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Original Article

Impact of age on EGFR mutations in never-smoking female lung adenocarcinoma patients with malignant pleural effusion

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Abstract: Our aim was to evaluate EGFR mutations in never-smoking female lung adenocarcinoma patients with malignant pleural effusion and to reveal the relationship between age and EGFR mutations. Never-smoking female lung adenocarcinoma patients were retrospectively studied, including 301 biopsy samples and 80 cytological specimens. Our results showed a significant increase of EGFR mutation prevalence by increase of age in cytological specimens, but not in biopsy samples. Our data suggests that age at the time of diagnosis may be associated with presence of EGFR mutations in patients with malignant pleural effusion.

Keywords: Age, EGFR, lung cancer, malignant pleural effusion

Introduction

Lung cancer is the leading cause of cancer-related deaths in the world [1]. Non-small-cell lung cancer (NSCLC) represents approximately 85% of all new lung cancer diagnoses [2]. Adenocarcinoma is the most common subtype of NSCLC and accounts for more than 50% of all NSCLC [3]. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have been recommended as the front-line therapy in adenocarcinoma NSCLC patients harboring activated EGFR mutations [4]. Malignant pleural effusion (MPE) is a common clinical phenomenon in lung cancer, especially in adenocarcinoma [5, 6]. In contrast to biopsy samples (surgically resected specimens and needle biopsy), MPE is easily accessible. EGFR mutation screening in MPE is also useful for prediction of response to TKI therapy [6-9].

Many studies regarding incidence of EGFR mutations have shown that smoking status and gender are significant predictors of mutation status in adenocarcinoma NSCLC patients [10-13]. Adenocarcinomas from those who have never smoked frequently contain mutations of EGFR and would be good candidates for personalized diagnostic and therapeutic approaches [14, 15]. Studies have also suggested that female NSCLC patients are predominantly never-smokers and are likely to have adenocarcinoma histology [16]. In particular, molecular studies have reported that female patients are associated with high incidence of EGFR mutations [13, 17]. Aging is one of the most common, but rarely referred, risk factors for many cancers, including NSCLC [18]. More recent studies on the relationship between age and EGFR mutation rates have remained controversial, regarding adenocarcinoma NSCLC patients with biopsy samples [19-22]. In addition, it has scarcely been evaluated whether age is solely associated with EGFR mutations in adenocarcinoma NSCLC patients with MPE. We, therefore, conducted a retrospective cohort study to evaluate the possible association between age and presence of EGFR mutations in never-smoking female lung adenocarcinomas with MPE.

Materials and methods

Samples

Biopsy samples from 301 patients (age 56.8± 10.9 years) and 80 cytological specimens (age 65.0±12.0 years) were selected for our study.

Table 1. EGFR mutations in never-smoking female lung adenocarcinoma patients with MPE or biopsy samples

	Mutant subtypes			Total mutations	Wild type	Total patients
	19-Del	L858R	Others			
MPE	19 (23.75)	23 (28.75)	11 (13.75)	53 (66.25)	27 (33.75)	80
Biopsy samples	83 (27.57)	95 (31.56)	22 (7.31)	200 (66.45)	101 (33.55)	301
Total	102	11	33	253	128	381

Data are presented as n (%) or n. EGFR, epidermal growth factor receptor. MPE, malignant pleural effusion. 19-Del, EGFR exon 19 deletion. L858R, EGFR exon 21 L858R mutation. Others, including EGFR insertion in exon 20, L861Q, G719X, G719X and S768I, 19-Del and T790M, L858R, and T790M. P>0.05 for MPE versus biopsy samples for EGFR mutations.

Table 2. EGFR mutation in two age groups in all patients

	EGFR mutations	Wild type	Total	*P*-value
<65 years	169 (63.77)	96 (36.23)	265	
≥65 years	84 (72.41)	32 (27.59)	116	P=0.10
Total	253	128	381	

Data are presented as n (%) or n. EGFR, epidermal growth factor receptor.

