INTERNAL MEDICINE JOURNAL

High proportion of rare and compound epidermal growth factor receptor mutations in an Australian population of non-squamous non-small-cell lung cancer

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Key words
non-small-cell lung cancer, epidermal growth factor receptor, receptor, epidermal growth factor/antagonist and inhibitor, DNA sequence analysis, mutation.

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Received 21 April 2014; accepted 7 September 2014.

doi:10.1111/imj.12587

Abstract

Background: Epidermal growth factor receptor (EGFR) mutation positivity in primary non-small-cell lung cancer (NSCLC) may confer increased sensitivity to EGFR tyrosine kinase inhibitor (TKI) therapy with improved progression-free survival over EGFR wild-type tumours. Some mutation subtypes may not confer such TKI sensitivity. The incidence of rare and compound subtypes in the Australian lung cancer population is not fully defined.

Aims: The aim of the study was to audit the incidence of EGFR mutation in serial cases of primary non-squamous NSCLC presenting to two multidisciplinary team meetings in metropolitan Sydney for incidence, type of mutation and phenotypic association with mutation positivity.

Methods: Serially presenting cases of primary non-squamous NSCLC were tested for EGFR mutation. The cases presented to either of two multidisciplinary team meetings in metropolitan Sydney and were referred for EGFR mutation testing on the basis of non-squamous NSCLC histopathology. Samples from the two sites were analysed for EGFR mutation at one of three different laboratories, each using a slightly different assay. Data on phenotypic characteristics, smoking history and clinicopathological features of the tumour were collected.

Results: There is a relatively high incidence of EGFR mutation in non-squamous NSCLC in a series of patients drawn from two metropolitan multidisciplinary team meetings in Sydney at a rate of 23.8%. A high proportion of rare and compound EGFR mutations were identified (6/32 mutation positive cases, 18.8%).

Conclusions: The incidence of EGFR mutation may be higher in Australian populations than in other populations of predominantly European origin. Rare and compound EGFR mutations may occur and may have implications for treatment that differ from classically activating mutations.

Introduction

The presence of specific driver mutations in primary lung cancer can dictate response to targeted therapy and may give the patient with advanced disease, at least initially, the chance for a radically different short-term outcome than that expected with previous therapeutic strategies. This is most well established in the case of epidermal growth factor receptor (EGFR) mutations where tumours may demonstrate complete radiological response to targeted therapy. Multiple first-line studies support the use of targeted therapy in EGFR mutation positive disease,[1–6] as does a recent comprehensive meta-analysis.[7] The role of EGFR mutational analysis and subsequent targeted therapy for mutation positive tumours is not in dispute, although international regulatory differences influence the routine availability of testing and targeted therapy. In Australia, in a relatively short time period, EGFR mutational analysis has gone from very limited availability at just a single institution

Funding: Roche Pty Ltd provided funding for EGFR analysis for a proportion of the St Vincent's Hospital EGFR mutation testing as a pilot project.
Conflict of interest: None.

(Peter MacCallum Cancer Institute, Victoria) to widespread availability in multiple institutions nationally with a subsequent reduction in turnaround time. Testing remains partially funded at the government level and first-line use of tyrosine kinase inhibitor (TKI) therapies has not always been based on the available evidence and has evolved rapidly over the 4 years since the IRESSA Pan-Asia Study[1] was published. This project combines EGFR mutational analysis data from two institutions, obtained during the course of separate projects over a similar time period. Both datasets were collected during a time in Australia when availability of EGFR mutational analysis was relatively limited. Rare and compound (double or complex) EGFR mutations have been reported in routine analysis of exons 18–21;[8] the incidence in the Australian setting is not established. This study aimed to audit EGFR testing in these two institutions, looking in particular at EGFR mutation incidence, types of mutation and phenotypic association with mutation positivity.

