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ORIGINAL ARTICLE

Relationship between molecular changes in epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) mutations in lung adenocarcinoma*

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Abstract

Objective This study aimed to analyze the relationship between the mutations in epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) and their impact on the prognosis and treatment of lung adenocarcinoma.

Methods A total of 158 cases of lung adenocarcinoma reported between January 2007 and January 2014 were retrospectively analyzed. These tumors were resected using radical pneumonectomy and underwent pathology-based diagnosis at our institution (Inner Mongolia People's Hospital, Hohhot, China). The tissue sections were evaluated using the updated World Health Organization classification of lung adenocarcinomas (2015 version), with each histological component recorded in 5% increments. The histological subtypes were classified, and any surviving cases were followed up. The reverse transcription-polymerase chain reaction (RT-PCR) and direct DNA sequencing were used to evaluate mutations in exons 18, 19, 20, and 21 in the *EGFR* gene, and the echinoderm microtubule-associated protein-like 4 gene-ALK variant (*EML4-ALK*) fusions were detected using sequencing.

Results Our cohort included 25 patients with pre-invasive adenocarcinoma, 13 patients with lepidic, 66 patients with acinar, 13 patients with papillary, and 25 patients with solid infiltrative adenocarcinoma with the remaining cases presenting with a variety of pathological subtypes. The prognosis of each histological subtype was different with the 5-year disease-free survival and 5-year overall survival (OS) of pre-invasion adenocarcinoma at 100%; the 5-year OS of lepidic, acinar, and papillary adenocarcinoma patients was only 84.6%, 72.7%, and 76.9%, respectively. The 5-year OS of solid and mucinous adenocarcinomas were 32.0% and 36.4%, respectively. *EGFR* mutation was detected in 69 cases with a mutation rate of 43.7% and majority of these mutations were found in exons 19 (50.6%) and 21 (37.9%), with women and non-smokers shown to experience a higher mutation rate ($P < 0.05$). However, histological subtype analysis showed that *EGFR* mutations were primarily found in adenocarcinomas. Most of these mutations were found in lepidic (53.8%) or acinar adenocarcinomas (50.0%), whereas these mutations were rare in both solid (28.0%) and mucinous adenocarcinoma (27.2%). The fusion mutation rate in the *EML4-ALK* gene was 5.69%, and was most common in young, nonsmoking patients ($P < 0.05$).

Conclusion The prognosis of patients in each lung adenocarcinoma subtype is different, and these outcomes are likely related to mutations in the *EGFR* and *EML4-ALK* genes. *EGFR* mutation rates are higher in lepidic and acinar adenocarcinomas, whereas *EML4-ALK* gene fusion mutations are more common in solid and mucinous adenocarcinoma. *EGFR* mutations are more common in female and non-smoking patients, whereas *EML4-ALK* fusions are more common in young, non-smoking patients.

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Key words: lung cancer; histological subtypes; prognosis; the echinoderm microtubule-associated protein-like 4 gene-ALK variant (*EML4-ALK*); epidermal growth factor receptor (*EGFR*)

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More than half of all newly diagnosed non-small cell lung cancer is lung adenocarcinoma that has variable genetic and morphological patterns and presents with a variety of clinicopathological characteristics. The World Health Organization (WHO) published a new classification system for lung tumors in 2015 which relies on histology-based classification terms [1]. Epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) have been identified as the critical genetic drivers for lung adenocarcinoma, and both act as critical prognostic factors in this disease. This study aimed to explore the relationship between the various histological types of lung adenocarcinoma and common gene mutations, as well as the clinicopathological characteristics of these patients in an effort to provide new insights into individualized treatments for this disease.

Materials and methods

Tissue samples

We collected the data from 158 cases of lung adenocarcinoma treated at the Department of Oncology, Inner Mongolia People's Hospital, Hohhot, China, between January 2007 and January 2014. All patients signed an informed consent and both the clinical and pathological data of the patients were reviewed, and the relevant information was recorded, including gender, age, smoking history, tumor size, tumor location, and tumor stage. Any patients receiving preoperative adjuvant radio- or chemotherapy were excluded, and staging (tumor, lymph node, and metastasis) was performed according to the guidelines established in the 7th edition of the United States Joint Committee on lung cancer staging system. The follow-up information collected for surviving patients included preoperative staging, surgical methods and the treatment outcome of patients after operation, etc.

