Int J Clin Exp Pathol 2017;10(7):7627-7631
www.ijcep.com /ISSN:1936-2625/IJCEP0053814

Original Article

Clinical and pathological findings of surgically resected patients for lung adenocarcinomas harboring uncommon EGFR mutations

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Received March 26, 2017; Accepted May 27, 2017; Epub July 1, 2017; Published July 15, 2017

Abstract: Background: This study aimed to examine the clinical and pathological characteristics of surgically resected lung adenocarcinoma patients harboring uncommon epidermal growth factor receptor (EGFR) mutations and provide a rational basis to provide postoperative adjuvant treatment for these patients. Methods: Data from 13 patients with lung adenocarcinomas harboring uncommon EGFR mutations were retrospectively collected, and the clinicopathological parameters and disease-free survival (DFS) data analyzed. Results: EGFR uncommon mutations commonly occurred in acinar predominant adenocarcinomas, females, and never smokers. Despite treatment, six patients relapsed. Conclusion: Patients with EGFR 20 insertion mutations relapsed quickly. Postoperative adjuvant treatment with chemotherapeutic drugs or new tyrosine kinase inhibitors for lung adenocarcinoma patients harboring EGFR 20 insertion mutations requires further evaluation.

Keywords: Lung adenocarcinoma, epidermal growth factor receptor, uncommon mutations, disease-free survival

Introduction

Lung cancer is the most common malignancy, globally, and a leading cause of cancer-related mortality. Non-small cell lung cancers (NSCLCs) account for 85% of all lung cancers, and more than half of NSCLCs are adenocarcinomas [1]. Although surgical resection is considered curative in the early stages, recurrence of the disease is common [2]. Adjuvant chemotherapy with cisplatin-based regimens has become the standard treatment for surgically resected NSCLC with documented improvement in 5-year overall survival (OS) of 5% [2, 3]. As a predictive biomarker of the efficacy of cisplatin-based chemotherapy, the usefulness of excision repair cross-complementation group 1 protein in NSCLC is limited [3]. The results of a recent randomized phase III trial of adjuvant chemotherapy with or without bevacizumab in resected early stage NSCLC did not improve the overall survival (OS) [4]. It is clear that novel therapeutic modalities based on the unique clinicopathological characteristics of early stage NSLC are required to improve survival outcomes.

Epidermal growth factor receptor (EGFR) mutations are common in NSCLCs. Commonly, deletion mutations in exon 19 or the Leu858Arg point mutations in exon 21, or both, are seen [5]. However, in about 10% of patients, tumors that harbor uncommon EGFR mutations have also been observed. Uncommon EGFR mutations include point mutations or duplications in exons 18-21, de-novo T790M mutations in exon 20 alone or in combination with other mutations, and exon 20 insertions. G719X, L861G, and S768I, alone or in combination with other mutations have been noted to be the most frequent uncommon mutations [5]. The sensitivity of these tumors to treatment with EGFR tyrosine kinases inhibitors (TKIs) has been evaluate in patients with resected stages I to III lung adenocarcinomas harboring mutations in EGFR exon 19 or 21, a study com-

Table 1. Clinicopathological features of all patients (n=13)

Clinical characteristic	Total	Exon 18 G719X (n=4)	Exon 21 L861Q (n=6)	Exon 20 Insertions (n=3)
Sex (male/female)	4/9	0/4	3/3	1/2
Median age (years old)	60	60	57	62
Stage				
IA (IA1/IA2/IA3)	4 (1/2/1)	2 (1/0/1)	1 (0/1/0)	1 (0/1/0)
IB	4	1	1	2
IIA	0	0	0	0
IIB	2	0	2	0
IIIA	3	1	2	0
Pathological type				
Lepidic predominant	2	0	2	0
Acinar predominant	6	2	3	1
Papillary predominant	3	0	1	2
Solid predominant	1	1	0	0
Minimally invasive	1	1	0	0
Smoking history				
Never smoker	11	4	4	3
Light smoker	0	0	0	0
Moderate smoker	0	0	0	0
Heavy smoker	2	0	2	0

pared the clinical outcomes in patients who did not receive adjuvant first-generation TKIs (erlotinib or gefitinib) against those who received these agents as adjuvant chemotherapy. A trend toward improvement in disease-free survival (DFS) was observed among individuals who received TKI therapy [6]. The efficacy of the second-generation TKI, afatinib, has been noted in NSCLC tumors that harbor certain types of uncommon EGFR mutations, especially the G719X, L861G, and S768I, but clinical benefit was noted to be lower in patients with de-novo T790M or exon 20 insertion mutations [6]. However, Heigener DF analyzed the activity of afatinib in patients with uncommon EGFR mutations and reported treatment benefit even in tumors harboring resistance-mediating exon 20 mutations [7]. Third-generation EGFR-mutant-selective TKIs, such as AZD9291 or rociletinib, which target T790M-mutant tumors, have entered clinical trials, and exciting, albeit preliminary, efficacy data have been reported so far [8, 9].