Methods

The investigators conducted an audit of cases of non-squamous non-small-cell lung cancer (NSCLC) presented to two metropolitan multidisciplinary team meetings between October 2010 and March 2013. The cases were all subject to prospective testing for EGFR mutation. At one of the institutions (Nepean Hospital), the data were then collected retrospectively as part of an audit. At the other institution (St Vincent's Hospital), the data were collected prospectively as part of an investigator-initiated study. All testing was done with patient consent. The data were collected with local ethical approval (Nepean Hospital Ethics Committee Approval Number HREC 12/30, St Vincent's Hospital Ethics Committee Approval Number HREC 11/130). Data points collected included type of tissue sample, histopathology, stage, ethnicity, gender, smoking status, presence of mutation and subtype and subsequent treatment. Samples were analysed in one of three laboratories with either real-time polymerase chain reaction (PCR) (St Vincent's Hospital), DNA sequencing (Healthscope Pathology) or high-resolution melting (HRM) then DNA sequencing (Peter MacCallum Cancer Centre). Real-time PCR analysis used the Cobas 4800 EGFR Mutation detection assay (Roche Australia, Sydney, NSW, Australia) as per the manufacturer's instructions. DNA sequencing was carried out following standard protocol for Sanger dideoxy-based sequencing (BigDye terminator sequencing kit, Life Technologies Ltd, Melbourne, Vic., Australia). The DNA fragments sequenced were either products of EGFR exon-specific nested PCR amplification or HRM analysis.[9] Exons 18–21 of the EGFR genes were analysed in all centres.

Table 1 Patient characteristics

Variable	All, *n* = 134 (%)	EGFR mutation detected, *n* = 32 (%)
Gender		
Female	72 (53.7)	24 (75.0)
Male	62 (46.3)	8 (25.0)
Median age		
Year (±SD, range)	66 (+12, 39–89)	65 (+12, 39–89)
Smoker		
Never	35 (26.1)	18 (56.2)
Ex or current	90 (67.3)	14 (43.8)
Missing data	9 (6.7)	0
Race		
Caucasian	117 (87.3)	25 (78.1)
Asian	6 (4.5)	3 (9.3)
Indian	2 (1.5)	2 (6.3)
Pacific Island	9 (6.7)	2 (6.3)
Stage		
Early (I–IIIA)	44 (32.8)	7 (21.9)
Advanced (IIIB–IV)	90 (67.2)	25 (78.1)
Biopsy type		
Surgical	51 (38.1)†	13 (40.6)
FNA	29 (21.6)‡	6 (18.8)
Core	31 (23.1)§	7 (21.9)
EBUS	18 (13.4)¶	5 (15.6)
Other	4 (3.0)††	1 (3.1)
Unconfirmed	1 (0.8)††	0

†2/51 (3.9%) surgical samples were insufficient for mutation analysis. ‡4/29 (13.8%) FNA samples were insufficient for mutation analysis. §3/31 (9.7%) core biopsy samples were insufficient for mutation analysis. ¶3/18 (16.7%) EBUS samples were insufficient for mutation analysis. ††All sufficient for mutation analysis. EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; EGFR, epidermal growth factor receptor; FNA, fine-needle aspiration; SD, standard deviation.

Results

One hundred and thirty-four cases of primary non-squamous NSCLC were analysed; 69 cases from St Vincent's and 65 from Nepean (Table 1). The average age of the patients was 66 (±12) years. Sixty-two were male (46.3%), 117 were confirmed Caucasian (87.3%) and 35 (26.1%) were confirmed non-smokers. Of the EGFR mutation positive cases, the classical phenotype of Asian, non-smoking female applied to 2/32 (6%). There were 44 (32.8%) cases of early-stage disease (I–IIIA); 90 cases (67.2%) were advanced disease (stage IIIB–IV). Biopsy samples were sufficient for molecular analysis in 122 (91.0%) cases. Of the tissue sample types, 51 were surgical resection specimens, 29 fine-needle aspiration (FNA) biopsy samples, 31 core biopsies, 18 endobronchial

Table 2 Mutation subtypes

	Exon	Mutation	*n* = 32 (%)
Classical activating,	19	Deletion	16 (50.0)
n = 22	21	p.L858R	6 (18.8)
TKI resistance, *n* = 4	20	Insertion	3 (9.4)
	20 + 21	p.[T790M(;)L858R]	1 (3.1)
Rare, *n* = 6	18	p.G719X	2 (6.3)
	20	p.V774delinsHC†	1 (3.1)
	20	p.A767_V769dup†	1 (3.1)
	20 + 20	p.[S768I(;)D770_N71insG]	1 (3.1)
	21	p.P848L‡	1 (3.1)

†Novel mutation. ‡Occurred with KRAS mutation Exon 2p.G12V. TKI, tyrosine kinase inhibitor.

