Original Article

Significance of thyroid transcription factor 1 and Napsin A for prompting the status of *EGFR* mutations in lung adenocarcinoma patients

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Background: To evaluate the prompting value of thyroid transcription factor 1 (TTF-1) and Napsin A for the status of epidermal growth factor receptor (*EGFR*) mutations in an independent cohort of lung adenocarcinomas (LUADs) when genetic testing is unavailable.

Methods: In this study, 976 untreated primary LUADs were retrospectively reviewed. The clinical and pathological data, including age, gender, smoking history, predictive values of TTF-1 and Napsin A, *EGFR* status, and tumor-node-metastasis (TNM) stage were obtained through medical records available in Shanxi Province Cancer Hospital. All patients were divided into 2 groups, a mutant group (n=362) and wild-type group (n=614), according to their *EGFR* status. The clinical data and the expression of TTF-1 and Napsin A were compared between the 2 groups. TTF-1 and Napsin A are detected by fully automated IHC.PCR was carried out to detect the EGFR mutation. Univariate and multivariate logistic regression analyses were undertaken to distinguish independent factors of *EGFR* mutations.

Results: A total of 362 cases (37.1%) of *EGFR* mutations were detected, which were more frequent in females, never smokers, lymphatic metastasis, distant metastasis, and the positive expression of TTF-1 and Napsin A. Multivariate analysis indicated that females [odds ratio (OR), 1.950; 95% confidence interval (CI): 1.2958 to 2.938; P=0.001], never smokers (OR, 2.040; 95% CI: 1.345 to 3.094; P=0.001), and the positive expression of TTF-1 (OR, 2.366; 95% CI: 1.440 to 3.887; P=0.001) and Napsin A (OR, 2.295; 95% CI: 1.448 to 3.638; P<0.001) were effective prompting for EGFR mutations.

Conclusions: The positive expression of TTF-1 and Napsin A had the prompting value for EGFR mutations in patients with LUAD, and the indicators could be combined with other clinical characteristics to enhance the prediction of the *EGFR* status in LUAD.

Keywords: Epidermal growth factor receptor (EGFR); lung adenocarcinoma (LUAD); thyroid transcription factor 1 (TTF-1); Napsin A; mutation

Submitted Aug 25, 2022. Accepted for publication Oct 28, 2022.
doi: 10.21037/jtd-22-1265
View this article at: https://dx.doi.org/10.21037/jtd-22-1265

Introduction

Lung cancer is the dominant source of cancer mortality worldwide (1). Non-small cell lung cancer (NSCLC) accounts for more than 80% of total lung cancers, and adenocarcinoma is the most common types of NSCLC. The total five-year overall survival (OS) rate of lung cancer after diagnosis is only 15.6% (2). Patients with lung adenocarcinoma (LUAD) usually have Epidermal growth factor receptor (*EGFR*) mutations and mutation-positive patients may have new treatment modalities, such as tyrosine-kinase inhibitors (TKIs). There are no clear factors associated with the EGFR mutations and it is still in the exploratory stage. One study concluded that Asians, women and nonsmokers had a much higher rate of TTF-1 positive expression and EGFR mutations. The outcomes of a particular cohort who responded to therapies were remarkably improved, and the treatment of NSCLC has enabled a significant paradigm shift (3-6). Many randomized clinical trials have indicated that progression-free survival (PFS) of *EGFR* mutations is longer when TKIs are used compared with chemotherapy (7-9). Furthermore, *EGFR* expression is closely related to the efficacy of TKIs. Therefore, molecular profiling [e.g., *EGFR* or anaplastic lymphoma kinase (*ALK*)] should be recommended for patients with advanced LUAD, regardless of race, smoking history, gender, or other clinical factors (10). Nevertheless, due to the deficiency of biopsy samples or the patients' health condition, the achievement of sufficient high-quality tumor tissues for *EGFR* testing has remained challenging in different conditions.

