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Original Article

TTF-1 and EGFR expression are related to EGFR mutation in lung adenocarcinoma

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Abstract: Thyroid transcription factor-1 (TTF-1) is routinely used in the diagnosis of lung carcinoma and the subclassification of non-small cell lung cancer (NSCLC) in combination with other markers. The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are particularly effective in NSCLC patients harboring active EGFR mutations. EGFR protein is a poor prognostic factor for NSCLC patients. The relationship between TTF-1 expression and EGFR mutation and EGFR expression has not been well documented. The aim of this study was to investigate the relationship between TTF-1 and EGFR expression and mutation, and the clinical significance in lung adenocarcinoma. We analyzed TTF-1 expression, EGFR expression and mutation in 213 cases of lung adenocarcinoma. TTF-1 and EGFR expression levels were detected by immunohistochemical staining with monoclonal antibodies. EGFR mutations in exon 18, 19, 20 and 21 were assayed by the scorpion amplification refractory mutation system (ARMS) method. Forty-eight patients with EGFR mutations in exon 19 or 21 were detected from 91 patients with TTF-1 strong positive expression (3+) (52.74%), and 35 patients were detected with either exon 19 or 21 mutations from 54 patients with both TTF1 and EGFR positive expression (64.81%). Our data indicate that TTF-1 expression was positively related to EGFR mutation ($P < 0.001$) and EGFR expression ($P < 0.001$). EGFR expression level was positively related to its mutation ($P = 0.003$). These results indicate TTF-1 and EGFR positive lung adenocarcinomas frequently harbor EGFR mutations.

Keywords: EGFR mutation, EGFR expression, NSCLC, TTF-1

Introduction

Lung cancer remains the leading cause of cancer deaths in the United States. An estimated 224,210 individuals will be diagnosed with lung cancer and approximately 159,260 will die of the disease [1, 2]. In China, lung cancer was the number one cause of cancer death. The registered lung cancer mortality rate increased by 733.3 per 100,000 in the past 3 decades [3]. Histologically, the main lung cancer types include adenocarcinoma (AD), small cell carcinoma, squamous cell carcinoma (SCC), and large cell carcinoma, which fall within two general categories: small cell lung carcinoma and non-small cell lung carcinoma (NSCLC). Adenocarcinoma represents the most common subtype of lung cancer. Although chemotherapy has produced modest survival benefits in patients with advanced stage disease, standard chemotherapy generates considerable toxicity. The great success of targeted therapy with EGFR TKI for treatment of lung adenocarcinoma has made targeted therapy become the most popular modality for human cancers [4-6]. For personalizing therapy, pathologists have been requiring to provide more detailed diagnoses, in particular separating squamous cell carcinoma from adenocarcinoma and large cell carcinoma. As morphology alone may be challenging, additional tools have been steadily integrated into the pathologist's diagnostic armamentarium to increase the consistency of morphologic diagnoses, among which immunohistochemistry has been gaining large popularity as a simple, relatively inexpensive, and reliable technique to unravel different cell lineages in lung cancer [7].

