Thyroid transcription factor-1 expression is significantly associated with mutations in exon 21 of the epidermal growth factor receptor gene in Chinese patients with lung adenocarcinoma

Objectives: The aim of this retrospective study was to investigate the relationship between thyroid transcription factor-1 (TTF-1) expression and epidermal growth factor receptor (EGFR) gene mutations in lung adenocarcinomas of Chinese patients.

Methods: There were 200 lung adenocarcinoma patients who were enrolled in this study. Tumor specimens of these patients were investigated for TTF-1 expression and mutations in EGFR using immunohistochemistry and a liquid chip platform for DNA analysis of slides with sections of formalin-fixed, paraffin-embedded specimens.

Results: The rates of TTF-1 expression and EGFR mutations were 81.5% and 45.5%, respectively, in the lung adenocarcinoma specimens of the recruited patients. Among female nonsmokers (n=72), 93.1% of specimens were positive for TTF-1 expression, and 63.9% had EGFR mutations. Of 89 patients with EGFR mutations, 83 (50.9%) specimens were simultaneously positive for TTF-1 expression. Kaplan–Meier analysis of all patient specimens found that postoperative survival time was not significantly associated with TTF-1 expression and the presence of EGFR mutations. However, patients with disease stages III–IV whose tumors were positive for TTF-1 expression and EGFR mutations had better postoperative survival than similar patients whose tumors were negative for TTF-1 expression and EGFR mutations.

Conclusion: Our study showed a significant association between TTF-1 positivity and the presence of EGFR mutations (exon 21) in the Chinese lung adenocarcinoma patients. We further identify that patients with disease stages III–IV who were positive for TTF-1 expression and EGFR mutations had a better postoperative survival than those patients who were negative for TTF-1 expression and EGFR mutations. Therefore, TTF-1 might be a potential prognostic biomarker for stages III–IV lung adenocarcinoma patients. In clinical practice, TTF-1 expression may be a marker for planning therapy for certain patients with lung adenocarcinoma, especially for selection of EGFR tyrosine kinase inhibitors.

Keywords: EGFR, lung adenocarcinoma, survival, TTF-1

Introduction

Lung cancer is a worldwide oncology-related disease, with an annually increasing rate of morbidity and mortality.1,2 Nonsmall-cell lung cancers (NSCLCs) make up 85% of lung cancers, with a 5-year survival rate of only 15%–17%.3 Adenocarcinoma of the lung is one of the main subtypes of NSCLC; recently, its morbidity has increased in both male and female patients. With the identification of epidermal growth factor receptor (EGFR) gene mutations in NSCLCs and the development of the EGFR

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tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib, the survival and quality of life of adenocarcinoma patients have improved greatly. The NEJ 002 clinical trial found that NSCLC patients with EGFR mutations treated with EGFR TKIs as first-line treatments had a median progression-free survival of 10.8 months and a median overall survival of 30.5 months. The current National Comprehensive Cancer Network (NCCN) guidelines indicate that genetic testing to evaluate EGFR mutation status is essential for patients with lung adenocarcinoma. However, for some patients, EGFR mutation status cannot be easily determined because of the expense or inadequate tumor specimen, leading to lack of supporting evidence for using EGFR TKI treatment. Therefore, identifying other markers that predict EGFR mutation status is necessary.

Along with EGFR mutations, thyroid transcription factor-1 (TTF-1), a biomarker for lung adenocarcinoma, was reported to have a much higher rate of expression in the lung adenocarcinoma specimens of Asian females and nonsmoking lung cancer patients. The NEJ 002 clinical trial also found that the rate of EGFR mutations was significantly higher in lung adenocarcinoma specimens that were positive for TTF-1 expression than in specimens that were TTF-1 negative. Therefore, clarifying whether there is a relationship between EGFR mutations and TTF-1 positivity in lung adenocarcinomas and whether TTF-1 can be a biomarker of EGFR mutation status is essential, especially for some patients with advanced lung cancer having inadequate specimen for evaluating the EGFR status.