Thyroid transcription factor 1 (TTF-1) is expressed in type II pneumocytes and pulmonary bronchiolar epithelial cells, retaining variable expression based on histological type. Napsin A, as a functional aspartic proteinase, is expressed in the cytoplasm of normal lung parenchyma (e.g., type II pneumocytes, alveolar macrophages, etc.), and it plays an important role in maturation of surfactant protein B (11,12). Together with TTF-1, Napsin A is extensively used to identify pulmonary adenocarcinomas from squamous cell carcinoma. Meanwhile, *EGFR* mutations are more frequently found in LUADs. Moreover, *EGFR* activates TTF-1/*Nkx2.1* gene expression when the H1975 cell line is used, and the high expression of Napsin A can reduce the resistance of TKIs in lung cancer cells (13,14). Hence, TTF-1 and Napsin A may be useful markers for predicting *EGFR* mutations. Therefore, the baseline TTF-1, and Napsin A, as well as *EGFR* status in 976 LUAD patients in the last 5 years were retrospectively reviewed in order to investigate whether TTF-1 and Napsin A could be valuable indicators for predicting *EGFR* mutations in LUAD. We present the following article in accordance with the STARD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1265/rc).

Methods

Patients and inclusion criteria

We retrospectively examined all untreated primary LUAD patients whose *EGFR* status, and TTF-1 and Napsin A were simultaneously detected during January 2013 and December 2017. In total, 1,372 cases without serious physical illnesses (e.g., thyroid disease, metabolic disease, etc.) were identified, and among them, 976 patients were finally enrolled. Furthermore, 396 individuals were excluded because of one of the following reasons: (I) 273 patients had received treatment before EGFR status or TTF-1 and Napsin A would be detected; (II) 65 patients had other malignant tumor history or mixed pathologic categories; (III) 43 cases had incomplete information; and (IV) 15 individuals had a combined exon mutation. Therefore, 976 individuals were finally analyzed in this study. The clinical and pathological data, including age, gender, smoking history, predictive values of TTF-1 and Napsin A, *EGFR* status, and tumor-node-metastasis (TNM) stage were obtained through medical records available in Shanxi Province Cancer Hospital. Patients who had smoked no more than 100 cigarettes totally or had never smoked were defined as never smokers. The patients were classified into 2 groups, including a mutant group (n=362) and a wild-type group (n=614) based on the *EGFR* status.

The TNM staging was based on the 8th edition of the TNM classification for lung cancer, and established on the basis of clinical workup procedures. All procedures were performed according to international guidelines and with at least 2 experienced clinicians. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Shanxi Province Cancer Hospital (No. 201835; Shanxi, China) and individual consent for this retrospective analysis was waived. Authors could identify individual participants during or after data collection according to their admission number.

Acquisition and analysis of TTF-1 and Napsin A

Tissue samples were obtained by surgical removal or biopsy of primary tumor, primary lymph node, or distant lymph node, and local or distant metastasis. Then, the samples were socked in 10% neutral formalin and subsequently inserted in paraffin wax. Slices (3 μm) of the samples were taken, and one of them was used for hematoxylin and eosin (H&E) staining, and the remaining were used for TTF-1 and Napsin A staining. In brief, the tissues were analyzed by the following antibodies: TTF-1 (clone 8G7G3/1, ZhongShan Jinqiao Biotech., Beijing, China) and Napsin A (multi-clone, Fuzhou Maixin Biotech Inc., Fuzhou, China). The procedure was performed by Ventana immunohistochemistry (IHC) which was a fully automated IHC assay. At the same time, positive and negative controls were used in each experiment. The results of pathological diagnosis and IHCs were interpreted by at least 2 skilled pathologists and positive for brown or tan nuclear appearance. Positive expressions of TTF-1 and Napsin A were defined as more than 5% and 50% of tumor cells with nuclear staining, respectively.

