

RESEARCH ARTICLE

Epidermal Growth Factor Receptor Mutations in Non-Small Cell Lung Cancers in a Multiethnic Malaysian Patient Population

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Abstract

Background: Mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) in non-small cell lung cancer (NSCLC) are predictive of response to EGFR-targeted therapy in advanced stages of disease. This study aimed to determine the frequency of EGFR mutations in NSCLCs and to correlate their presence with clinical characteristics in multiethnic Malaysian patients. **Materials and Methods:** In this prospective study, EGFR mutations in exons 18, 19, 20 and 21 in formalin-fixed paraffin-embedded biopsy specimens of consecutive NSCLC patients were assessed by real-time polymerase chain reaction. **Results:** EGFR mutations were detected in NSCLCs from 55 (36.4%) of a total of 151 patients, being significantly more common in females (62.5%) than in males (17.2%) [odds ratio (OR), 8.00; 95% confidence interval (CI), 3.77-16.98; $p < 0.001$] and in never smokers (62.5%) than in ever smokers (12.7%) (OR, 11.50; 95% CI, 5.08-26.03; $p < 0.001$). Mutations were more common in adenocarcinoma (39.4%) compared to non-adenocarcinoma NSCLCs (15.8%) ($p = 0.072$). The mutation rates in patients of different ethnicities were not significantly different ($p = 0.08$). Never smoking status was the only clinical feature that independently predicted the presence of EGFR mutations (adjusted OR, 5.94; 95% CI, 1.94-18.17; $p = 0.002$). **Conclusions:** In Malaysian patients with NSCLC, the EGFR mutation rate was similar to that in other Asian populations. EGFR mutations were significantly more common in female patients and in never smokers. Never smoking status was the only independent predictor for the presence of EGFR mutations.

Keywords: EGFR mutation - multiethnic - non-small cell lung cancer - smoking - Malaysian

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Introduction

Lung cancer is the leading cause of cancer death worldwide including Malaysia (Parkin et al, 2005; National Cancer Registry, Malaysia, 2006). In Malaysia, lung cancer accounts for 13.8% of all cancers in males and 3.8% of all cancers in females (National Cancer Registry, Malaysia, 2006) and is the leading cause of cancer death in men and ranks fifth overall in women (Department of Statistics, Malaysia, 2006). Non-small-cell lung cancer (NSCLC) histologies (adeno-, squamous cell- and large-cell carcinoma) account for 80-85% of all lung cancers (Jemal et al., 2006). In recent years, the incidence of lung adenocarcinoma has increased at the expense of squamous cell carcinoma worldwide and in Malaysia (Rivera et al., 2001; Liam et al., 2006).

The epidermal growth factor receptor (EGFR) signalling pathway has been identified as a therapeutic target in lung cancer (Herbst et al., 2008). The finding that 'classical' activating mutations in the tyrosine kinase

domain of the EGFR gene are predictive of treatment response to EGFR tyrosine kinase inhibitors such as gefitinib has led to a paradigm shift in the management of advanced NSCLC (Mok et al., 2009). These specific EGFR mutations are also reported to be associated with favourable clinical prognostic features in NSCLC which include East Asian ethnicity, female gender, never-smoking status and adenocarcinoma histology (Lynch et al. 2004; Paez et al., 2004; Cortes-Funes et al., 2005; Han et al., 2005; Mitsudomi et al., 2005; Takano et al., 2005; Taron et al., 2005).

This study aimed to determine the frequency of EGFR mutations in Malaysian patients with NSCLC since this has not been reported. The multiethnic Malaysian population consisting of three major ethnic groups of Malays (54.6%), Chinese (24.6%) and Indians (7.3%), plus other ethnic minorities (13.5%) (Department of Statistics, Malaysia, 2010) provides a unique opportunity to determine the influence of ethnicity, gender, age and smoking status on the frequency of such mutations.