Materials and methods

Materials and patients

This retrospective study enrolled 200 patients with histologically confirmed primary lung adenocarcinoma who underwent lung cancer surgery at Tianjin Medical University General Hospital between January 2008 and May 2013. All evaluated samples were obtained from resected lung cancer tissue. Surgical procedures included partial lobectomy, lobectomy, pneumonectomy, and partial resection of the superior vena cava with artificial blood vessel replacement. Neither chemotherapy nor radiotherapy was administered prior to surgery. Basically, the NSCLC patients with EGFR mutations (exon 19 or exon 21 mutations) were given four or six cycles of chemotherapy after surgery with a rigorous follow-up every 3 months. EGFR TKIs were administered upon disease progression of the patients. If EGFR TKIs did not work, other treatment alternatives were adopted according to the individual’s condition, including surgery, radiotherapy, and chemotherapy. The treatment flowchart is depicted in Figure 1.

Clinical information on each patient was obtained from the hospital medical records database. The clinical

Figure 1 Treatment flowchart of lung adenocarcinoma patients with EGFR mutations in this study.

Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.
characteristics of participants are shown in Table 1. There were 76 male and 124 female patients, with a median age of 61 years, ranging from 32 years to 78 years. There were 101 nonsmokers and 99 current or former smokers (>400 cigarettes/year), 75 patients with tumors located in the left lung, and 125 with tumors in the right. The seventh lung cancer TNM classification and staging system released by the Union for International Cancer Control (UICC) in 2009 was used for pathological staging. The pathological stages of all patients were evaluated, and included 87 patients with stages I–II and 113 with stages III–IV of the disease.

Follow-up was performed by telephone. The postoperative survival time was defined from the date of operation to the date of follow-up or the date of death. Up to July 25, 2014, there were 119 survivors, 55 deaths, and 26 patients lost to follow-up. The mean survival time was 935.9 days (range, 16–2,243 days).

All patients signed an informed consent before enrollment. The Institutional Ethics Committee of Tianjin Medical University General Hospital approved the study.

Methods of detection
Immunohistochemistry was used to assess TTF-1 expression and a liquid chip platform was used to identify EGFR mutations on separate slides of formalin-fixed, paraffin-embedded patient specimens.

TTF-1 detection
The tissue specimens were fixed using 10% formaldehyde. After standard processing, the paraffin-embedded specimens were cut into a 4 μm thick section and serial sections were generally used for the following staining. The sections were stained using hematoxylin–eosin stain and immunohistochemical staining employing mouse-anti TTF-1 monoclonal antibody (diluted at 1:100) from Fuzhou Maixin Biotechnology Company, according to the instructions.

Histopathologic diagnosis was performed by two experienced pathologists who used the World Health Organization tumor histological analysis method to classify cell types. Nuclei staining tan or brown after staining for TTF-1 expression were considered positive for TTF-1 expression, as shown in Figure 2. A tumor was considered positive or negative for TTF-1 based on the percentage of positive cells. As described by Shanzhi et al a sample was considered negative (−) for TTF-1 expression, if 0%–10% of tumor cells were positive, partially positive (+) if 10%–50% of tumor cells were positive, and positive (+) if >50% of tumor cells were positive. To facilitate statistical comparisons, tumors classified as strongly positive or partially positive for TTF-1 expression were uniformly classified positive.

Detection of EGFR mutations
All fixed samples with slides were sent to the Guangzhou Yishan Company for mutation analysis using the SurPlex-xTAG70plex-EGFR liquid chip. The procedure includes five major steps in sequence: 1) multiplex PCR was used to amplify the regions of target genes; 2) excess nucleotides and primers of PCR mixture were removed by exonuclease and shrimp alkaline phosphatase; 3) the left product was subjected to the process of allele specific primer extension,
in which 70 universal tags were, respectively, linked to a specific primer sequence complementary to a specific gene of interest. Primers that only matched the templates were extended by the Tsp DNA; 4) the allele specific primer extension products were made to hybridize to specific anti-tag probes precoated on the polystyrene microspheres; and 5) the products of hybridization were subjected to the Luminox 200 and median fluorescence intensity (MFI) was read and analyzed. The sensitivity and specificity of EGFR liquid chip that we exploited in this study is very high compared to the real mutation status. When the real EGFR mutation rate is as low as 1%, the sensitivity and specificity of EGFR mutation by liquid chip is almost 100%. And the assay is under rigorous quality control.