EGFR mutation analysis

We detected *EGFR* mutations according to the principle of the amplification refractory mutation system (ARMS). Also, 4 paraffin-embedded wax slices with a continuous 7 μm thickness were taken to extract DAN using the QIAamp DNA FFPE Kit (Qiagen, Venlo, The Netherlands) on the basis of the manufacturer's instructions. After that, polymerase chain reaction (PCR) was carried out to detect the *EGFR* mutation using the EGFR 29 Mutations Detection Kit (Amoy Diagnostics, Xiamen, China). All the steps were completed according to the manufacturer's instructions. The results of *EGFR* mutations were interpreted by at least 2 experienced pathologists.

Statistical analysis

All data were analyzed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Chi-squared test and *t*-test were used to compare clinical characteristics according to EGFR status. statistically significant was defined as P values less than 0.05. Logistic regression analysis was used to identify independent predictors of *EGFR* mutation. Clinical characteristics and IHC with P<0.05 in the univariate analysis were further included in multivariate regression analysis and the variables of P<0.05 in which were regarded to be independent, the odds ratio (OR) and 95% confidence interval (CI) of the independent predictors were also taken into account.

Results

Patients and tumor characteristics

Among the 976 LUAD patients detected between January 2013 and December 2017, 362 cases were mutant patients, and 614 participants were wild-type patients based on the *EGFR* status, in which the mutant rate of *EGFR* was 37.1%. *Table 1* presents the basic situation for the two groups.

The 976 patients were aged from 30 to 87 years, with an average age of 59.34±9.84 years, and 489 (50.1%) cases were non-smokers. The cohort comprised 585 women (59.9%) and 391 men (40.1%). Additionally, there were 789 (80.8%) individuals with positive expression of TTF-1, and 750 (76.8%) cases with positive expression of Napsin A. Moreover, the mutant group contained 18 cases with exon 18 mutation, 168 cases with exon 19 mutation, 23 cases with exon 20 mutation, and 153 cases with exon 21 mutation, which accounted for 5.0%, 46.4%, 6.4%, and 42.3% of all mutations, respectively (*Table 1*).

Association between the two groups

In order to clarify the association between the clinical data and EGFR status, we compared the characteristics of the 2 groups. We detected *EGFR* mutations more common in females (59.9% *vs.* 40.1%, P<0.001), never smokers (70.7% *vs.* 29.3%, P<0.001), and those with positive expressions of TTF-1 (92.3% *vs.* 7.7%, P<0.001) and Napsin A (90.9% *vs.* 9.1%, P<0.001), lymphatic metastasis (24.6% *vs.* 75.4%,

Table 1 Association between the two groups

Variables	*EGFR*		Total	P value
	Mutant group (N=362)	Wild-type group (N=614)		
Age (years), mean ± SD [range]	59.52±9.61 [31–86]	59.24±9.97 [30–87]	59.34±9.84 [30–87]	0.658[a]
Age (years) (n, %)				0.981[b]
<60	186 (51.4)	315 (51.3)	475 (48.7)	
≥60	176 (48.6)	299 (48.7)	501 (51.3)	
Gender (n, %)				<0.001[b]
Male	145 (40.1)	440 (71.7)	585 (59.9)	
Female	217 (59.9)	174 (28.3)	391 (40.1)	
Smoking status (n, %)				<0.001[b]
Never smoker	256 (70.7)	233 (37.9)	489 (50.1)	
Ever smoker	106 (29.3)	381 (62.1)	487 (49.9)	
TTF-1 (n, %)				<0.001[b]
Negative	28 (7.7)	159 (25.9)	187 (19.2)	
Positive	334 (92.3)	455 (74.1)	789 (80.8)	
Napsin A				<0.001[b]
Negative	33 (9.1)	193 (31.4)	226 (23.2)	
Positive	329 (90.9)	421 (68.6)	750 (76.8)	
Tumor statement (n, %)				0.516[b]
1	61 (16.9)	104 (16.9)	165 (16.9)	
2	161 (44.5)	255 (41.5)	416 (42.6)	
3	66 (18.2)	136 (22.1)	202 (20.7)	
4	74 (20.4)	119 (19.4)	193 (19.8)	
Nodal involvement (n, %)				0.034[b]
0	89 (24.6)	136 (22.1)	225 (23.1)	
1	56 (15.5)	61 (9.9)	117 (12.0)	
2	114 (31.5)	216 (35.2)	330 (33.8)	
3	103 (28.5)	201 (32.7)	304 (31.1)	
Metastatic statement (n, %)				0.011[b]
0	151 (41.7)	308 (50.2)	459 (47.0)	
1	211 (58.3)	306 (49.8)	517 (53.0)	
Stage (n, %)				0.320[b]
I, II	81 (22.4)	121 (19.7)	202 (20.7)	
III, IV	281 (77.6)	493 (80.3)	774 (79.3)	
EGFR mutations (n, %)				
Exon 18	18 (5.0)			
Exon 19	168 (46.4)			
Exon 20	23 (6.4)			
Exon 21	153 (42.3)			