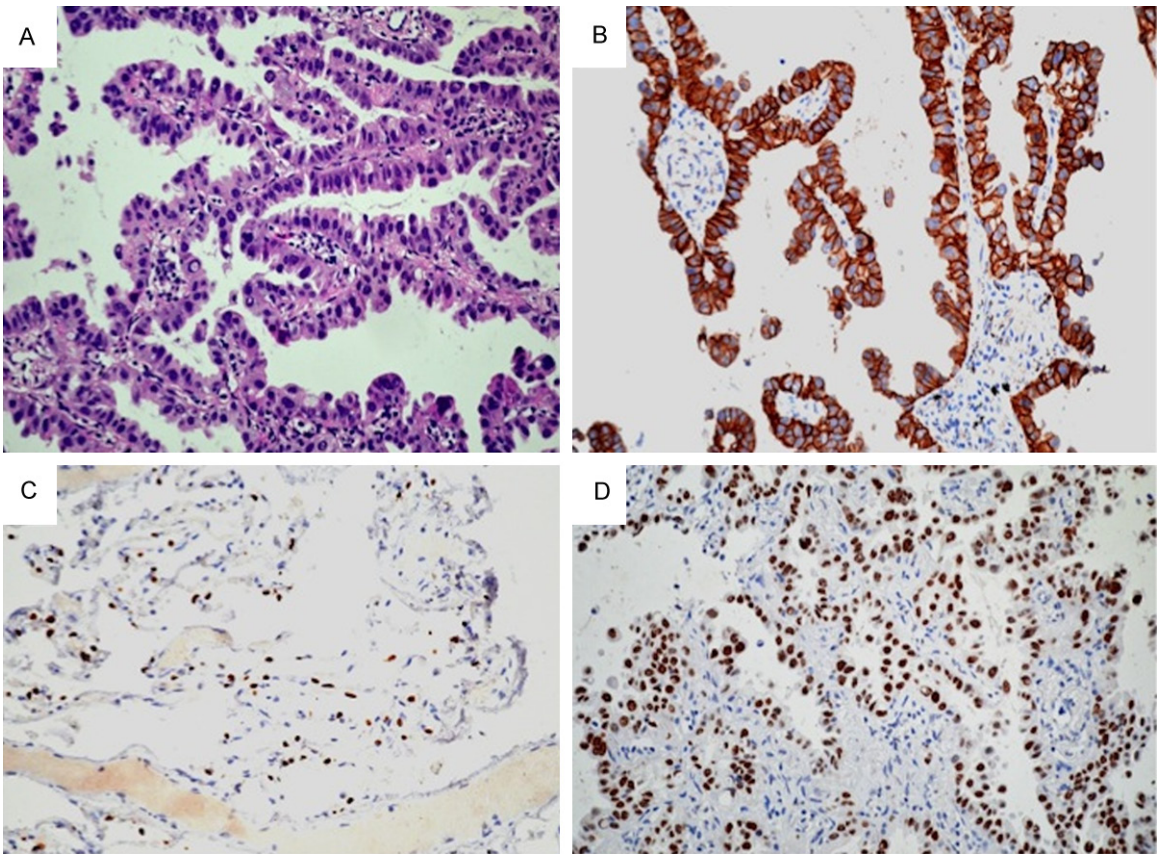

Figure 1. Representative examples of EGFR and TTF-1 expression in lung tissues. A. H&E staining of lung adenocarcinoma (400×). B. EGFR protein positively expressed in lung adenocarcinoma (400×). C. TTF-1 positively expressed in normal lung epithelial cells (400×). D. TTF-1 positively expressed in lung adenocarcinoma (400×).

TTF-1, otherwise known as NKX2.1 or T/EBP, is a homeodomain nuclear protein that belongs to the NK2 family of transcription factor [8-10]. TTF1 expression is restricted to the thyroid, lung, and parts of the fetal brain during embryonic development. Expression of TTF1 is found throughout lung development in the respiratory epithelial cells in both the conducting and peripheral airways [11]. In the 2015 World Health Organization (WHO) classification of lung tumors, TTF1 is recommended as one of a panel of lineage-specific immunohistochemical markers for adenocarcinoma differentiation [12]. TTF-1 plays a pivotal role in lung development and is also implicated in the carcinogenesis of lung adenocarcinoma. In adult human tissues it is expressed in the distal bronchial epithelium, including type II alveolar epithelial cells and terminal respiratory epithelial cells. In lung cancer, TTF-1 is expressed frequently in small cell carcinoma and in adenocarcinoma, whereas it is expressed infrequently in squamous cell cancers and large cell carcinomas. TTF-1 is routinely used in the diagnosis of lung carcinoma and the subclassification of NSCLC combination of other markers (napsin A and p40) [13].

The EGFR protein has been implicated in the proliferation and survival of cancer cells. Aberrant expression of EGFR has been detected in many human epithelial maliganancies including NSCLC. The availability of EGFR small molecule TKIs (including erlotinib and gefitinib) for treatment of lung adenocarcinoma has made targeted therapy the most popular treatment modality [4-6]. In patients with advanced non-small cell lung cancer, response to EGFR TKI is significantly higher in females, never-smokers, patients with Asian ethnicity, adenocarcinoma histology, and tumors harboring activating EGFR mutations.

Individual studies on the EGFR expression, mutations, and expression of TTF-1 in lung cancers have been documented [14-16]. However,

Table 1. The relationship between TTF-1 expression and EGFR mutation

	No.	EGFR mutation (19 + 21 exon)	EGFR wild type	Mutation rate	*P* value
TTF-1					
0-1+	67	15	52	22.40%	< 0.001
2+	55	22	33	40.00%	
3+	91	48	43	52.74%	
Total	213	85	128	40.84%	

Table 2. The relationship between TTF-1 expression and EGFR expression

	No.	EGFR expression			*P* value
		0-1+	2+	3+	
TTF-1					
0-1+	48	10	13	25	< 0.001
2+	40	5	10	25	
3+	65	2	9	54	
Total	153	17	32	104	

comprehensive studies on the relationship between TTF-1 expression and EGFR expression and mutations in lung cancer is lacking. In this study, we investigated the association between TTF-1 expression and EGFR mutations and expression in a set of lung adenocarcinomas.