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Materials and Methods

Patients

In this prospective study, consecutive biopsy confirmed NSCLC patients who attended the University of Malaya Medical Centre and Hospital Tengku Ampuan Afzan, two centres that treat a relatively large number of lung cancer patients in Malaysia, from 1st August 2010 to 30th December 2011 were recruited. The study was approved by the respective hospital ethics committee and written informed consent was obtained from all patients. The patients were categorised as never-smokers if they had smoked less than 100 cigarettes in their lifetime, former smokers if they had smoked more than 100 cigarettes in their lifetime but had stopped smoking at least one year, or current smokers if they had smoked more than 100 cigarettes in their lifetime and were still smoking or had stopped smoking less than a year (Ebbert et al., 2003; Sequist et al., 2011). The patient's performance status at diagnosis was categorised in accordance to the Eastern Cooperative Oncology Group (ECOG) performance status (Oken et al., 1982). All patients underwent baseline clinical examination and computed tomography examination (with contiguous slices of 10 mm) of the thorax and upper abdomen upon diagnosis. Staging of disease was based on the 2009 International Staging System for Lung Cancer (Goldstraw, 2009). CT scan of the brain was performed if the patient had neurological symptoms or signs.

EGFR mutation detection

Genomic deoxyribonucleic acid (DNA) was extracted from 10% formalin-fixed paraffin embedded biopsy specimens from the patients. Routine histopathological confirmation and typing of the NSCLC was performed and the presence of adequate tumour tissue was verified by the pathologists (JP and PR). Macrodissection (i.e. scraping using a scalpel) was carried out on unstained paraffin embedded slides of sections of the tumour in the Pathology Laboratory, Sime Darby Medical Centre to enrich tumour DNA, and therefore reducing contamination by normal DNA. Tumour DNA was extracted using QIAamp[®] DNA FFPE Tissue Kit (QIAamp[®] DNA FFPE Tissue Handbook, 2007) (50) Cat. No 56404. (QIAGEN GmbH, D-40724 Hilden, Germany) at the Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Malaysia. The commercially available, QIAGEN EGFR RGQ PCR Kit (EGFR RGQ PCR Kit Handbook, 2011) (24) Cat. No. 870101 (QIAGEN Manchester Ltd., UK) was used for EGFR mutation detection. The kit allowed the detection of 29 most prevalent EGFR somatic mutations in the known EGFR oncogene (Herbst et al., 2008; Maheswaran et al., 2008; Liang et al., 2010). The excellent sensitivity of the kit enabled the detection of low-level somatic mutations in the sample. Nineteen deletions in exon 19 (the kit detected the presence of any of 19 deletions but did not distinguish between them); T790M; L858R; L861Q; G719X (the kit detected the presence of G719S, G719A or G719C but did not distinguish between them); S768I; 3 insertions in exon 20 (the kit detected the presence of

any of 3 insertions but did not distinguish between them) could be detected by this kit in real-time polymerase chain reaction (PCR) on the Rotor-Gene Q instrument based on Scorpions[®] and Amplification Refractory Mutation System (ARMS[®]) technologies. The methods used in this kit were highly selective and were able to detect DNA copies of even below 10 copies and with the presence of only 1% of somatic mutant in a background of wild-type genomic DNA (Wang et al., 2010).

Statistical analysis

Results are expressed as the mean±standard deviation (SD) or the median and range for continuous variables or as a percentage for categorical variables. In univariate analyses, differences between groups were tested for significance with chi-square test with Yates' correction or Fisher exact test for categorical variables and Student's t-test for continuous variables. Variables associated with EGFR mutation positivity and with p values less than 0.25 in univariate analyses were used simultaneously in multivariate logistic regression analyses to determine demographic or clinical characteristics independently associated with EGFR mutation positivity. Results of logistic regression analyses were reported as odds ratios (ORs) with 95% confidence intervals (CI) and p values, taking p values <0.05 as the level of statistical significance. All significance testing was two sided. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS for windows version 19.0, SPSS Inc., Chicago, IL, USA).

Results

Patient demographic and clinical characteristics

The demographic and clinical characteristics of 120 patients and 31 patients with NSCLC studied at the University of Malaya Medical Centre and Hospital Tengku Ampuan Afzan, respectively are shown in Table 1. The Chinese was the predominant ethnic group followed by the Malays, Indians, Singhalese (two patients) and Orang Asli (indigenous people) (one patient). Almost all female patients [59/64 (92.2%)] compared to 13 (14.9%) of 87 male patients were never-smokers (OR, 67.17; 95%CI, 22.69-199.11; p<0.001). Adenocarcinoma was the predominant cell type accounting for 87.4% of the cases. The majority of the patients had stage IV disease (81.5%) and good performance status (ECOG 0 or 1) (69.5%) at diagnosis.

