RESEARCH ARTICLE

Epidermal Growth Factor Receptor Mutations in Japanese Men with Lung Adenocarcinomas

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Abstract

Background: Epidermal growth factor receptor (*EGFR*) mutations play a vital role in the prognosis of patients with lung adenocarcinoma. Such somatic mutations are more common in women who are non-smokers with adenocarcinoma and are of Asian origin. However, to our knowledge, there are few studies that have focused on men. Materials and Methods: One hundred and eighty-four consecutive patients (90 men and 94 women) of resected lung adenocarcinoma were studied retrospectively. Results: *EGFR* mutations were positive in 48.9% and negative (wild type) in 51.1%. Overall mutation was significant in women (66.0% *vs.* 32.2%) compared with men (p<0.001). For overall patients, *EGFR* mutation status was associated with gender, pStage, pT status, lepidic dominant histologic subtype, pure or mixed ground-glass nodule type on computed tomography and smoking status. However, in men, *EGFR* mutation status was only associated with lepidic dominant histologic subtype and not the other variables. Interestingly, the Brinkman index of men with mutant *EGFR* also did not differ from that for the wild type (680.0±619.3 *vs.* 813.1±552.1 p=0.1077). Conclusions: The clinical characteristics of men with lung adenocarcinoma related to *EGFR* mutation are not always similar to that of overall patients. Especially we failed to find the relationship between *EGFR* mutations and smoking status in men.

Keywords: *EGFR* mutation - men - lung adenocarcinoma - Brinkman index

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Introduction

Epidermal growth factor receptor (*EGFR*), a transmembrane glycoprotein is involved in the cancer cell proliferation, angiogenesis, and resistance to apoptosis (Olayioye et al., 2000; Pinkas-Kramarski et al., 1996). Recently *EGFR* mutation status for non-small cell lung cancer (NSCLC) has become one of the most important factors for selecting treatment with *EGFR*-tyrosine kinase inhibitor, such as gefitinib or erlotinib (Kosaka et al., 2004; Shigematsu et al., 2005; Mitsudomi et al., 2006). The two most common *EGFR* mutations, exon 19 deletion and L858R in exon 21, represent 85 to 90% of *EGFR* mutations (Pan et al., 2005).

The *EGFR* mutation has been reported to be strongly related with never-smoker, women, adenocarcinoma and Asians (Kosaka et al., 2004; Shigematsu et al., 2005; Mitsudomi et al., 2006). Therefore it might demonstrate the role of histology, sex, lifestyle and ethnicity as variables associated with *EGFR* mutation. To our knowledge, however, there is only one study that focused on *EGFR* mutation in men and smokers (D'Angelo et al., 2011). Of great interest, they concluded that a large number of *EGFR* mutations are found in adenocarcinoma tumor specimen from men and people who smoked cigarettes. In the present study, we focused on the men with adenocarcinoma patients, and we examined the relationship between *EGFR* mutation and clinical characteristics.

Materials and Methods

One hundred and eighty-four consecutive patients (90 men and 94 women) of resected lung adenocarcinoma who underwent surgery from 2007 to 2012 in our hospital and for whom *EGFR* mutation status were available were enrolled into the present retrospective study.

The preoperative serum CEA level was measured using the two-site immunoenzymometric assay; the normal upper limit for this assay was 5.0ng/ml. Surgical samples were analyzed for *EGFR* mutation using Cycleave polymerase chain reaction (PCR) method by SRL Inc. (Tokyo, Japan) (Yatabe et al., 2006). The lifetime consumption of cigarette smoke was assessed using the Brinkman index, calculated by the numbers of cigarettes smoked per day multiplied by the smoking years (Brinkman et al., 1963). Pathological (p) tumor-node-metastasis (TNM) staging was recorded in all patients based on the 7th edition of the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC)

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classification. Histologic subtype was also recorded based on International association for the study of lung cancer/ american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma (Travis et al., 2011).

The baseline characteristics are summarized in Table 1. Follow-up information, including cause of death, was ascertained through a review of clinic notes and direct or family contact. The chi-square test with Yates' correction was applied to test any association between the clinical characteristics and *EGFR* mutation status. Paired t-test was applied to assess any significant differences in the Brinkman index. Statistical calculations were conducted with JMP (SAS Institute Inc. Cary, NC, USA) and values of p less than 0.05 were accepted as being significant.