Table 3. EGFR mutation in two age groups in patients with biopsy samples

	EGFR mutations	Wild type	Total	*P*-value
<65 years	149 (65.64)	78 (34.36)	227	
≥65 years	51 (68.92)	23 (31.08)	74	*P*=0.60
Total	200	101	301	

Data are presented as n (%) or n. EGFR, epidermal growth factor receptor. Biopsy samples, surgically resected specimen, and needle biopsy.

Table 4. EGFR mutation in two age groups in patients with MPE

	EGFR mutations	Wild-type	Total	*P*-value
<65 years	20 (52.63)	18 (47.37)	38	
≥65 years	33 (78.57)	9 (21.43)	42	*P*=0.01
Total	53	27	80	

Data are presented as n (%) or n. EGFR, epidermal growth factor receptor. MPE, malignant pleural effusion.

All patients were never-smoking female lung adenocarcinoma patients, between January 2011 and January 2018, at Guizhou Provincial People's Hospital. Age at the time of initial diagnosis and smoking status were obtained from hospital medical records. This study was approved by the Ethics Committee of Guizhou Provincial People's Hospital.

DNA extraction and EGFR mutation detection

DNA from paraffin-embedded tissue sections and cell blocks (carcinoma cytology specimens) was extracted by AmoyDx DNA FFPE tissue kit (Amoy Diagnostics Co., Ltd., Xiamen, China). AmoyDx EGFR mutation test kit (Amoy Diagnostics Co., Ltd., Xiamen, China) was used to detect 29 EGFR mutation hotspots in exon 18-21. DNA samples were amplified by Real Time PCR (Agilent Stratagene Mx3000P). Upon completion, results were analyzed according to criteria defined by the manufacturer's instructions.

Statistical analysis

Data are presented as mean ± SD and analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Relationships between EGFR mutation and age were compared using Chi-square test, Fisher's exact probability test, or linear-by-linear association, as appropriate. Statistical significance was defined as P<0.05.

Results

EGFR mutations in patients

Never-smoking female lung adenocarcinoma patients were divided into two categories: patients with MPE and patients with biopsy samples. Mutation rates of EGFR were similar between MPE (66.25%) and biopsy samples (66.45%) (**Table 1**, P>0.05). EGFR exon 19 deletion (19-Del) and EGFR exon 21 L858R mutation (L858R) were the main mutation subtypes in MPE (23.75% and 28.75%) and biopsy samples (27.57% and 31.56%) (**Table 1**). Mutation rates of EGFR subtypes were also similar between MPE and biopsy samples (**Table 1**, all P>0.05).

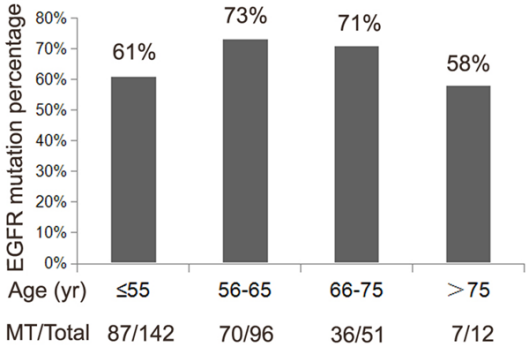

Figure 1. EGFR mutations in different age segments in patients with biopsy samples (P=0.29). Number of patients with EGFR mutations/number of total patients in each group. Mt: mutation.

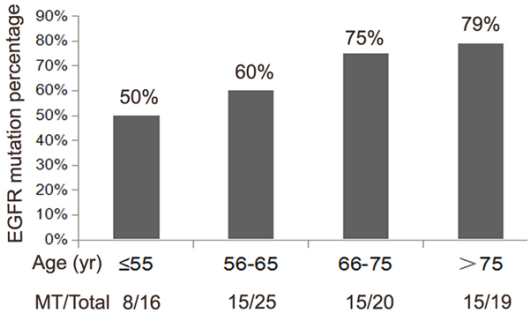

Figure 2. EGFR mutations in different age segments in patients with malignant pleural effusion (P=0.04). Number of patients with EGFR mutations/number of total patients in each group. Mt: mutation.