Materials and methods

Patients and specimens

Two hundred thirteen lung adenocarcinoma specimens (168 biopsy and 45 resection samples) diagnosed at the Affiliated Jiangyin Hospital of Southeast University Medical College, China from 2013 to 2014 were included in this study. The 213 patients included 122 men and 91 women with an average age of 60.6 years (median, 61 years; range, 32-90 years). This study was approved by institutional review board of the Affiliated Jiangyin Hospital of Southeast University Medical College. The histopathologic classification of the specimens was done based on both 2004 WHO classification system and new adenocarcinoma classification by IASLC/ATS/ERS [17].

EGFR and TTF-1 IHC

Tissue samples were fixed in 10% formalin and embedded in paraffin. Four-micron-thick sections were cut, deparaffinized and incubated in a citrate buffer (10 mM sodium-citrate monohydrate, pH 6.5) at 120°C for 20 min in an autoclave. The sections were reacted for 1 hour with antibodies of EGFR and TTF-1 (monoclonal antibody, Denmark DAKO products) and then incubated with a commercially available detection kit (DAKO EnVision Plus-HRP, Dako, Glostrup, Denmark) following the manufacturer's instructions. The specificity of all reactions was checked replacing the primary antibody with a nonrelated mouse immunoglobulin at a comparable dilution or using normal serum alone. Positive and negative controls were used as appropriate. Immunoreactivity was rendered semiquantitatively on a scale from negative to 3+. Tumors were graded for TTF1 protein as negative (5%), weak positive (5-30%), and strong positive (30%), based on the percentage of positively stained tumor cells [18]. Immunohistochemical staining for EGFR was scored according to the criteria previously reported. Briefly, 0, no discernible staining or presence of background staining only; 1+, equivocal discontinuous membrane staining; 2+, unequivocal membrane staining with moderate intensity; and 3+, strong and complete plasma membrane staining. Samples with more than 10% of the tumor cells showing membranous staining at the 2+ and 3+ staining level were considered to be positive.

EGFR mutation analysis

The tumor specimens were fixed with formalin and embedded in paraffin. Genomic DNA was extracted with the use of a QIAamp DNA FFPE Tissue kit (Qiagen Strasse 1, 40724 Hilden, German) according to the manufacturer's instructions. EGFR mutations of exon 18, 19, 20, and 21 were detected using the ARMS EGFR mutation test kit (AmoyDx, Xiamen, China). Twenty-nine of the most common somatic mutations of the EGFR gene can be detected by the kit. The sensitivity of ARMS EGFR mutation test was 1%.

Statistical analysis

Statistical analyses were performed using Spearman test and the Chi square test. Statistical significance was determined by *P* values less than 0.05. All analyses were performed by SPSS software (version 16.0, Chicago, IL).

Table 3. The relationship between EGFR mutation and EGFR expression

	No.	EGFR mutation		EGFR wild type	Mutation Rate %	*P* value
		19 exon	21 exon			
EGFR protein						
0-1+	17	1	2	14	17.60	0.003
2+	32	4	7	21	34.40	
3+	104	27	27	50	51.92	
Total	153	32	36	85	44.44	

Table 4. The study population of EGFR mutation

	EGFR mutation status		*P* value
	Wild type	Mutation	
Gender			
Male	84	38	0.003
Female	44	47	
Age (years)			
< 55	27	26	0.145
≥ 55	101	59	

Results

TTF-1 expression in lung adenocarcinomas

TTF-1, a member of the homeodomain-containing transcription factor family, was positively expressed in normal lung peripheral bronchial epithelial cells of the alveoli and parts of lung adenocarcinoma with a nuclear pattern of immunostaining (**Figure 1**). Ninety-one (42.7%) of the 213 cases of lung adenocarcinoma showed TTF-1 expression levels corresponding to 3+, 55 (25.8%) cases showed TTF-1 expression level of 2+, and 67 (31.5%) cases showed TTF-1 expression level of 1+ or negative (**Table 1**).

EGFR mutations in lung adenocarcinoma

Overall, EGFR mutations were examined in 213 cases of lung adenocarcinoma (exons 18, 19, 20, and 21, including T790M, see **Table 1**). The overall frequency of EGFR mutation in lung adenocarcinoma was 39.9% (85 of 213). Forty-two (19.71%) patients had an in-frame deletion in the exon 19, and 43 patients (20.19%) had missense mutation (L858R) in exon 21. One patient had mutations in both exon 19 and exon 21.

Association between TTF-1 expression level and status of EGFR mutation

The relationship between TTF-1 expression levels and EGFR mutation status was statistically analyzed. As showed in **Table 1**, TTF-1 expression was positively related to occurrence rate of EGFR mutations ($P < 0.001$).