This study aimed to further clear the current clinical and pathological characteristic features of surgically resected lung adenocarcinoma patients with tumors harboring uncommon EGFR mutations, and provide a rational basis to carry out postoperative adjuvant treatment for this subset of patients.

Materials and methods

Patient characteristics

Specimens from 13 NSCLC patients (9 females and 4 males) were collected from the Zhejiang Cancer Hospital in China between 2008 and 2014. All specimens were obtained from previously resected adenocarcinomas and retrospectively analyzed. The median age of the patients was 60 years (age range 47 to 74 years). The pathological diagnosis was based on the standard criteria defined by the World Health Organization. The sex, median age, stage, pathological type, smoking history, and status of EGFR mutation are included in **Table 1**. The methodology of evaluating mutations was by using the amplification refractory mutation system (ARMS). Four patients with EGFR exon 18 G719X mutations, six patients with exon 21 L861Q mutations, and three patients with EGFR exon 20 insertion mutations were included (**Figure 1**). Among the patients with the exon 18 G719X mutations, only one patient with stage IIIA received adjuvant chemotherapy. Among the patients with the exon 21 L861Q mutations, two patients with stage IA and IB did not receive chemotherapy. None of the patients with exon 20 insertions received adjuvant chemotherapy. This study was approved by the Medical Ethical Committee of Zhejiang Cancer Hospital.

Follow-up

All included patients were followed-up until December 31, 2016. The survival time was calculated from the date of pathological diagnosis.

Statistical analyses

All data were analyzed using the statistical package for the social sciences software version 15.0. Overall data were screened using

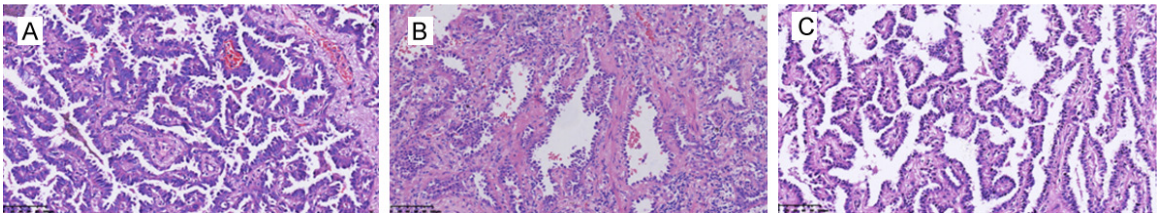

Figure 1. The pathology of three lung adenocarcinoma patients with EGFR exon 20 insertions using H and E staining. A. Case 1 was a 62-year-old female with stage IA (pT1aN0M0) NSCLC. B. Case 2 was a 74-year-old female with stage IB (pT2aN0M0) NSCLC. C. Case 3 was a 61-year-old male with stage IB (pT2aN0M0) NSCLC. Staging was done according to the eighth edition of the TNM classification for lung cancer.

Table 2. Follow-up findings of the 13 surgically resected patients for NSCLC adenocarcinoma harboring uncommon EGFR mutations

Case	Follow-up
Exon 18 G719X	
Case 1	Relapse (7 months)
Case 2	No relapse (42 months)
Case 3	No relapse (40 months)
Case 4	No relapse (29 months)
Exon 21 L861Q	
Case 1	Relapse (47 months)
Case 2	Relapse (16 months)
Case 3	No relapse (46 months)
Case 4	No relapse (42 months)
Case 5	No relapse (32 months)
Case 6	Relapse (28 months)
Exon 20 Insertions	
Case 1	No relapse (44 months)
Case 2	Relapse (13 months)
Case 3	Relapse (15 months)

the Chi-square test, and the survival curves were calculated using the Kaplan-Meier method with a statistical significance ($P<0.05$). The survival curves were compared by using the log-rank test.

Results

EGFR uncommon mutations occurred easily in the acinar predominant adenocarcinomas (46.2%), females (69.2%), and never smokers (84.6%). Six patients relapsed after the initial pathological diagnosis despite treatment, and no patients were lost to follow-up (**Table 2**). Only one patient with Exon 18 G719X mutation relapsed quickly (7 months). Three patients suffered relapse harboring the Exon 21 L861Q mutation, but took much longer. In contrast, two patients with EGFR 20 insertion mutations relapsed after the pathological diagnosis (13 and 15 months). The DFS of the patients is illustrated in **Figure 2**.