Results

The number of current or former smokers was 85/90 in men, whereas that was 8/94 in women. There was a significant difference in smoking status between men and women (p<0.001). *EGFR* mutation was positive in 48.9% and negative (wild type) in 51.1%. Overall mutation was significant in women (65.96% *vs*. 32.22%) compared with men (p < 0.001). The rates of exon18 G719X point mutations (G719X, including G719C, G719S, G719A), exon 19 deletion mutation and exon 21 L858R point mutation (L858R) in overall patients were 3.3% (3/91), 40.66% (37/91) and 56.04% (51/91), respectively. These mutation subtypes were found in 10.34% (3/29), 31.03% (9/29) and 58.62% (17/29) in men, while 0% (0/62), 45.16% (28/62) and 54.86% (34/62) in women, respectively. There was a difference between the exon carrying mutation and gender (p=0.0243).

The relationship between *EGFR* mutation status and clinical characteristics in overall patients was in Table 2. Based on the previous study by Lee et al. (2013), the histologic subtype was subdivided into 2 groups: lepidic dominant histologic subtype, including adenocarcinoma in situ, minimally invasive adenocarcinoma, and lepidic predominant invasive adenocarcinoma versus other subtypes. *EGFR* mutation status was associated with gender, pStage, pT status, lepidic dominant histologic subtype, pure or mixed ground-glass opacity (GGO) on computed tomography and smoking status. On the other hand, pN status and serum CEA level were not related to *EGFR* mutation status. However, when study patients were limited to men with adenocarcinoma, *EGFR* mutation status was only associated with lepidic dominant histologic subtype (Table 3). Furthermore there was a trend towards an association between *EGFR* mutation and pure or mixed GGO but this did not reach statistical significance. Other factors were not related to *EGFR* mutation status in men (Table 3). Since we failed to find the *EGFR* mutation status and smoking status in

Table 1. Clinical Characteristics of Study Participants

		Number of patients
Age	<65	69
	≥65	115
Gender	Men	90
	Women	94
pStage	I	131
	II-IV	53
pT status	pT1	121
	pT2-3	63
pN status	pN0	146
	pN1-2	38
Histology	Minimally invasive Adenocarcinoma	24
	Invasive AD, lepidic predominant	28
	Invasive AD, acinar predominant	31
	Invasive AD, papillary predominant	69
	Invasive AD, micropapillary predominant	5
	Invasive AD, solid predominant	19
	Others	8
Smoking	Current/former	93
	Never	91
CEA	Normal	128
	High	56

*AD: adenocarcinoma; CEA: carcinoembryonic antigen

Table 2. Comparison of Clinical Characteristics of All Patients Based on *EGFR* Mutation Status

		Wild type	Mutant *EGFR*	P
Age	<65	58	57	0.97
	≥65	35	34	
Gender	Men	61	29	<0.0001
	Women	32	62	
pStage	I	58	74	0.004
	II-IV	35	17	
pT status	pT1	52	69	0.0042
	pT2-3	41	22	
pN status	pN0	70	76	0.1657
	pN1-2	23	15	
Histology	Lepidic dominant	15	38	0.0001
	Others	78	53	
CT findings	Pure/mixed GGO	19	49	<0.0001
	Solid	74	42	
Smoking status	Current/former	63	30	<0.0001
	Never	30	61	
CEA	Normal	60	66	0.2416
	High	33	25	

*GGO: ground-glass opacity

Table 3. Comparison of Clinical Characteristics of Men Based on *EGFR* Mutation Status

		Wild type	Mutant *EGFR*	P
Age	<65	24	17	0.854
	≥65	37	12	
pStage	I	36	22	0.112
	II-IV	25	7	
pT status	pT1	31	20	0.101
	pT2-3	30	9	
pN status	pN0	46	24	0.426
	pN1-2	15	5	
Histology	Lepidic dominant	8	10	0.021
	Others	53	19	
CT findings	Pure/mixed GGO	9	9	0.078
	Solid	52	20	
Smoking status	Current/former	59	26	0.189
	Never	2	3	
CEA	Normal	36	18	0.782
	High	25	11	