EGRR expression in lung adenocarcinoma

Positive EGFR expression manifests as a strong membrane staining in lung carcinoma cells (**Figure 1**). In our study, 153 out of 213 samples were evaluated for expression of EGFR protein (**Table 2**). Other samples were not detected because of limited tissues. Seventeen (11.11%) of the 153 cases showed EGFR expression levels corresponding to 0 or 1+; 32 (20.92%) cases showed EGFR expression levels of 2+; and 104 (67.98%) cases showed EGFR expression levels of 3+.

Relationship between TTF-1 and EGFR protein expression

The relationship between TTF-1 expression and EGFR expression was analyzed as Spearman's rank correlation coefficient. There was a significant relationship between TTF-1 expression and EGFR expression in 153 patients with lung adenocarcinoma. The expression level of TTF-1 was positively related to expression level of EGFR ($P < 0.001$).

Association of EGFR protein expression with mutation status of EGFR

As shown in **Table 3**, EGFR expression was more robust in lung adenocarcinoma with EGFR mutations than in tumors without EGFR mutations ($P = 0.003$).

The population analysis of EGFR mutation

As shown in **Table 4**, we analyzed the distribution of gender and age of patients in EGFR mutations. There were male patients 122 and female 91. Thirty-eight out of 122 (31.14%) male patients harbored EGFR mutation, while 47 out of 91 (51.64%) female patients harbored mutation ($P = 0.003$). No significant relation on age and mutation was found ($P = 0.145$).

Discussion

TTF-1 is a tissue-specific transcription factor expressed in normal lung cells of the terminal respiratory unit and malignant lung tumors.

TTF-1 and p63 (a p53-homologus nuclear protein) immunostaining in histologic samples is a valuable aid for separating small cell lung cancer and poorly differentiated pulmonary squamous cell carcinomas [19]. In patients with lung adenocarcinoma, TTF-1 expression is a predictor of good outcome. Patients with no TTF-1 expression or TTF-1 gene amplification tend to have a significantly worse prognosis than patients with TTF-1 expression and no TTF-1 gene amplification [20].

In the past decade, major progress has been made toward personalized medical treatment of NSCLC through the discovery of EGFR mutations. EGFR TKIs are shown to be superior to chemotherapy in first-line therapy of advanced NSCLC patients with EGFR mutation. Although EGFR mutations are strongly correlated to clinical response to TKIs, the correlation is not absolute. Studies implied that mechanisms other than mutations of EGFR, for example, KRAS mutation may be involved [21, 22]. Previous studies showed that patients with wild-type EGFR could also derive significant survival benefit from treatment. El-Zammar *et al* assessed EGFR status in lung adenocarcinoma with FISH, PCR, and IHC and found that 87% of IHC positive cases were also positive by FISH [23]. This indicated a concordance of the EGFR amplification and protein expression in NSCLC. Yatabe *et al* suggested that the presence of an EGFR mutation is specific for terminal respiratory unit-type adenocarcinoma, characterized by the expression of TTF-1 [24].

Because a low proportion (< 30%) of patients respond to EGFR TKI, patient selection is very important for initiating EGFR TKI therapy. EGFR gene copy number determined by FISH, EGFR protein expression determined by IHC, and EGFR tyrosine kinase mutations are all potential markers to be used as selection criteria in EGFR-targeted therapy. Liang *et al* examined the relationship between EGFR expression, amplification and mutation, and found that EGFR protein expression may predict EGFR gene status (including copy number and mutation) to some extent [25]. Currently, EGFR protein expression is not recommended for predicting outcome to EGFR TKIs due to controversial published results [26]. The mutations that predict response to EGFR TKIs are localized in the kinase domain or intracellular domain (ID). The antibodies commonly used in detection of EGFR protein are specific antibodies binding to the EGFR external domain (ED) as we used in this study. They do not discriminate between active or inactive receptors. This may explain why assessment of protein expression of EGFR using ED-specific antibody is not a consistent predictor of response to EGFR TKIs. In the present study, we investigated the relationship between TTF-1 expression and EGFR mutations and expression. To our knowledge, this is the first comprehensive study investigating the relationship between TTF-1, EGFR mutation, and EGFR expression. Our data indicate that TTF-1 is positively associated with EGFR mutation and expression. Forty-eight patients with EGFR mutations in exon 19 or 21 were detected in 91 patients with TTF-1 strong positive (3+) (52.74%), and 35 patients were detected either exon 19 or 21 mutations from 54 patients with both TTF1 and EGFR positive expression (64.81%). These results suggest that lung adenocarcinomas strongly expressing TTF-1 and EGFR protein may harbor EGFR mutations, which are responsive to EGFR TKIs.

Disclosure of conflict of interest

None.

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