EGFR mutations in two age groups of patients

Never-smoking female lung adenocarcinoma patients were divided into two groups: over 65 patients and under 65 patients. Mutation rate of EGFR was higher in over 65 group (72.41%) than under 65 group (63.77%). The difference, however, was not statistically significant (**Table 2**, P=0.10). Patients were further subdivided into two age groups: with MPE or biopsy samples patients (**Tables 3**, **4**). Mutation rates of EGFR was close between over 65 group (68.92%) and under 65 group (65.64%) in patients with biopsy samples (**Table 3**, P=0.60). Meanwhile, the mutation rate of EGFR was significantly higher in over 65 patients (78.57%) than in under 65 patients (52.63%) with MPE (**Table 4**, P=0.01).

Correlation between EGFR mutations and age segments

Patients were subdivided into 4 different age segments (under 55, 56-65, 66-75, and over 75). In patients with biopsy samples, there were no differences of EGFR mutations among different age segments (**Figure 1**, P=0.29).

However, in patients with MPE, over 75 year-old patients had the highest rate of mutation (79%) while under 65 year-old patients only had the lowest rate of mutation (50%). Mutation rates of EGFR were escalated with aging in patients with MPE (**Figure 2**, P=0.04).

Discussion

Presence of EGFR mutations is known to be associated with female sex, never-smoking status, adenocarcinoma histology, and East Asian ethnicity in NSCLC [10-13, 23]. However, association between age at diagnosis and presence of EGFR mutations remains controversial. Some studies have shown that older patients are more likely to have EGFR mutations than younger patients and older age at diagnosis was an independent predictor of EGFR mutations in NSCLC [19, 22-24]. Other studies have suggested that younger age was associated with increased likelihood of EGFR mutations and was an underappreciated clinical biomarker in NSCLC [20, 21, 25-27]. Other results have even suggested that there was no association between presence of mutation and age at diagnosis in NSCLC [28]. The reason for these controversial results may be that EGFR mutations are affected by multiple confounding factors including smoking status, sex, age, histology, and race [10-13, 23, 27]. Therefore, studying the relationship between age and EGFR mutations in NSCLC patients is challenging. We only selected never-smoking female lung adenocarcinoma patients to analyze the relationship between age and mutations of EGFR. Our results showed that, in all patients, the difference of EGFR mutation was not statistically significant between over 65 age group and under 65 age group (**Table 2**, P=0.10). Considering that lung adenocarcinoma with MPE shows distinct clinical features, patients were further subdivided into two groups: patients with MPE and patients with biopsy samples. Further studies revealed that EGFR mutations were not different between MPE and biopsy samples, including mutation rates of EGFR subtypes (**Table 1**, all P>0.05). However, in MPE groups, the mutation rate of EGFR was significantly higher in over 65 year-old group (78.57%) than in under 65 year-old group (52.63%) (**Table 4**, P=0.01). Meanwhile, a significant difference

was not shown in patients with biopsy samples (**Table 3**, P=0.60). Furthermore, patients were subdivided into four different age segments (under 55, 56-65, 66-75, and over 75). Our results demonstrated that the mutation rate of EGFR was escalated with aging in patients with MPE (**Figure 2**, P=0.04). On the basis of these studies, we hypothesized that aging may be predictive of EGFR mutations in never-smoking female lung adenocarcinoma patients with MPE, but not in biopsy samples. Further large-scale studies should be undertaken to validate these findings.

In conclusion, we found a significant increase of EGFR mutation prevalence with increase of age in never-smoking female lung adenocarcinoma patients with MPE.

Disclosure of conflict of interest

None.

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