*GGO: ground-glass opacity

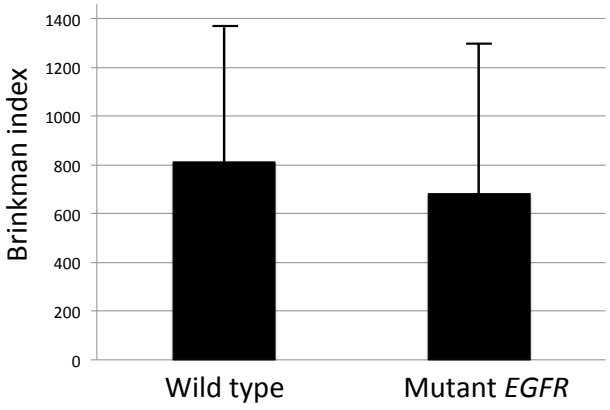

Figure 1. Brinkman Index Based on the *EGFR* Mutation Status

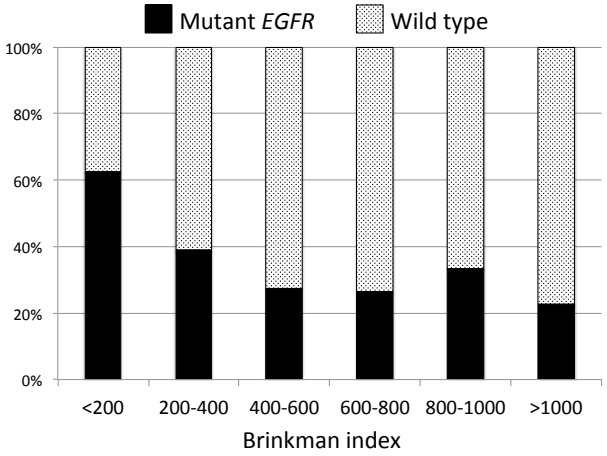

Figure 2. *EGFR* Mutation Ratio Based on the Brinkman Index

men. We also compared the Brinkman index. As shown in Figure 1, the Brinkman index of men with mutant *EGFR* was not also different from that of men with wild type (679.97±619.29 *vs*. 813.13±552.08 p=0.8357). The rate of exon18 G719X point mutations, deletions in exon 19 and the L858R mutation in exon 21 was not also related to the Brinkman index in men (p=0.3445: data not shown). Figure 2 also shows the *EGFR* mutation ratio based on the Brinkman index. Although there was a trend that the ratio of *EGFR* mutation was higher in men with Brinkman index <200, there were no statistical differences among these groups (p=0.4078).

Discussion

We demonstrated a high prevalence of *EGFR* mutations in our study population (48.9%), which was consistent with several other studies showing high incidence of *EGFR* mutation in Asian patients (Kosaka et al., 2004; Shigematsu et al., 2005; Mitsudomi et al., 2006).

Is has been well accepted that never-smoking status, women, adenocarcinoma and Asians ethnicity have been considered the most important factors associated with *EGFR* mutations in NSCLC (Kosaka et al., 2004; Shigematsu et al., 2005; Mitsudomi et al., 2006). In addition, previous studies reported that pure or mixed GGO and lepidic dominant histologic subtype could be better predictors for *EGFR* mutation in lung adenocarcinoma (Hsieh et al., 2005; Yano et al., 2006; Lee et al., 2013). In our results, both lepidic dominant histologic subtype and pure or mixed GGO were related to *EGFR* mutation in overall patients. In men, we also found that lepidic dominant histologic subtype was related to *EGFR* mutation. However there was a trend towards an association between *EGFR* mutation and pure or mixed GGO in men but this did not reach statistical significance. Our result of no association between *EGFR* mutation and GGO could be because the small sample size, because it has been reported the association between lepidic pattern and GGO (Ambrosini-Spaltro et al., 2014). Therefore we believe that both GGO and lepidic dominant histologic subtype are related to *EGFR* mutation in men.

