am J Pathol.* 2004; 164:35–42. [PubMed: 14695316]
15. Endo K, Konishi A, Sasaki H, Takada M, Tanaka H, Okumura M, et al. Epidermal growth factor receptor gene mutation in non-small cell lung cancer using highly sensitive and fast TaqMan PCR assay. *Lung Cancer.* 2005; 50:375–84. [PubMed: 16199108]
16. Molina-Vila MA, Bertran-Alamillo J, Reguart N, Taron M, Castellà E, Llatjós M, et al. A sensitive method for detecting EGFR mutations in non-small cell lung cancer samples with few tumor cells. *J Thorac Oncol.* 2008; 3:1224–35. [PubMed: 18978556]
17. Noronha V, Prabhash K, Thavamani A, Chougule A, Purandare N, Joshi A, et al. EGFR Mutations in Indian Lung Cancer Patients: Clinical Correlation and Outcome to EGFR Targeted Therapy. *PLoS One.* 2013; 8:e61561. [PubMed: 23620765]
18. Cortes-Funes H, Gomez C, Rosell R, Valero P, Garcia-Giron C, Velasco A, et al. Epidermal growth factor receptor activating mutations in Spanish gefitinib-treated non-small-cell lung cancer patients. *Ann Oncol.* 2005; 16:1081–6. [PubMed: 15851406]
19. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med.* 2009; 361:947–57. [PubMed: 19692680]
20. Pan-Chyr, Yang, Y, S., Joseph, Siu-kieAu, et al. Molecular epidemiological prospective study of EGFR mutations from Asian patients (pts) with advanced lung adenocarcinoma. *J Clin Oncol.* 2012; 30 (suppl; abstr 1534)).
21. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst.* 2005; 97:339–46. [PubMed: 15741570]
22. Jang TW, Oak CH, Chang HK, Suo SJ, Jung MH. EGFR and KRAS mutations in patients with adenocarcinoma of the lung. *Korean J Intern Med.* 2009; 24:48–54. [PubMed: 19270482]
23. Noronha V, Dikshit R, Raut N, Joshi A, Pramesh CS, George K, et al. Epidemiology of lung cancer in India: Focus on the differences between non-smokers and smokers: A single-centre experience. *Indian J Cancer.* 2012; 49:74–81. [PubMed: 22842172]
24. Sun MH, Yang F, Shen L, Zhang L, Chen Y, Cai X, et al. Detection of epidermal growth factor receptor mutations in non-small-cell lung carcinoma by direct sequencing and correlations with clinicopathological characteristics and sample types. *Zhonghua Bing Li Xue Za Zhi.* 2011; 40:655–9. [PubMed: 22321541]
25. Huang SF, Liu HP, Li LH, Ku YC, Fu YN, Tsai HY, et al. High frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. *Clin Cancer Res.* 2004; 10:8195–203. [PubMed: 15623594]
26. Li M, Zhang Q, Liu L, Liu Z, Zhou L, Wang Z, et al. The different clinical significance of EGFR mutations in exon 19 and 21 in non-small cell lung cancer patients of China. *Neoplasma.* 2011; 58:74–81. [PubMed: 21067269]

Table 1

Geographical distribution of EGFR mutations within India

EGFR mutations	Region				
	Central	East	North	South	West
Positive (%)	8 (19.5)	65 (22.8)	47 (23.4)	4 (26.7)	131 (27.5)
Wildtype	33	220	154	11	345
Total	41	285	201	15	476

EGFR=Epidermal growth factor receptor

Table 2

Frequency and overall mutation rate of different variables in NSCLC (1018)

Variables	Mutations	Total	Percentage	Significance
Over all mutation rate	255	1018	25.0	
Male	146	700	21.0	
Female	109	318	34.0	
Smoking status				
Smokers	61	382	16	
Never-smokers	188	597	31.5	
Not available	6	39	15.0	
Histopathology				
Adenocarcinomas	245	885	27.7	<i>P</i> <0.001
Squamous	6	107	5.6	
Others	4	26	15.0	
ADC Never-smoker female	107	288	37.2	
Incidence of subtypes of EGFR mutations				
Exon 19	135	255	52.9	
Exon 21	97	255	38.0	
Exon 18	15	255	5.8	
Exon 20	06	215	2.8	
Both (exon 19,21)	02	255	0.8	

ADC=Adenocarcinoma; EGFR=Epidermal growth factor receptor; NSCLC=Non-small cell lung cancer

Table 3

Correlation of EGFR mutations with histopathology

Reports	Pathology reports (N=1018)			
	Adenocarcinoma (N=885)	Squamous (N=107)	Adenosquamous (N=13)	Others (N=13)
Exon 18	15	0	0	0
Exon 19	129	3	3	0
Exon 20	5	1	0	0
Exon 21	94	2	1	0
Both	2	0	0	0
Wildtype	640	101	9	13

EGFR=Epidermal growth factor receptor

Table 4

Correlation of EGFR mutations with smoking status

N=1018	Male (N=700)			Female (N=318)		
	Smoker (n=372)	Never-smoker (n=293)	NA (n=35)	Smoker (n=10)	Never-smoker (n=304)	NA (n=4)
Both	1	1	0	0	0	0
18.0	8	2	1	0	4	0
19.0	23 (39%)	49 (62%)	2	0	60 (57%)	1
21.0	27 (46%)	27 (34%)	2	0	41 (39%)	0
Subtotal	59	79	5	0	105	1
Wildtype	313	214	30	10	199	3

EGFR=Epidermal growth factor receptor; NA=Not available