Pathological examinations

Hematoxylin and eosin (H&E)-stained sections from each of the tissue samples were re-examined by two experienced pathologists and classified using the new WHO lung adenocarcinoma classification system (2015). These reexaminations included a reevaluation of the e morphological standard and the histological subtype of the tumor which were then classified as: *in situ* adenocarcinoma (AIS), minimal invasive adenocarcinoma (MIA), lepidic adenocarcinoma (LPA), acinar adenocarcinoma, papillary adenocarcinoma, solid adenocarcinoma with mucus formation, micropapillary adenocarcinoma, invasive mucinous adenocarcinoma, enteric adenocarcinoma, and colloidal carcinoma. Each histological component was recorded in 5% increments and the degree of tumor differentiation, tumor thrombus, mitotic number (/10 hpf), necrosis and the relationship between the tumor and pleura were evaluated and recorded. All diagnostic criteria were then omitted.

Specimen preparation

All samples were fixed in neutral formaldehyde and embedded in paraffin. The blocks with tumor tissue were selected and each paraffin block was cut into 4-micron-thick sections, subjected to H&E staining and evaluated under a microscope. A block with a tumor component ratio of greater than 75% was selected for each case. Four to five 5 μM thick sections were placed into a 1.5 mL tube and then subjected to genetic evaluation. Genetic analysis of the tumor was performed in 158 patients. *EGFR* mutations were detected by Sanger sequencing or amplification refractory mutation system, as previously described. *ALK* alterations were detected by fluorescence *in situ* hybridization with ALK break apart probes and/or immunohistochemistry (IHC) staining with Ventana anti-ALK antibody as previously described. Mutations in exons 18, 19, 20 and 21 of the *EGFR* gene and the echinoderm microtubule-associated protein-like 4 gene-ALK variant *(EML4-ALK)* fusions were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and direct DNA sequencing.

Statistical analysis

SPSS 22.00 (IBM Corp., Armonk, NY, USA) was used to analyze the data. Chi-square analysis was used to evaluate the correlation between *EGFR* and *ALK* mutations and associated information, and the log-rank test was used to evaluate the survival rate in each group. A P value of < 0.05 was set as statistically significant.

Results

Our cohort comprised of 70 males and 88 females at an average age of 59.1 years (30–77 years), with these 158 participants including 120 nonsmokers (75.9%). Our participants spanned various tumor types and stages, with 63 participants in stage I, 44 in stage II, 41 in stage III, and 10 cases at stage IV. These included 25 cases of pre-invasion adenocarcinomas, including lepidic adenocarcinoma (13 cases), acinar adenocarcinoma (66 cases), papillary adenocarcinoma (13 cases), solid adenocarcinoma (25 cases), and other pathological subtypes. The follow-up time spanned between 22–112 months, and four cases (one lepidic, two acinar, one papillary) were lost to follow up. A total of 119 patients survived their initial disease with 13 experiencing tumor recurrence and metastasis, and 35 dying before the end of the study. The prognosis of each histological subtype was different (Table 1), with the 5-year disease-free survival and overall survival (OS) at 100% for *in situ* and minimally invasive adenocarcinoma, and 84.6%, 72.7%, and 76.9% for lepidic, acinar, and

papillary adenocarcinoma, respectively. The 5-year OS rates in solid and mucinous adenocarcinoma were only 32.0% and 36.4%, respectively.

Of the 158 samples, 69 exhibited detectable mutations in the *EGFR* gene, making the overall mutation rate for this gene 43.7%. The majority of these mutations were found in exons 19 (50.6%) and 21 (37.9%), and these mutation rates were significantly higher in female patients (54.5% for women and 30% for men; $P < 0.05$). The mutation rate in the non-smoking group (49.2%) was higher than that of the smoking group (26.3%), with this difference also demonstrating statistical significance ($P < 0.05$). However, age, tumor size, stage, pleural involvement, and lymph node metastasis were

Table 1 Survival and histological subtype of lung adenocarcinomas

Histological subtype	*n*	5-year DFS (%)	5-year OS (%)
Precancerous adenocarcinoma			
Adenocarcinoma *in situ*	5	100	100
Minimal invasive adenocarcinoma	20	100	100
Infiltrating adenocarcinoma			
Mainly lepidic	13	69.2	84.6
Mainly acinar	66	51.5	72.7
Mainly papillary	13	53.8	76.9
Micropapillary	5	0	60.0
Mainly solid	25	0	32.0
Mucinous adenocarcinoma	11	36.4	36.4