Table 3 EGFR mutation subtype according to assay

Assay	Exon	Mutation	Number (% per assay)
DNA sequencing,	18	p.G719A	1 (9.1)
n = 11	19	Deletion	4 (36.4)
	20	Insertion	3 (27.2)
	20	p.V774delinsHC†	1 (9.1)
	20 + 20	p.[S768I(;)D770_N71insG]†	1 (9.1)
	20 + 21	p.[T790M(;)L858R]	1 (9.1)
HRM + DNA	19	Deletion	5 (62.5)
sequencing,	20	p.A767_V769dup†	1 (12.5)
n = 8	21	p.L858R	2 (25.0)
RT-PCR, *n* = 13	18	p.G719X †	1 (7.7)
	19	Deletion	7 (53.8)
	21	p.P848L†	1 (7.7)
	21	p.L858R	4 (30.8)

†Rare mutation. EGFR, epidermal growth factor receptor; HRM, high-resolution melting; RT-PCR, reverse transcription polymerase chain reaction.

ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) biopsies and four were other types of sample, including transbronchial biopsy and pleural fluid. One sample was of unconfirmed origin. Insufficient samples for mutation analysis ($n = 12$) came from surgical samples (2/51), FNA (4/29), core biopsy (3/31) and EBUS-TBNA (3/18).

EGFR mutation analysis detected the presence of mutation in 32/134 (23.8%) cases. Of the 32 positive cases, mutation subtypes (Table 2) showed that 22 (68.8%) were classical activating mutations, four (1.52%) were non-activating/resistance mutations and six (18.8%) were rare mutations. Two mutations presented are not in the Catalogue of Somatic Mutations in Cancer database[10] or other locus-specific databases and are therefore considered to be novel: the p.V774delinsHC mutation and the p.A767_V769dup mutation. One EGFR mutation occurred in conjunction with a KRAS mutation, p.G12V. The two institutions used different EGFR mutation assays (Table 3). All cases from Nepean were analysed by DNA sequencing with a positive mutation rate of 11/65 (16.9%). Cases from St Vincent's were analysed by HRM plus DNA sequencing (30 cases, 8/30 positive, 26.7%) or by real-time PCR (39 cases, 13/39 positive, 33.3%) with a total EGFR mutation rate of 21/69 (30.4%). All three detection methods identified rare or compound mutations (Table 3).

Patients received treatment according to attending physician (Table 4). Of 22 cases with classical activating mutations, 11 (50.0%) received first-line TKI therapy, two (9.1%) received second-line TKI therapy and seven (31.8%) received no TKI therapy. A further two cases (9.1%) were lost to follow up. Of the seven cases that received no TKI therapy, four were early-stage disease treated surgically, one received chemotherapy, one received radiotherapy only and one case had comorbidities that prevented use of TKI therapy. Of the four cases with non-classical activating/resistance mutations, all received first-line TKI. Of the six cases with rare and compound mutations, three received first-line TKI therapy, one received second-line TKI and two did not receive TKI therapy because of comorbidities.

Discussion

EGFR mutation status is now a standardised part of primary lung adenocarcinoma diagnosis. The prognostic implications and response to targeted therapy in mutation positive tumours are clear, at least for classically activating mutations. Our groups actively test all cases of locally advanced or metastatic primary lung adenocarcinoma, in line with international practice, but at least initially ahead of policy in this country. In the study cohorts presented here, early-stage disease was also tested for EGFR mutation. The overall incidence in this study, completed across two demographically distinct parts of a major metropolitan centre, was 23.8%, slightly higher than other predominantly Caucasian populations

Table 4 Treatment received

Mutation	First-line TKI	Second-line TKI	No TKI	Lost to follow up	Total
Classical activating	11	2	7†	2	22
TKI resistance	4	0	0	0	4
Rare	3	1	2	0	6
Total	18	3	9	2	32