Statistical analysis

The χ² or the Fisher’s exact tests were used to analyze the relationships between EGFR mutations, TTF-1 expression, and clinical factors. The Kaplan–Meier method and Cox regression analysis were used to assess the relationship between postoperative survival, TTF-1 expression, and EGFR mutation status. All significant levels were two-sided. A P-value <0.05 was considered statistically significant. SPSS software (Version 17.0) was used for analysis.

Results

General characteristics of patients with tumors positive for EGFR mutations and TTF-1 expression

As shown in Table 2, 89 of 200 adenocarcinoma specimens (44.5%) had EGFR mutations, which included 44 specimens with exon 19 deletion mutations (22%) and 45 with exon 21 mutations (22.5%). EGFR mutations occurred in specimens from 40 patients older than 61 years (42.6%) and 49 patients 61 years of age or younger (46.2%). In 124 female participants, EGFR mutations were detected in specimens from 66 of 124 (53.2%) women and 23 of 76 men (30.3%, P<0.001). EGFR mutations were detected in specimens from 56 of 99 (56.6%) nonsmokers and 33 of 101 (32.7%, P=0.001) smokers. EGFR mutations were detected in 32 of 75 (42.7%) left lung specimens and 57 of 125 (45.6%, P=0.686) right lung specimens. EGFR mutations were detected in specimens from 36 of 87 (41.4%) patients with disease stages I–II and 53 of 113 (39.8%, P=0.436) patients with disease stages III–IV. EGFR mutations were detected in specimens from 46 of 72 (63.9%) female nonsmokers, of whom 24 (33.3%) had mutations in exon 19 and 22 (30.6%) had mutations in exon 21. The EGFR mutation rate in female nonsmokers was significantly higher than in the rest of the patients.

TTF-1 positive expression was found in 163 of 200 (81.5%) specimens. TTF-1 positive expression was found in specimens from 75 (79.8%) patients older than 61 years and 88 patients of age (83.0%) 61 years or younger. TTF-1 positive expression was found in specimens from 109 of 124 (88.0%) women and 54 of 76 men (71.1%, P=0.004). TTF-1 positive expression was found in specimens from 89 of 99 (89.9%) nonsmokers and 74 of 101 (73.3%, P=0.002) smokers. TTF-1 positive expression was found in 63 of 75 (84.0%) left lung specimens and 100 of 125 (80.0%, P=0.481) right lung specimens. TTF-1 positive expression was found in specimens from 69 of 87 (79.3%) patients with disease stages I–II and 94 of 113 (83.2%, P=0.484) patients with disease stages III–IV. TTF-1 positive expression was found in specimens from 67 of 72 (93.1%) female

| Category                | TTF-1 Positive (%) | TTF-1 Negative (%) | P-value | EGFR Positive (%) | EGFR Negative (%) | P-value |
|-------------------------|--------------------|--------------------|---------|-------------------|-------------------|---------|
| Age                     |                    |                    |         |                   |                   |         |
| >61 years               | 75 (79.8)          | 19 (20.2)          | 0.557   | 40 (42.6)         | 54 (57.4)         | 0.602   |
| ≤61 years               | 88 (83.0)          | 18 (17.0)          |         | 49 (46.2)         | 57 (53.8)         |         |
| Sex                     |                    |                    |         |                   |                   |         |
| Male                    | 54 (71.1)          | 22 (28.9)          | 0.004   | 23 (30.3)         | 53 (69.7)         | 0.000   |
| Female                  | 109 (88.0)         | 15 (22.0)          |         | 66 (53.2)         | 58 (46.8)         |         |
| Smoking                 |                    |                    |         |                   |                   |         |
| Ever                    | 74 (73.3)          | 27 (26.7)          | 0.002   | 33 (32.7)         | 68 (67.3)         | 0.001   |
| Never                   | 89 (89.9)          | 10 (10.1)          |         | 56 (56.6)         | 43 (43.4)         |         |
| Tumor location          |                    |                    |         |                   |                   |         |
| Left                    | 63 (84.0)          | 12 (16.0)          | 0.481   | 32 (42.7)         | 43 (57.3)         | 0.686   |
| Right                   | 100 (80.0)         | 25 (20.0)          |         | 57 (45.6)         | 68 (54.4)         |         |
| Pathological stage      |                    |                    |         |                   |                   |         |
| I–II                    | 69 (79.3)          | 18 (20.7)          | 0.484   | 36 (41.4)         | 51 (58.6)         | 0.436   |
| III–IV                  | 94 (83.2)          | 19 (16.8)          |         | 53 (39.8)         | 60 (60.2)         |         |

Abbreviations: EGFR, epidermal growth factor receptor; TTF-1, thyroid transcription factor-1.
nonsmokers. The TTF-1 positive expression rate in female nonsmokers was also significantly higher than in the rest of the patients.