[a], *t*-test calculation; [b], chi-square calculation. EGFR, epidermal growth factor receptor; SD, standard deviation; TTF-1, thyroid transcription factor 1.

P=0.034), and distant metastasis (58.3% *vs.* 22.4%, P=0.011) (*Table 1*).

Prompting of the EGFR mutation

To further identify the prompting factors of *EGFR* mutation, univariate and multivariate logistic regression analyses were performed. The univariate regression indicated that gender (OR, 3.784; 95% CI: 2.878 to 4.977; P<0.001), smoking status (OR, 3.949; 95% CI: 2.988 to 5.220; P<0.001), TTF-1 (OR, 4.168; 95% CI: 2.723 to 6.381; P<0.001), Napsin A (OR, 4.570; 95% CI: 3.075 to 6.794; P<0.001), and distant metastasis (OR, 1.406; 95% CI: 1.082 to 1.828; P=0.011) were correlated with *EGFR* mutations. After the univariate regression, the inclusion indicators altogether in the multivariate regression indicated that gender, smoking status, and the positive expression of TTF-1 and Napsin A is still an independent factors for prompting the status of EGFR mutation. Females (OR, 1.950; 95% CI: 1.2958 to 2.938; P=0.001), status of never smoker (OR, 2.040; 95% CI: 1.345 to 3.094; P=0.001), the positive expression of TTF-1 (OR, 2.366; 95% CI: 1.440 to 3.887; P=0.001) and Napsin A (OR, 2.295; 95% CI: 1.448 to 3.638; P<0.001) were effective predictors of the status of *EGFR* mutation (*Table 2*).

Here, 362 *EGFR* mutant individuals were analyzed to evaluate the prompting value of each factor. Briefly, univariate regression analysis demonstrated that gender, smoking status, the positive expression of TTF-1 and Napsin A were correlated with each type of *EGFR* mutation. However, the factors failed to predict the type of *EGFR* mutation (*Table 3*).

Discussion

Predominantly, *EGFR* mutations appear in LUAD, which is the predominant subtype of lung cancer (15). Previous studies have reported that TKIs play an important role to prolong PFS in patients with *EGFR* mutations (7-9). Thus, molecular profiling (e.g., *EGFR*) was recommended as the standard care for patients with advanced-stage adenocarcinoma regardless of race, smoking history, gender, or other clinical characteristics (10). Nevertheless, due to the deficiency of biopsy samples or the patients' health condition, the achievement of sufficient high-quality tumor tissues for EGFR testing has remained challenging. In this study, we revealed that the positive expression of TTF-1 and Napsin A is of great significance for predicting the status of EGFR mutations in patients with LUAD, and the indicators could be combined with other clinical characteristics to enhance the prediction of the EGFR status in LUAD patients.