Overall mutation rate was significant in women compared with men. This result was consistent with several other studies (Kosaka et al., 2004; Shigematsu et al., 2005; Mitsudomi et al., 2006). Our result also showed an association between the exon carrying mutation and gender. The reason for such gender difference has not been clarified in detail. However previous studies reported some possible gender related differences in NSCLC. For example, first, the frequency of gastrin-releasing peptide receptor expression was reported to be higher in women and it increased with the extent exposure to tobacco (Shriver et al., 2000). Second, estrogen status, sex-related hormone, is reported to be a factor in lung cancer risk among woman (Si*EGFR*ied et al., 2001). Third, woman's domestic work, including burning coal or other smoke-producing fuel for cooking, might be a risk of lung cancer (Luo et al., 1996). These might be related the gender difference in *EGFR* mutation, at least in part.

Furthermore the well-known difference between men and women was smoking habits. Our result also showed this difference. As described above, previous studies reported that NSCLC patients characterized by female gender, never-smoking status and adenocarcinoma histology were more likely to harbor *EGFR* mutations (Kosaka et al., 2004; Shigematsu et al., 2005; Mitsudomi et al., 2006). On the other hand, some studies showed that *EGFR* mutation was significantly associated with adenocarcinoma and light-smoking but not gender (Tanaka et al., 2010; Hsiao et al., 2014). The majority of women with NSCLC, particularly in Asian populations, have no or slight history of smoking. In our results, the majority of women (86/94) are also never-smoker. Therefore, in some population, the variables affected by smoking may show a seeming gender difference. In other words, there is a possibility that the *EGFR* mutational frequency among men and women was not significantly different when patients were stratified into never- and ever-smokers.

Previous studies reported that increasing smoke exposure was inversely related to the rate of *EGFR* mutation (Kosaka et al., 2004; Hsieh et al., 2005; Shigematsu et al., 2005; Mitsudomi et al., 2006; D'Angelo et al., 2011). Thus, it has been suggested that *EGFR* mutations may play a key role in the development of NSCLC in patients with a low exposure to cigarette smoking. Of great interest, however, we failed to find the relationship between *EGFR* mutation status and smoking status in men. D'Angelo et al. also concluded that a large

number of *EGFR* mutations are found in adenocarcinoma tumor specimens from men and people who smoked cigarettes (D'Angelo et al., 2011). In addition, this study demonstrated that the Brinkman index does not have a potential predictive value for the presence of *EGFR* mutations in men. In view of these results, it can be considered that smoking status does not always play a key role for *EGFR* mutation in men, and some mechanism of *EGFR* mutation might not be always identical among men and women. The *EGFR* mutation may therefore be an interesting model to pursue the gender difference of lung adenocarcinoma.

Recently, some metabolic polymorphisms are widely studied in order to understand the inheritance of cancer and individuals tolerance to tobacco carcinogens. These include cytochrome P4501A1 (CYP1A1) ile/val and glutathione-S-transferase M1 (GSTM1) null/present gene polymorphisms (Saeed et al., 2013). These genes encode such enzymes that play major roles in detoxification pathway of several carcinogens, including polycyclic aromatic hydrocarbons that are present in tobacco smoke (Shukla et al., 2013). Shukla et al. showed the GSTT1 null polymorphism to be associated with smoking-induced lung cancer and the GSTM1 null polymorphism to have a link with non-smoking related lung cancer (Shukla et al., 2013). In spite of the effects of genetic polymorphisms in CYP1A1 and GSTM1 on lung cancer risk, the relationship between these genetic polymorphisms and *EGFR* mutation has been unknown. Further studies should be needed.

Usuda et al. reported that mutant *EGFR* is significantly associated with smaller tumor diameter in chest CT in addition to gender, pure or mixed GGO, adenocarcinoma, never-smoker (Usuda et al., 2014). Our results also showed that pStage and pT status were related to *EGFR* mutation in overall patients, but not men. This result might also show the gender difference in the *EGFR* mutation. Further studies in this area are warranted.

In conclusions, the clinical characteristics of men with lung adenocarcinoma that related to *EGFR* mutation were not always similar to that of overall patients. Especially, the smoking status was not related to *EGFR* mutation status in men. Therefore we believe that all patients with lung adenocarcinoma should undergo *EGFR* mutation testing, regardless of clinical characteristics.