**TTF-1 expression and EGFR mutations**

EGFR mutations were detected in 83 of 163 (50.9%) TTF-1-positive specimens and six of 37 (16.2%, \( P < 0.001 \)) TTF-1-negative specimens (Table 3). EGFR mutations were detected in 46 of 67 (63.9%, \( n = 72 \)) TTF-1-positive specimens from female nonsmokers. Rates of TTF-1 expression positivity and EGFR mutations were higher in specimens from female nonsmokers than in the rest of the patients.

There was a significant association of TTF-1 expression with EGFR mutations by the \( \chi^2 \) test (\( P < 0.001 \)). Table 3

**Table 3** Relationship between TTF-1 expression and EGFR gene mutation status in tumors of different categories of patients

| Category                        | Patient, n | TTF-1 expression Status of TTF-1 expression and EGFR mutation | EGFR(+) | EGFR(-) | Total | P-value |
|---------------------------------|------------|---------------------------------------------------------------|---------|---------|-------|---------|
| Overall status                  | 200        | TTF-1(+) 83 80 163                                           |         |         |       | 0.000   |
|                                 |            | TTF-1(-) 6 31 37                                            |         |         |       |         |
|                                 |            | Total 89 111 200                                             |         |         |       |         |
| Age                             | 94         | TTF-1(+) 38 37 75                                           |         |         |       | 0.002   |
| >61 years                       |            | TTF-1(-) 2 17 19                                            |         |         |       |         |
|                                 |            | Total 40 54 94                                              |         |         |       |         |
| ≤61 years                       | 106        | TTF-1(+) 45 43 88                                           |         |         |       | 0.025   |
|                                 |            | TTF-1(-) 4 14 18                                            |         |         |       |         |
|                                 |            | Total 49 57 106                                             |         |         |       |         |
| Sex                             | 76         | TTF-1(+) 19 35 54                                           |         |         |       | 0.080   |
| Male                            |            | TTF-1(-) 4 18 22                                            |         |         |       |         |
|                                 |            | Total 23 53 76                                              |         |         |       |         |
| Female                          | 124        | TTF-1(+) 64 45 109                                          |         |         |       | 0.001   |
|                                 |            | TTF-1(-) 2 13 15                                            |         |         |       |         |
|                                 |            | Total 66 58 124                                             |         |         |       |         |
| Smoking history                 | 101        | TTF-1(+) 29 45 74                                           |         |         |       | 0.021   |
| Ever                            |            | TTF-1(-) 4 23 27                                            |         |         |       |         |
|                                 |            | Total 33 68 101                                             |         |         |       |         |
| Never                           | 99         | TTF-1(+) 54 35 89                                           |         |         |       | 0.034   |
|                                 |            | TTF-1(-) 2 8 10                                             |         |         |       |         |
|                                 |            | Total 56 43 99                                              |         |         |       |         |
| Tumor location                  | 75         | TTF-1(+) 32 31 63                                           |         |         |       | 0.001   |
| Left                            |            | TTF-1(-) 0 12 12                                            |         |         |       |         |
|                                 |            | Total 32 43 75                                              |         |         |       |         |
| Right                           | 125        | TTF-1(+) 51 49 100                                          |         |         |       | 0.015   |
|                                 |            | TTF-1(-) 6 19 25                                            |         |         |       |         |
|                                 |            | Total 57 68 125                                             |         |         |       |         |
| Clinical stage                  | 87         | TTF-1(+) 34 35 69                                           |         |         |       | 0.003   |
| I–II                            |            | TTF-1(-) 2 16 18                                            |         |         |       |         |
|                                 |            | Total 36 51 87                                              |         |         |       |         |
| III–IV                          | 113        | TTF-1(+) 49 45 94                                           |         |         |       | 0.013   |
|                                 |            | TTF-1(-) 4 15 19                                            |         |         |       |         |
|                                 |            | Total 53 60 113                                             |         |         |       |         |
| Sex, smoking history            | 87         | TTF-1(+) 45 22 67                                           |         |         |       | 0.054   |
| Female nonsmokers               |            | TTF-1(-) 1 4 5                                             |         |         |       |         |
|                                 |            | Total 46 26 72                                              |         |         |       |         |
| Others                          | 113        | TTF-1(+) 49 45 94                                           |         |         |       | 0.013   |
|                                 |            | TTF-1(-) 4 15 19                                            |         |         |       |         |
|                                 |            | Total 53 60 113                                             |         |         |       |         |

