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’Angeló 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)
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                                                    |
| History                                               |
| 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                                       |
| History                                   |
| 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                           |
| 22                                       |
| 15                                       |
| 0.189                                    |
| Never                                     |
| 39                                       |
| 14                                       |
| CEA                                       |
| Normal                                    |
| 36                                       |
| 18                                       |
| 0.782                                    |
| High                                      |
| 25                                       |
| 11                                       |

*GGO: ground-glass opacity
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 (SiEGFRied 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 \textit{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 \textit{EGFR} mutations in men. In view of these results, it can be considered that smoking status does not always play a key role for \textit{EGFR} mutation in men, and some mechanism of \textit{EGFR} mutation might not be always identical among men and women. The \textit{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 \textit{EGFR} mutation has been unknown. Further studies should be needed.

Usuda et al. reported that mutant \textit{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 \textit{EGFR} mutation in overall patients, but not men. This result might also show the gender difference in the \textit{EGFR} mutation. Further studies in this area are warranted.

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

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