References

Ambrosini-Spaltro A, Ruiu A, Seebacher C, et al (2014). Impact of the IASLC/ATS/ERS classification in pN0 pulmonary adenocarcinomas: a study with radiological-pathological comparisons and survival analyses. *Pathol Res Pract*, **210**, 40-6.

Brinkman GL, Coates EO, Jr. (1963). The effect of bronchitis, smoking, and occupation on ventilation. *Am Rev Respir Dis*, **87**, 684-93.

D'Angelo SP, Pietanza MC, Johnson ML, et al (2011). Incidence of *EGFR* exon 19 deletions and L858R in tumor specimens from men and cigarette smokers with lung adenocarcinomas. *J Clin Oncol*, **29**, 2066-70.

Hsiao SH, Lin SE, Chou YT, et al (2014). Histological subtype and smoking status, but not gender, are associated with mutations in non-small-cell lung cancer. *Mol Clin Oncol*, **2**, 252-8.

Hsieh RK, Lim KH, Kuo HT, et al (2005). Female sex and bronchioloalveolar pathologic subtype predict *EGFR* mutations in non-small cell lung cancer. *Chest*, **128**, 317-21.

Kosaka T, Yatabe Y, Endoh H, et al (2004). Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res*, **64**, 8919-23.

Lee HJ, Kim YT, Kang CH, et al (2013). Epidermal growth factor receptor mutation in lung adenocarcinomas: relationship with CT characteristics and histologic subtypes. *Radiology*, **268**, 254-64.

Luo RX, Wu B, Yi YN, et al (1996). Indoor burning coal air pollution and lung cancer--a case-control study in Fuzhou, China. *Lung Cancer*, **14**, 113-9.

Mitsudomi T, Kosaka T, Yatabe Y (2006). Biological and clinical implications of *EGFR* mutations in lung cancer. *Int J Clin Oncol*, **11**, 190-8.

Olayioye MA, Neve RM, Lane HA, et al (2000). The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J*, **19**, 3159-67.

Pan Q, Pao W, Ladanyi M (2005). Rapid polymerase chain reaction-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas. *J Mol Diagn*, **7**, 396-403.

Pinkas-Kramarski R, Soussan L, Waterman H, et al (1996). Diversification of Neu differentiation factor and epidermal growth factor signaling by combinatorial receptor interactions. *Embo J*, **15**, 2452-67.

Saeed HM, Alanazi MS, Nounou HA, et al (2013). Cytochrome P450 1A1, 2E1 and GSTM1 gene polymorphisms and susceptibility to colorectal cancer in the Saudi population. *Asian Pac J Cancer Prev*, **14**, 3761-8.

Shigematsu H, Lin L, Takahashi T, et al (2005). Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst*, **97**, 339-46.

Shriver SP, Bourdeau HA, Gubish CT, et al (2000). Sex-specific expression of gastrin-releasing peptide receptor: relationship to smoking history and risk of lung cancer. *J Natl Cancer Inst*, **92**, 24-33.

Shukla RK, Tilak AR, Kumar C, et al (2013). Associations of CYP1A1, GSTM1 and GSTT1 polymorphisms with lung cancer susceptibility in a Northern Indian population. *Asian Pac J Cancer Prev*, **14**, 3345-9.

Si*EGFR*ied JM (2001). Women and lung cancer: does oestrogen play a role? *Lancet Oncol*, **2**, 506-13.

Tanaka T, Matsuoka M, Sutani A, et al (2010). Frequency of and variables associated with the *EGFR* mutation and its subtypes. *Int J Cancer*, **126**, 651-5.

Travis WD, Brambilla E, Noguchi M, et al (2011). International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol*, **6**, 244-85.

Usuda K, Sagawa M, Motono N, et al (2014). Relationships between *EGFR* mutation status of lung cancer and preoperative factors - are they predictive? *Asian Pac J Cancer Prev*, **15**, 657-62.

Yano M, Sasaki H, Kobayashi Y, et al (2006). Epidermal growth factor receptor gene mutation and computed tomographic findings in peripheral pulmonary adenocarcinoma. *J Thorac Oncol*, **1**, 413-6.

Yatabe Y, Hida T, Horio Y, et al (2006). A rapid, sensitive assay to detect *EGFR* mutation in small biopsy specimens from lung cancer. *J Mol Diagn*, **8**, 335-41.