**Abbreviations:** EGFR, epidermal growth factor receptor; TTF-1, thyroid transcription factor-1.
shows the relationship between TTF-1 expression and EGFR mutations according to different groups of patients. There was a significant association between TTF-1 expression and EGFR mutations in specimens from female patients, but not from male patients. In addition, of 72 female nonsmokers, 45 (62.5%) cases were found to have dual expression of TTF-1 positivity and EGFR mutation, which was higher than the dual negative expression rate (5.6%, 4/72).

**Association between TTF-1 expression and EGFR mutation subtypes**

The rate of mutations in exons 19 and 21 were 22.0% (44/200) and 22.5% (45/200), respectively. No mutations were detected in exons 18 and 20. As shown in Table 4, of 163 specimens positive for TTF-1 expression, exon 21 mutations were detected in 44 (27.0%), which was significantly higher than the rate (2.7%, P=0.001) detected in TTF-1-negative specimens. There was a significant association between exon 21 mutations and TTF-1 positivity by the Fisher’s exact test (P=0.001). The rate of exon 19 mutations in TTF-1-positive specimens was 23.9% (39/163), which was higher than the rate detected in TTF-1-negative specimens (13.5%, P=0.167).

**Relationship between postoperative survival time, EGFR mutations, and TTF-1 expression**

This study was followed up for each patient by phone. The postoperative survival time was defined as from the date of operation to the day of follow-up or the day of patients’ death. Until July 25, 2014, 119 patients survived, 55 patients died, and 26 patients were lost for the follow-up. The mean survival time was 935.9 days (range, 16–2,243 days).

**Table 4 Relationship between TTF-1 expression and EGFR gene mutation status subtypes**

| TTF-1 | Exon 19 | Exon 21 |
|-------|---------|---------|
|       | Mut (+), Mut (-), n (%) | P-value | Mut (+), Mut (-), n (%) | P-value |
| Positive (+) | 39 (23.9) 124 (76.1) | 0.167 | 44 (27.0) 119 (73.0) | 0.001 |
| Negative (-) | 5 (13.5) 32 (86.5) | 0.2 (7.3) | 36 (97.3) |
| Total | 44 (22.0) 156 (78.0) | 45 (22.5) 155 (77.5) |

Abbreviations: EGFR, epidermal growth factor receptor; Mut (+), with mutation; Mut (-), without mutation; TTF-1, thyroid transcription factor-1.

Kaplan–Meier analysis showed that there was no association between postoperative survival time and TTF-1 expression or EGFR mutation status (P=0.353 and P=0.106), respectively (Figure 3).

Table 5 shows the association between postoperative survival time, EGFR mutations, and TTF-1 expression among different patient subgroups, and the Kaplan–Meier method was used to analyze postoperative survival in relation to TTF-1 expression and EGFR mutation status among the different subgroups. The survival curves showed significant association between postoperative survival and TTF-1-positive specimens of patients with disease stages III–IV (P=0.032, Figure 4).

Cox regression analysis was used to evaluate the association of postoperative survival time, EGFR mutations, and TTF-1 expression among different subgroups of specimens and specimens of patients with disease stages III–IV: 1) TTF-1(+)/EGFR(+); 2) TTF-1(+)/EGFR(−); 3) TTF-1(−)/EGFR(+); and 4) TTF-1(−)/EGFR(−). There were no significant associations found when subgroups of all specimens were compared (Table 6). There was no significant difference between the risk of death for TTF-1(+)/EGFR(+) specimens and TTF-1(−)/EGFR(−) specimens (hazard ratio

![Figure 3](#) Kaplan–Meier estimates for postoperative survival according to EGFR gene mutation status and TTF-1 expression.