Note: DFS, disease-free survival; OS, overall survival

Table 2 Mutational analysis of 158 cases of lung adenocarcinoma [*n* (%)]

Item	*n*	*EGFR* (+)	*EGFR* (–)	*P*	*ALK* (+)	*ALK* (–)	*P*
Gender				0.0000			0.2701
Male	70	21 (30.0)	49 (70.0)		4 (5.7)	66 (94.3)	
Female	88	48 (54.5)	40 (45.5)		5 (5.7)	83 (94.3)	
Age (years)				0.5944			0.0000
< 55	60	26 (43.3)	34 (56.7)		6 (10.0)	54 (90.0)	
≥ 55	98	42 (42.9)	56 (57.1)		3 (3.1)	95 (96.9)	
Smoking status				0.0000			0.0049
Non smoking	120	59 (49.2)	99 (40.8)		8 (6.7)	112 (93.3)	
Smoking	38	10 (26.3)	28 (73.7)		1 (2.6)	37 (97.4)	
Histological subtype				0.3369			0.0019
Precancerous adenocarcinoma							
AIS	5	3 (60.0)	2 (40.0)		0 (0.0)	5 (100.0)	
MIA	20	9 (45.0)	11 (55.0)		1 (5.0)	19 (95.0)	
Infiltrating adenocarcinoma							
Lepidic	13	7 (53.8)	6 (46.2)		0 (0.0)	13 (100.0)	
Acini	66	33 (50.0)	33 (50.0)		2 (3.1)	64 (96.9)	
Papillae	13	5 (38.5)	8 (61.5)		0 (0.0)	13 (100.0)	
Mainly solid	25	7 (28.0)	18 (72.0)		3 (12.0)	22 (88.0)	
Micro nipple	5	2 (40.0)	3 (60.0)		0 (0.0)	5 (100.0)	
Mucous adenocarcinoma	11	3 (27.2)	8 (92.8)		3 (27.2)	8 (92.8)	
Tumor size (cm)				0.0311			
≤ 3	107	51 (47.7)	56 (52.3)				
> 3	51	14 (27.5)	37 (72.5)				
Pleural invasion				0.6771			
Yes	88	34 (38.7)	54 (61.3)				
No	70	29 (41.4)	41 (58.6)				
Lymph node metastasis				0.8313			
Yes	40	16 (40.0)	24 (60.0)				
No	118	52 (44.1)	66 (55.9)				
Tumor stage				0.9121			
Stage I	63	36 (57.1)	27 (42.9)				
Stage II	44	25 (56.8)	19 (43.2)				
Stage III	41	18 (43.9)	23 (56.1)				
Stage IV	10	6 (60.0)	4 (40.0)				

Note: *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; AIS, *in situ* adenocarcinoma; MIA, minimal invasive adenocarcinoma

not found to be related to *EGFR* mutation rates in these patients. Histological subtype analysis showed that *EGFR* mutation was more common in lepidic (53.8%) and acinar type adenocarcinomas (50.0%), whereas these mutations were rarer in solid tumors (28.0%) and mucinous adenocarcinomas (27.2%). Fusion mutation, *EML4-ALK*, was identified in 5.69% of the samples and was found to be more common in young and non-smoking patients (all $P < 0.05$). This fusion was also more common in mucinous and solid adenocarcinomas (Table 2).

Discussion

This study demonstrates that the new classification method for lung adenocarcinomas may be predictive for curative effect, prognosis, and tumor metabolism [2–5], and suggests that this new classification method can be used to supplement clinical treatment decision-making using tumor staging. The prognosis of each histological subtype of lung adenocarcinoma is different. Both *in situ* and minimally invasive adenocarcinoma have a 5-year disease-free survival of 100% and a 5-year total survival of 100%, suggesting that these patients may only need surgical resection without adjuvant treatment; the prognosis of lepidic, acinar, and papillary adenocarcinoma is moderate; however, solid and mucinous adenocarcinomas have a 5-year OS of 32.0% and 36.4%, respectively, with very poor prognosis. This suggests that some of these patients may need adjuvant treatment after surgery and a growing number of studies have confirmed that both the micro papilla and solid components are directly related to poorer prognosis [6–7], which has the added value of helping to predict the survival odds of patients independent of tumor stage. The appearance of solid components also indicates that the tumor is more invasive, rendering it necessary to include the micropapillary and solid elements in the pathological report using 5% increments.