EGFR mutation analysis results

EGFR mutations were detected in the NSCLC of 55 (36.4%) patients. Of these 55 patients, deletion mutations in exon 19 were detected in 40 (72.7%) and substitution mutations in exon 21 were detected in 15 (27.3%). Mutations at exon 18 and exon 20 were not detected and there were no multiple mutations. Of the 132 patients with adenocarcinoma, EGFR mutations were detected in 52 (39.4%) patients.

Comparison of the demographic and clinical characteristics of the patients according to the EGFR mutation status (Table 1) showed that EGFR mutations

Table 1 Demographic and Clinical Characteristics of 151 Patients and Comparison of These Characteristics According to EGFR Mutation Status

Characteristic		No. of patients (N=151)	EGFR mutation		p value of univariate analysis
			Positive (n=55)	Negative (n=96)	
Gender, No. (%)	Female	64	40 (62.5%)	24 (37.5%)	<0.001
	Male	87	15 (17.2%)	72 (82.8%)	
Age (year)	Mean (\pm SD)	61.3 \pm 11.2	59.5 \pm 12.1	62.3 \pm 10.5	0.14
	Range	29-92			
Age group, No. (%)	<65 years	87	35 (40.2%)	52 (59.8%)	0.336
	\geq 65 years	64	20 (31.2%)	44 (68.8%)	
Ethnicity, No. (%)	Chinese	87	34 (39.1%)	53 (60.9%)	0.08
	Malay	54	16 (29.6%)	38 (70.4%)	
	Indian	7	5 (71.4%)	2 (28.6%)	
	Other	3	0 (0%)	3 (100%)	
Smoking status, No. (%)	Never smoker	72	45 (62.5%)	27 (37.5%)	<0.001
	Former smoker	44	8 (18.2%)	36 (81.8%)	
	Current smoker	35	2 (5.7%)	33 (94.3%)	
Histological subtype of NSCLC, No. (%)	Adenocarcinoma	132	52 (39.4%)	80 (60.6%)	0.265
	Squamous cell carcinoma	11	1 (9.1%)	10 (90.9%)	
	Adenosquamous carcinoma	1	0 (0%)	1 (100.0%)	
	Large cell carcinoma	1	0 (0%)	1 (100.0%)	
	NSCLC not otherwise specified (NOS)	6	2 (33.3%)	4 (66.7%)	
ECOG performances status, No. (%)	0	26	12 (46.2%)	14 (53.8%)	0.642
	1	79	28 (35.4%)	51 (64.6%)	
	2	23	9 (39.1%)	14 (60.9%)	
	3	13	4 (30.8%)	9 (69.2%)	
	4	10	2 (20.0%)	8 (80.0%)	
Disease stage, No. (%)	IA	4	3 (75.0%)	1 (25.0%)	0.059
	IB	4	0 (0%)	4 (100.0%)	
	IIA	2	0 (0%)	2 (100.0%)	
	IIB	2	1 (50.0%)	1 (50.0%)	
	IIIA	7	2 (28.6%)	5 (71.4%)	
	IIIB	9	0 (0%)	9 (100.0%)	
	IV	123	49 (39.8%)	74 (60.2%)	

were significantly more common in females (62.5%) than in males (17.2%) (OR, 8.00; 95% CI, 3.77-16.98; $p < 0.001$) and in never smokers (62.5%) than in ever smokers (12.7%) (OR, 11.50; 95% CI, 5.08-26.03; $p < 0.001$). The mean age of patients with EGFR mutation-positive and that of those with EGFR mutation-negative tumours were not significantly different. However, patients with exon 19 mutation-positive tumours were significantly younger [mean, 57.4 (\pm 12.6) years] than those with exon 21 mutation-positive tumours [mean, 65.1 (\pm 8.7) years] [mean difference, 7.8 (\pm 3.6) years; 95% CI, 0.7-14.9; $p = 0.033$]. The intensity of smoking (in pack-years) was not significantly different between patients with exon 19 mutation-positive tumours [4.7 (\pm 20.3) pack-years] and those with exon 21 mutation-positive tumours [8.3 (\pm 17.8) pack-years] [mean difference, 3.6 (\pm 6.3) pack-years; 95% CI, 9.1-16.2; $p = 0.575$].