Notes: (A) Survival function in EGFR; (B) survival function in TTF-1.

Abbreviations: EGFR, epidermal growth factor receptor; TTF-1, thyroid transcription factor-1.
(HR) 1.706, 95% confidence interval (CI) 0.752–3.869; P=0.201; Table 6).

However, patients with disease stages III–IV and TTF-1(+)/EGFR(+) tumors had better survival than patients with disease stages III–IV and TTF-1(−)/EGFR(−) tumors (HR 2.616, 95% CI 1.085–6.306; P=0.027; Figure 5 and Table 6).

**Discussion**

TTF-1 is an important member of the NKX2-1 family, and is mainly expressed in the thyroid, brain, lung, and other organs.9 A previous study found that TTF-1 was a significant biomarker for lung adenocarcinoma, with 100% specificity and 94.6% sensitivity.10 Therefore, TTF-1 is an important clinical indicator for differentiating lung adenocarcinoma from squamous cell carcinoma. TTF-1 can also be useful for differentiating primary from metastatic lung cancer, because TTF-1 is expressed predominantly in primary lung adenocarcinoma, but minimally in other metastatic tumors.11 Central and peripheral lung adenocarcinomas have been found to have different origins. Peripheral lung adenocarcinomas mainly originate from the terminal bronchioles, Clara cells, and type II alveolar epithelial cells; whereas central airway adenocarcinomas mainly originate from bronchial basal cells. Research has found that TTF-1 expression was always positive in peripheral adenocarcinomas and negative in central adenocarcinomas. A previous study has found that TTF-1 expression was associated with regulation of gene expression of surfactant protein-A (SPA), surfactant protein-B (SPB), and Clara cell antigen.12 TTF-1 was confirmed to have a strong correlation with the pulmonary surfactant protein, and was highly expressed in adenocarcinomas of female nonsmokers, with less frequent Rb loss, and negative p53 expression.13 Berghmans et al14

**Table 5** Postoperative survival and TTF-1 expression and EGFR gene mutation status in different categories of patients

| Category         | TTF-1 χ² | P-value | EGFR χ² | P-value |
|------------------|----------|---------|---------|---------|
| Age              |          |         |         |         |
| >61 years        | 0.096    | 0.757   | 2.330   | 0.127   |
| ≤61 years        | 3.729    | 0.053   | 0.763   | 0.382   |
| Sex              |          |         |         |         |
| Male             | 0.381    | 0.537   | 1.062   | 0.303   |
| Female           | 0.281    | 0.596   | 2.134   | 0.144   |
| Smoking          |          |         |         |         |
| Ever             | 0.068    | 0.795   | 2.011   | 0.156   |
| Never            | 1.224    | 0.269   | 0.427   | 0.514   |
| Tumor location   |          |         |         |         |
| Left             | 0.000    | 0.999   | 2.889   | 0.089   |
| Right            | 1.562    | 0.211   | 0.487   | 0.485   |
| Pathological stage |        |         |         |         |
| I–II             | 0.889    | 0.346   | 1.638   | 0.201   |
| III–IV           | 4.578    | 0.032   | 2.075   | 0.150   |