Discussion

Adjuvant cisplatin-based chemotherapy is now an accepted standard for completely resected stage II and IIIA NSCLC. Since the survival rates continue to be less than 50% at 5 years for stages II and III, there is a clear need to improve clinical outcomes in early stage NSCLC [10-12]. The administration of gefitinib following pemetrexed and carboplatin adjuvant therapy showed significant improvement in DFS in patients with resected stage IIIA-N2 NSCLC harboring EGFR mutations (either exon 19 deletion or L858R point mutation) [13]. Gefitinib and erlotinib are active in patients with G719X/L861Q/S768I mutations, however, less effective than in those with common mutations [14]. Uncommon EGFR mutations constitute a unique part of the whole spectrum of EGFR mutations. Their composition and sensitivity to EGFR-TKIs are heterogeneous and exon 20 insertions lack sensitivity [15, 16].

In our cohort, the DFS for the patients with EGFR exon 20 insertions was short and disease recurrence occurred easily. Preclinical models have shown that the most prevalent EGFR exon 20 insertion mutated proteins are resistant to clinically achievable doses of reversible (gefitinib, erlotinib) and irreversible (neratinib, afatinib, PF00299804) EGFR TKIs [17]. Advanced lung adenocarcinoma harboring EGFR exon 20 insertions do not respond to erlotinib therapy, and standard chemotherapy should be used as first-line therapy [18]. Compared to those with classic activating EGFR mutations,

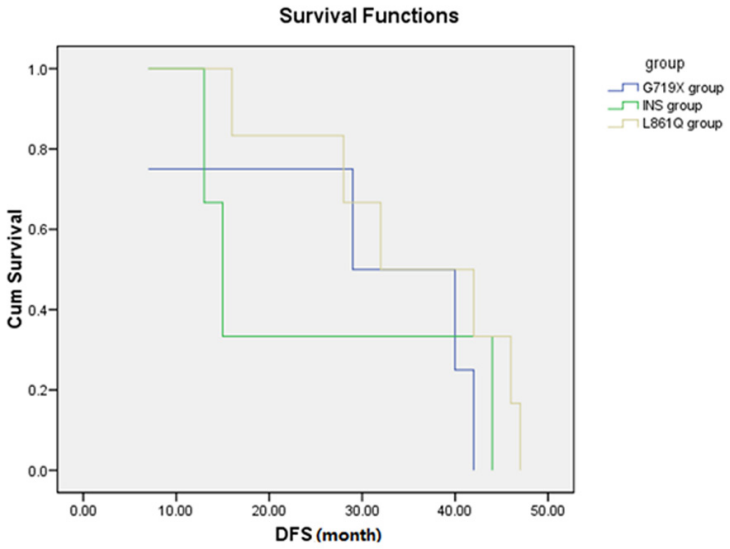

Figure 2. Comparison for disease-free survival (DFS) of lung adenocarcinoma patients with EGFR exon 18 G719X mutation (n=4), exon 21 L861Q (n=6), and EGFR exon 20 insertions (n=3) (*P*=0.421).

exon 20 insertion mutations were characterized in one study by a diagnosis at a significantly younger age, shorter relapse-free survival, higher ratio in never smokers, less dependence on EGFR status, and different pathway activation patterns [19, 20], but another report showed that patients with EGFR exon 20 insertions have similar clinical characteristics but a poorer prognosis [21]. EGFR exon 20 insertion mutations have been reported to be present in 2%-3% of lung adenocarcinomas and 4%-10% of all the EGFR-mutant lung adenocarcinomas [18-21]. Of the three patients with EGFR exon 20 insertions (IB 2 cases; IA 1case) in our study cohort, none of the patients received adjuvant chemotherapy. Two patients with stage IB with EGFR exon 20 insertions relapsed in a short time; this response to chemotherapy is similar for lung adenocarcinoma patients with EGFR exon 20 insertions and with EGFR exon 19del/L858R [18].

In conclusion, patients with EGFR 20 insertion mutations relapsed easily. Adjuvant chemotherapy might be further evaluated for resected lung adenocarcinoma patients with IA or IB with tumors harboring EGFR exon 20 insertion mutations. AP32788, a potent selective inhibitor of EGFR mutation including exon 20 insertions, has been approved by the United States Food and Drug Administration to carry out clinical trials. Adjuvant AP32788 therapy is worth pursuing in lung adenocarcinomas with EGFR exon 20 insertion mutations.

Acknowledgements

This work was supported by the 1022 Talent Training Program of Zhejiang Cancer Hospital.

Disclosure of conflict of interest

None.

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