†4/7 had early stage disease treated surgically, 1/7 had standard chemotherapy, 1/7 had radiotherapy only and 1/7 had comorbidities that prevented TKI. TKI, tyrosine kinase inhibitor.

published in other studies[11–13] and similar to data on incidence on the Indian subcontinent.[14] There was a difference between the two centres in this study; the more suburban centre (Nepean) had an incidence of EGFR mutation positivity of 11/65 (16.5%) as expected in Caucasian populations; the inner city centre (St Vincent's) had an incidence of 21/69 (30.4%). In the whole study cohort, 87.3% of patients were of confirmed Caucasian background. Nepean had 55/65 (86.2%) Caucasian patients; St Vincent's had 61/69 (88.4%) Caucasian patients. Only 2/32 (6.3%) of the EGFR mutation positive cases were Asian, non-smoking females. There is no clear reason for this relatively high incidence of EGFR mutation in a predominantly Caucasian population; however, differences in testing and possible differences in genetic admixture need to be considered.

The different methodologies used to detect EGFR mutations at the two centres may have contributed to the different incidence of EGFR mutations observed. The two methodologies used for samples from St Vincent's Hospital can detect mutations at a lower mutation load than that from the Nepean Hospital. As a result, the false negative rate among the Nepean Hospital patients may have been higher than that from St Vincent's Hospital. Samples adequate for testing were derived from various biopsy types, including cytological specimens from fine-needle aspirates and pleural aspirates. A small number of samples was inadequate for testing, including two surgical samples, despite multiple attempts at testing, thought to relate to imperfect tissue handling early in the EGFR testing programme at the relevant institution.

This study identified a relatively high incidence of rare and compound EGFR mutations with poorly understood clinical implications. Keam *et al.*[15] identify varying responses to TKI between three groups of mutations (classic, compound with classic + rare, rare alone) and found that progression-free survival (PFS) was poorer in rare and compound mutations. We report one case with a rare EGFR mutation (p.P848L, COSM22943) in association with a KRAS mutation (p.G12V). Occasional cases have combined EGFR/KRAS mutations with variable responses to TKI therapy.[16,17] TKI resistance mutations, such as p.T790M, are associated with a reduced response to TKI therapy.[18] The implication of rare and compound mutations is not well established, although the mutations appear to be associated with poorer PFS than classically activating mutations in the limited available reports.[15] Yang *et al.*[19] analysed data from three phase III studies of afatinib, an irreversible EGFR TKI. Seventy-five patients with uncommon mutations were identified with variable response to therapy; lower response rates were identified in patients with de novo p.T790M mutations and exon 20 insertions. Three of six rare and compound mutations identified in our study were exon 20 insertions.

This study has several strengths in the investigation of EGFR mutation incidence in our population. EGFR mutation analysis was done prospectively on unselected serial cases of primary non-squamous NSCLC. Cases were drawn from two subpopulations of a large metropolitan centre, one outer suburban and one inner urban, with varying incidences of EGFR mutation positivity. Ethnic diversity was evident across the study population and did not appear to be strongly associated with EGFR mutation positivity. Due to the investigational nature of the study, EGFR testing was not limited to advanced disease, giving a possibly more complete picture of the incidence of mutation in our population. There are several limitations to this study. One limitation of the study derives from the different EGFR mutation assays used across the study group. Real-time PCR has high sensitivity and a low limit of detection (smaller tissue sample required). However, this technique only detects specific mutations; it may not differentiate which mutation is present and may not detect rare mutations. HRM followed by DNA sequencing has high sensitivity and a low limit of detection and can differentiate between mutations detected. However, it could potentially miss some mutations (if not detected by HRM analysis). DNA sequencing alone has high sensitivity but a higher limit of detection (i.e. need more tumour present in sample) and therefore limited sensitivity in low tumour percentage samples. However, this method can detect all mutations and can differentiate between them.

Conclusion

The incidence of EGFR mutation in primary lung adenocarcinoma/non-squamous NSCLC in an Australian population is slightly higher than other predominantly Caucasian populations. There is a relatively high incidence in this study cohort of rare and compound EGFR mutations, including a previously unreported EGFR/KRAS combined mutation.

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