**Abbreviations:** EGFR, epidermal growth factor receptor; TTF-1, thyroid transcription factor-1.

**Table 6** Postoperative survival analysis of patients with different EGFR and TTF-1 expression status

| Groups                      | Total Hazard ratio | Confidence interval (95%) | P-value | Stages III–IV Hazard ratio | Confidence interval (95%) | P-value |
|-----------------------------|---------------------|---------------------------|---------|----------------------------|---------------------------|---------|
| EGFR                        | 0.636               | 0.369–1.097               | 0.104   | 0.644                      | 0.352–1.178               | 0.153   |
| TTF-1                       | 0.743               | 0.391–1.413               | 0.365   | 0.479                      | 0.241–0.954               | 0.036   |
| EGFR(+)TTF-1(+) vs others   | 0.574               | 0.326–1.009               | 0.054   | 0.571                      | 0.305–1.067               | 0.079   |
| EGFR(+)TTF-1(−) vs others   | 1.644               | 0.512–5.282               | 0.404   | 1.642                      | 0.503–5.365               | 0.412   |
| EGFR(−)TTF-1(+) vs others   | 1.421               | 0.835–2.417               | 0.195   | 1.098                      | 0.601–2.004               | 0.762   |
| EGFR(−)TTF-1(−) vs others   | 1.223               | 0.597–2.505               | 0.583   | 2.116                      | 0.975–4.590               | 0.058   |
| EGFR(+)TTF-1(+) vs EGFR(−)TTF-1(−) | 1.706   | 0.752–3.869               | 0.201   | 2.616                      | 1.085–6.306               | 0.027   |

**Abbreviations:** EGFR, epidermal growth factor receptor; TTF-1, thyroid transcription factor-1.
and Tang et al\textsuperscript{13} showed that TTF-1 was an independent prognostic factor for patients with lung cancer, especially those with lung adenocarcinoma.\textsuperscript{14,15} A recent study showed that TTF-1 expression (HR = 0.340, 95% CI 0.143–0.811; \(P = 0.015\)) was an independent predictor of survival in lung adenocarcinoma patients.\textsuperscript{10} Zamecnik and Kodet,\textsuperscript{16} Sakuma et al,\textsuperscript{17} and Zhao et al\textsuperscript{18} reported TTF-1 positive expression rates of 75\%, 84.6\%, and 80\%, respectively, in lung adenocarcinoma patients. Our study found a TTF-1 expression rate of 81.5\% (163/200), which is comparable to the results from the other investigators, confirming that TTF-1 is a specific marker for lung adenocarcinoma.

\textit{EGFR} mutations play an important role in the pathogenesis of lung adenocarcinoma and are markers for patients with lung adenocarcinoma who take \textit{EGFR} TKIs as the first-line treatment.\textsuperscript{19} \textit{EGFR} gene mutations account for ~10\% of NSCLC in western patients and 50\% in Asian patients.\textsuperscript{20} Previous studies have reported that mutations mainly occur in exons 19 and 21 of \textit{EGFR} in lung adenocarcinomas, especially among Asian female patients who are never smokers.\textsuperscript{21,22} According to retrospective reports, ~90\% of patients who had a good response from TKIs were found to have \textit{EGFR} mutations, and patients that did not respond to TKIs did not have \textit{EGFR} mutations.\textsuperscript{21,23} Moreover, a study found that patients with \textit{EGFR} mutations had a better response rate to erlotinib than to chemotherapy.\textsuperscript{24} The 2010 NCCN recommended \textit{EGFR} mutation testing if first-line therapy for NSCLC was \textit{EGFR} TKI, with Category 2A or 2B level of evidence.\textsuperscript{25} The 2011 NCCN guidelines were somewhat different. Testing for \textit{EGFR} mutation status was strongly recommended for lung adenocarcinoma patients with Category 1 level of evidence.\textsuperscript{26} The proportion of tumor cells and normal cells in tissue samples is very important for detection of \textit{EGFR} mutations, but currently there is no established requirement for the number of cells. The percentage of tumor cells needed for DNA sequencing has been >50\%. Therefore, many investigators recommend as many tumor cells as possible for \textit{EGFR} mutation testing. However, at times it has been difficult to obtain sufficient tissue, especially from fine needle and bronchial biopsies. Therefore, other biomarkers are needed that can predict which patients have tumors with \textit{EGFR} mutations.

Two studies have recently demonstrated that TTF-1 expression and \textit{EGFR} mutations in lung adenocarcinomas were associated with sex (\(P = 0.003, P = 0.002\), respectively) and smoking history (\(P = 0.002, P = 0.001\), respectively), and were significantly more frequent in nonsmoking female patients.\textsuperscript{8,27} Further analysis showed that the rate of TTF-1 expression in tumors of female patients was 88.0\% (109/124) and 77.1\% in male patients (54/76). Similarly, the \textit{EGFR} mutation rate in tumors of female patients was 53.2\% (66/124) and 30.3\% in male patients (23/76). In addition, the rates of TTF-1 expression and \textit{EGFR} mutations in non-smokers were higher than in smoking patients (89.9\% vs 73.3\% and 56.6\% vs 32.7\%, respectively). More importantly, consistent with the results of Sun et al\textsuperscript{22} and Shanzi et al, we found that 83 tumors with \textit{EGFR} mutations were also positive for TTF-1 expression (93.3\%, 83/89; \(P < 0.001\); Table 3).\textsuperscript{8} We further analyzed the association between TTF-1 expression