The EGFR mutation rates of 39.1% in 87 Chinese, 29.6% in 54 Malay, 71.4% in seven Indian and 0% in three patients of other ethnicities were not significantly different ($p = 0.080$). Six of the seven Indian patients were females and all were never-smokers and all had adenocarcinoma subtype. There was no significant difference in the proportions of Indian patients [5 of 7 (71.4%)] and non-Indian patients [50 of 144 (34.7%)] with EGFR mutation-positive tumours (OR, 4.70; 95% CI, 0.88-25.10; $p = 0.100$).

EGFR mutations were detected in 52 (39.4%) of 132 adenocarcinomas, one (9.1%) of 11 squamous cell carcinomas, none of one adenosquamous carcinoma, none of one large cell carcinoma and two (33.3%) of six

NSCLCs not otherwise specified ($p = 0.265$). The EGFR mutation rate was higher in adenocarcinoma (39.4%) compared to non-adenocarcinoma NSCLC (15.8%) (OR, 3.47; 95% CI, 0.97-12.49; $p = 0.072$). Of the 132 patients with adenocarcinoma, 43 (63.2%) of 68 never smokers and nine (14.1%) of 64 ever smokers had EGFR mutation-positive tumours ($p < 0.0001$). The only EGFR mutation-positive squamous cell carcinoma was from a never smoker while the squamous cell carcinomas from all 10 smokers were EGFR mutation-negative.

The ECOG performance status at the time of diagnosis was not significantly different between patients with EGFR mutation-positive [40 (72.7%) of 55 with good ECOG PS of 0-1] and mutation-negative tumours [65 (76.7%) of 96 with good ECOG PS of 0-1] ($p = 0.645$). Similarly, the stage of disease at diagnosis was not significantly different between patients with EGFR mutation-positive [49 (89.1%) of 55 had stage IIIB or IV disease] and mutation-negative tumours [83 (86.5%) of 96 had stage IIIB or IV disease] ($p = 0.830$).

Multivariate analysis with gender (female versus male), smoking history (never smoker versus ever smoker), ethnicity (Indian versus non-Indian), and NSCLC histological subtype (adenocarcinoma versus non-adenocarcinoma) as covariates showed that only a never smoking status (adjusted OR, 5.94; 95% CI, 1.94-18.17; $p = 0.002$) but not gender, ethnicity or NSCLC histological subtype was independently associated with EGFR mutation positivity.

Discussion

The EGFR mutation frequency of 36.4% in the NSCLC of our patients who comprised of Chinese, Malay, Indian and other ethnicities is similar to that of 30% to 50% reported in East Asian populations including Chinese and Korean patients (Huang et al., 2004; 2011; Sun et al., 2012). On the other hand, EGFR mutation rates are lower in Western population ranging from 13% to 19% in European (Pallis et al., 2007; Rosell et al., 2009), 13% in North American (Sequist et al., 2011) and 32.2% Latin-American NSCLC patients (Arrieta et al., 2011).

The demographic and clinical characteristics of our patients with EGFR mutations were similar to those described by previous studies, with a preponderance of female patients, patients with adenocarcinoma and never-smokers (Lynch et al., 2004; Kosaka et al., 2004; Paez et al., 2004; Cortes-Funes et al., 2005; Han et al., 2005; Mitsudomi et al., 2005; Takano et al., 2005; Taron et al., 2005; Tsao et al., 2005; Rosell et al., 2009; Sun et al., 2012). Significantly higher EGFR mutation rates have been found among female (45-46%), never-smoker (48.6-64.2%) and adenocarcinoma patients (38.1-55.0%) in both East Asian (Huang et al., 2004; 2011) and Western (Pallis et al., 2007; Rosell et al., 2009; Sequist et al., 2011) study populations. East Asian studies with a high proportion of never smokers (61.5-100%) and female patients (49.1-78.7%) reported high mutation rates of 51.3-75.3% (Sun et al., 2010; 2012; Li et al., 2011) compared to mutation rates of 29-38.6% in other East Asian studies (Huang et al., 2004; 2011) with lower proportion of never smokers (36.4-59.8%) and female patients (33.1-44.6%).