Both of TTF-1 and Napsin A have rarely been used to predict the status of *EGFR* mutations, and this study investigated a relationship between TTF-1, Napsin A, and *EGFR* mutations. Briefly, the positive expression of TTF-1 and Napsin A have traditionally been used to distinguish the type of lung cancer or the primary and metastatic lung cancer (12,16,17). As an essential mammalian protein, TTF-1 regulates transcription, replication fork arrest, DNA damage repair, chromatin remodelling, and so on; TTF1 plays a crucial role in maintaining normal cellular physiology, and its dysregulation has been reported to induce oncogenic transformation of the cells (18). Previous data have shown that the positive expression of TTF-1 was also associated with the prognosis of adenocarcinoma (19). Moreover, the positive expression of TTF-1 was associated with *EGFR* mutations, and few data have shown a relationship between Napsin A and *EGFR* mutations in NSCLC patients (20-22). However, high expression of Napsin A could improve the sensitivity of lung carcinoma cells to TKIs (14). In the present study, untreated primary adenocarcinomas were selected to evaluate the predictive values of TTF-1 and Napsin A for the status of *EGFR* mutations. The findings were consistent with the results previously reported (19), where the positive expression of TTF-1 was an independent indicator for predicting the status of *EGFR* mutations, and the positive expression of Napsin A as an independent factor was also investigated in this study.

This study bore some similarities to previously conducted studies. First, in terms of clinical features, gender and smoking status were regarded as the important factors associated with *EGFR* mutations. In this study, females had higher *EGFR* mutation rates than males, and never smokers had more *EGFR* mutations than ever smokers, which aligns with previous studies (15,22). Second, previous data had demonstrated, the positive expression of TTF-1 has a significant positive correlation with *EGFR* mutations in patients with LUADs, and if the expression of TTF-1 was negative, there was a 95% chance of wild-type *EGFR* mutation (17-19). In the current study, among 362 patients, there were 329 cases (92.3%) with the positive expression of TTF-1. Moreover, about 10–18% of all *EGFR* mutations were "uncommon" and primarily consist of exons 18 and 20, as well as complex mutations (23). Complex *EGFR* mutations were excluded from our study, in which 18 and 23 cases had

Table 2 Univariate and multivariate analyses of various predictive indicators for predicting the EGFR status

Characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Age		0.997		
<60	Reference			
≥60	0.981 (0.769–1.293)			
Gender		<0.001		0.001
Male	Reference		Reference	
Female	3.784 (2.878–4.977)		1.950 (1.295–2.938)	
Smoking status		<0.001		0.001
Never	3.949 (2.988–5.220)		2.040 (1.345–3.094)	
Ever	Reference		Reference	
TTF-1		<0.001		0.001
Negative	Reference		Reference	
Positive	4.168 (2.723–6.381)		2.366 (1.440–3.887)	
Napsin A		<0.001		<0.001
Negative	Reference		Reference	
Positive	4.570 (3.075–6.794)		2.295 (1.448–3.638)	
Tumor statement				
1	Reference			
2	1.076 (0.742–1.562)	0.698		
3	0.827 (0.537–1.274)	0.390		
4	1.060 (0.690–1.628)	0.789		
Nodal involvement				
0	Reference			
1	1.403 (0.894–2.201)	0.141		
2	0.806 (0.568–1.145)	0.229		
3	0.783 (0.548–1.120)	0.180		
Metastatic statement		0.011		
0	Reference			
1	1.406 (1.082–1.828)			
Stage		0.320		
I, II	Reference			
III, IV	0.851 (0.620–1.169)			

EGFR, epidermal growth factor receptor; OR, odds ratio; CI, confidence interval; TTF-1, thyroid transcription factor 1.

Table 3 Univariate and multivariate analyses of various predictive indicators for predicting the status of *EGFR* subtypes in mutation group

Characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Exon 18				
Gender		<0.001		
Male	Reference			
Female	3.784 (2.878–4.977)			
Smoking status		<0.001		
Never	3.949 (2.988–5.220)			
Ever	Reference			
TTF-1		<0.001		
Negative	Reference			
Positive	4.168 (2.723–6.381)			
Napsin A		<0.001		
Negative	Reference			
Positive	4.570 (3.075–6.794)			
Exon 19				
Gender		<0.001		
Male	Reference			
Female	3.784 (2.878–4.977)			
Smoking status		<0.001		
Never	3.949 (2.988–5.220)			
Ever	Reference			
TTF-1		<0.001		0.054
Negative	Reference		Reference	
Positive	4.168 (2.723–6.381)		2.299 (0.985–5.365)	
Napsin A		<0.001		
Negative	Reference			
Positive	4.570 (3.075–6.794)			
Exon 20		0.320		
Gender		<0.001		
Male	Reference			
Female	3.784 (2.878–4.977)			
Smoking status		<0.001		
Never	3.949 (2.988–5.220)			
Ever	Reference			
TTF-1		<0.001		
Negative	Reference			
Positive	4.168 (2.723–6.381)			