![Figure 5](https://www.dovepress.com/doi-content.pdf)

**Figure 5** Cox regression analysis for postoperative survival according to TTF-1 expression and \textit{EGFR} mutation status in stage III–IV patients. **Notes:** Patients with TTF-1(+)\textit{EGFR}(+) tumors showed better survival than those with TTF-1(-)\textit{EGFR}(-) tumors. (A) Survival function; (B) cumulative hazard function. **Abbreviations:** \textit{EGFR}, epidermal growth factor receptor; TTF-1, thyroid transcription factor-1.
and EGFR mutation status in different subgroups of tumors, and found that TTF-1 had a significant association with EGFR mutation in all subgroups, but not in tumors of male patients ($P=0.080$, Table 3). In addition, we found mutation rates in exons 19 and 21 of 22% (44/200) and 22.5% (45/200), respectively (Table 4). Further analysis showed that there was a significant association between exon 21 mutations and TTF-1 expression ($P=0.001$, Table 4). We also observed that more tumors positive for TTF-1 expression had exon 19 mutation than tumors negative for TTF-1 expression, but the difference was not significant ($P=0.167$; Table 4).

As mentioned previously, EGFR mutation testing is very important for the diagnosis, treatment, and prognosis of patients with NSCLC, especially for those with lung adenocarcinoma. Investigators recently found that patients with tumors positive for EGFR mutations had better response to EGFR TKIs and chemotherapy than patients with tumors with wild-type EGFR. The findings suggest that testing patients with NSCLC for EGFR mutations status is crucial. However, the relationship between postoperative survival and EGFR mutation and/or TTF-1 expression has not been clearly elucidated. We found that postoperative survival time was not significantly associated with EGFR mutations or TTF-1 expression ($P=0.106$ and $P=0.353$, Figure 3). Further analysis showed significant association between postoperative survival time and TTF-1 expression in patients with disease stages III–IV disease ($P=0.032$, Figure 4). The postoperative survival of patients with disease stages III–IV and TTF-1(+)/EGFR(+) specimens was better than the survival of those with TTF-1(−)/EGFR(−) specimens ($P=0.027$, Figure 5), which may be a reflection of the fact that patients with TTF-1(+)/EGFR(+) tumors may be deriving benefit from targeted therapy. The associations between survival time and EGFR mutations and/or TTF-1 expression need further investigation.

Sometimes the status of EGFR mutations cannot be detected easily because of the difficulty in obtaining adequate tumor samples. This will result in the uncertain application of EGFR TKI treatment for adenocarcinoma patients and affect patient outcome. We found that the rate of EGFR mutations was higher in tumors of female patients (58.7%, 64/109) that were also positive for TTF-1 expression, and even higher in tumors of nonsmoking female patients with positive TTF-1 expression (67.2%, 45/67). These results may be accounted for by the high rate of EGFR mutations in lung cancers of Chinese patients. Therefore, both TTF-1 expression and EGFR mutations can be used as diagnostic markers and predictors of response to treatment for patients with lung adenocarcinoma. If the EGFR mutation status cannot be easily determined, testing for TTF-1 may be used to guide EGFR TKI therapy. For example, chemotherapy may be preferable for those lung adenocarcinoma patients with TTF-1-negative tumors, and unknown EGFR mutation status.

**Conclusion**

In summary, our study has demonstrated that TTF-1 expression in lung adenocarcinomas is significantly associated with the presence of EGFR mutation. Further investigation into the mechanisms for this relationship may contribute to greater understanding of the oncogenic role of TTF-1 in lung adenocarcinomas, and to studies on EGFR TKIs and the mechanisms of drug resistance. In clinical practice, TTF-1 expression may be a marker used for planning therapy for patients with lung adenocarcinomas and unknown EGFR mutation status, especially guiding therapy with EGFR TKIs.

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**Disclosure**

The authors report no conflicts of interest in this work.

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