Other than differences in demographic characteristics and distribution of NSCLC subtypes, the variation in the EGFR mutation rate in the different study populations may also be due to different sensitivity of different methods used in EGFR mutation detection (Arrieta et al., 2011). Real-time PCR using the scorpion ARMS methods has been found to be more sensitive than direct sequencing in detecting EGFR mutations (Kimura et al., 2006) and this could partly explain the higher mutation rate in our patients compared with some previous studies that used the latter method.

The EGFR mutation rate can be as high as 59.7% (Mok et al., 2009) in East Asian studies that target patients with clinical features associated with higher likelihood of EGFR mutation such as never or former light smokers and adenocarcinoma. The EGFR mutation rate in adenocarcinoma of our patients was 39.4% which is consistent with findings of other studies in East Asian patients reporting mutation rates ranging from 38.1% to 59% in this histological subtype (Huang et al., 2004; 2011; Sonobe et al., 2005; Sun et al., 2012).

EGFR mutations are rare in squamous cell carcinoma with a reported frequency of less than 5% (Perez-Moreno et al., 2012) as demonstrated by our finding that the only EGFR mutation-positive squamous cell carcinoma was from a never smoker while squamous cell carcinoma from all 10 smokers were EGFR mutation-negative. Expert panels (Salto-Tellez et al., 2011) and guidelines (Lindeman et al., 2013) recommend that apart from nonsquamous

NSCLC, EGFR mutation testing should be performed in squamous cell carcinoma patients with clinical features associated with higher prevalence of EGFR mutations such as a lack of smoking history. Furthermore, adenosquamous carcinomas and solid adenocarcinomas, in which EGFR mutations have been reported (Baik et al., 2013), can mimic squamous cell carcinoma in small biopsy samples (Paik et al., 2012).

The prevalence of EGFR mutations in our patients of Chinese ethnicity is similar to that reported in East Asians. Of great interest is that we did not find any significant difference in the prevalence of EGFR mutation between the Chinese and the Malays. This is the first report on the prevalence of EGFR mutation in NSCLC in the Malays. In Malaysia, lung cancer is less common among the Malays than among the Chinese (Department of Statistics, Malaysia, 2006; Liam et al., 2006). The age-standardised incidence of lung cancer for the Chinese is more than twice that of the Malays and Indians for both sexes (Department of Statistics, Malaysia, 2006). The Chinese are also reported to be the predominant ethnic group with lung cancer in Singapore which, like Malaysia, has a multiracial population comprising of Chinese, Malays and Indians (Wagenknecht et al., 1990). Although a relatively high mutation rate of 51.8% was found in a study on 220 Indian patients with NSCLC in India by Sahoo et al (Sahoo et al., 2011), the very high mutation rate of 71.4% in our seven patients of Indian ethnicity could have been confounded by their small number and the fact that six of them were female and all were never-smokers and all had adenocarcinoma. In the study on Indian patients in India, 44.1%, 52.7% and 80%, respectively were female, never smokers and had adenocarcinoma (Sahoo et al., 2011). Although the female Indian patients have a higher mutation rate than male patients (59.8% versus 45.5%), the proportion of male Indian patients with EGFR mutations was higher than male patients from other Asian studies (32.1-34.3%) (Huang et al., 2004; Huang et al., 2011; Sun et al., 2012).

Similar to reports by others (Shigematsu et al., 2005), we did not find any significant age difference between patients with EGFR-positive and those with EGFR-negative NSCLC. Consistent with other studies (Kosaka et al., 2004; Sharma et al., 2007; Rosell et al., 2009; Lund-Iversen et al., 2012), mutations in exon 19 were more common than those in exon 21 in our patients. Interestingly, our exon 19 mutation-positive patients were significantly younger than exon 21 mutation-positive patients as reported by another study (Lund-Iversen et al., 2012). Since mutations in exons 18 and 20 are relatively rare, it is not surprising that we did not find any of our patients with these two mutations because of our small sample size.

In conclusion, the EGFR mutation rate in NSCLC in our multiethnic Malaysian patients is similar to that of other East Asian populations. There was no significant difference in the mutation rates between the different ethnicities. EGFR mutations are significantly more common in female patients, never smokers and adenocarcinoma. However, never smoking status is the only independent predictor for the presence of EGFR

mutations in our patients.

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