Table 3 *(continued)*

Table 3 *(continued)*

Characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Napsin A		<0.001		
Negative	Reference			
Positive	4.570 (3.075–6.794)			
Exon 21				
Gender		<0.001		
Male	Reference			
Female	3.784 (2.878–4.977)			
Smoking status		<0.001		
Never	3.949 (2.988–5.220)			
Ever	Reference			
TTF-1		<0.001		
Negative	Reference			
Positive	4.168 (2.723–6.381)			
Napsin A		<0.001		
Negative	Reference			
Positive	4.570 (3.075–6.794)			

EGFR, epidermal growth factor receptor; OR, odds ratio; CI, confidence interval; TTF-1, thyroid transcription factor 1.

exons 18 and 23, respectively. The "uncommon" *EGFR* mutations accounted for 11.4% of all *EGFR* mutations in this study. These similarities may depend on the natural features or the pathways of the *EGFR* gene (13,24,25).

Nevertheless, there were some disparities in our study. Based on the results, the rate of *EGFR* mutations was 37.1% in the present study, which was slightly lower than the data previously reported. In Chinese cohorts, the rate of *EGFR* mutations accounted for 49.8% in LUADs patients or 50.2% in patients with advanced LUAD (15,22). This difference may associate with the selection of patients. A dominant mutant crowd may take TKIs in blind without detection of *EGFR* mutations, and all stages of LUADs, except for advanced LUAD, were included, resulting in a low rate of *EGFR* mutations in the included patients. Additionally, studies reported a relationship between the clinical features and subtypes of EGFR mutations (26). Exon 20 mutation was associated with clinicopathological features, which was similar to this study as well. However, exon 19 and 21 mutations were associated with gender and never smokers (26), that was different form our study. After regression analysis of the mutant patients, each exon mutation failed to associate with clinical or pathological features. In addition to the characteristics of the subtypes of EGFR mutations, different subtypes of EGFR mutations and expression of TTF-1 and Napsin A showed different levels of prognosis (27-29). The variable results may be due to the heterogeneity of EGFR mutations and relatively small samples with complex genetic background, demonstrating that the different mechanisms lead to a variety of EGFR mutations (26,30).

In conclusion, this study demonstrated that the positive expression of TTF-1 and Napsin A would be feasible indicators to prompt the status of EGFR mutations in LUAD when genetic testing is unavailable. Further studies are required to validate results achieved in multiple centers, and the prompting value of TTF-1 and Napsin A should be fully elucidated in different types of lung cancer.

Acknowledgments

We thank the staff at Department of Pathology, Shanxi

Province Cancer Hospital for their great contribution.
Funding: This work was supported by the Non-profit Central Research Institute Fund of Chinese Academy of Medical Science (No. 2020-PT320-005), Key R&D projects of Shanxi Province (No. 201903D321201), the Youth Foundation of Science and Technology Commission of Shanxi Province (No. 202103021223447), and the Natural Science Foundation of Shanxi Province (No. 202103021224407).

Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1265/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1265/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1265/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Shanxi Province Cancer Hospital (No. 201835; Shanxi, China) and individual consent for this retrospective analysis was waived.

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Cite this article as: Ren X, Wen X, Ren YJ, Liu X, Wang J, Hao M, Ma Q, Ren J, Jin B, Qiao X, Li B, Wu J, Li X, Liu Z. Significance of thyroid transcription factor 1 and Napsin A for prompting the status of *EGFR* mutations in lung adenocarcinoma patients. J Thorac Dis 2022;14(11):4395-4404. doi: 10.21037/jtd-22-1265