Recent evaluations have continued to expand our understanding of the genetic origins and drivers of lung cancer and molecular typing, based on genetic characteristics, helps to take the treatment of advanced lung cancer into the realm of individualized treatment. *EGFR* mutations are an important predictor of targeted therapy in lung adenocarcinoma and have been widely applied in clinical intervention trials. The *EGFR* mutation rate in lung cancers in Caucasian patients is approximately 10%, whereas in Asian patients, it can be as high as 30% to 40% [8]. Most of these mutations occur in young, female, non-smokers and the majority of these *EGFR* mutations have been linked to the clinical characteristics of the tumor, including histology, race, gender, etc. The results of this study showed that the *EGFR* mutation rate in this cohort was 43.7%, with majority of mutations in exons 19 and 21. Women and non-smokers had a higher mutation rate, which is similar to other reports, but there were no correlations between *EGFR* mutation and age, tumor size, stage, pleural involvement or lymph node metastasis. Histological subtype analysis showed that majority of *EGFR* mutations were identified in lepidic and acinar adenocarcinoma, whereas these mutations were rarer in the solid and mucinous adenocarcinoma samples. Histological features such as lepidic structure and acinar structure may predict *EGFR* mutation better than other clinical parameters. It was also found that the *EGFR* mutation rate in mucinous adenocarcinoma was lower (27.2%) than that of non-mucinous adenocarcinomas with lepidic structure (53.8%).

ALK fusions have been shown to define a unique molecular subtype in lung adenocarcinomas. Our data revealed a 5.69% occurrence of *ALK* fusion mutations in the lung adenocarcinomas of this cohort, with the average age of these patients being significantly lower than the *ALK*-negative group, which was consistent with other results in the literature [9]. The relationship between smoking history and *ALK* fusions is controversial, with several studies suggesting that *ALK* fusions are more common in nonsmokers, but several other papers reporting that smoking history is not a significant factor in *ALK* fusion mutations. This study shows that *ALK* fusions were significantly more common in nonsmokers in this cohort, but the specific mechanism underlying this correlation requires further study. There was no significant difference in the *ALK* fusion rate between the sexes which is significantly different from the *EGFR* gene, where these mutations are generally believed to be more common in women. Studies from western countries have suggested that *ALK* fusion-positive cases are more common in acinar like tumors [10], whereas others have found that Japanese patients with mucus secretion and cribriform-like lung adenocarcinomas have higher *ALK*-positive rates [11]. However, studies in China show that the *ALK* mutation rates are significantly higher in infiltrating mucinous and solid infiltrating adenocarcinomas in Chinese patients. There were no fusion mutations in pre-invasion adenocarcinomas, suggesting that these fusions may be a late event in the development of lung cancer. Early detection is one of the most relevant limiting factors in the treatment of lung cancer. This work may contribute to the early detection of lung cancer.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Travis WD, Brambilla E, Nicholson AG, *et al*. The 2015 World Health Organization Classification of Lung Tumors: Impact of genetic, clinical and radiologic advances since the 2004 Classification. J Thorac

Oncol, 2015, 10: 1243–1260.
2. Kadota K, Colovos C, Suzuki K, *et al*. FDG-PET SUVmax combined with IASLC/ATS/ERS histologic classification improves the prognostic stratification of patients with stage I lung adenocarcinoma. Ann Surg Oncol, 2012, 19: 3598–3605.
3. Chiu CH, Yeh YC, Lin KH, *et al*. Histological subtypes of lung adenocarcinoma have differential ^{18}F-fluorodeoxyglucose uptakes on the positron emission tomography/computed tomography scan. J Thorac Oncol, 2011, 6: 1697–1703.
4. Kadota K, Nitadori JI, Sarkaria IS, *et al*. Thyroid transcription factor-1 expression is an independent predictor of recurrence and correlates with the IASLC/ATS/ERS histologic classification in patients with stage I lung adenocarcinoma. Cancer, 2013, 119: 931–938.
5. Warth A, Muley T, Meister M, *et al*. The novel histologic International Association for the study of Lung Cancer/American Thoracic Society/European Respiratory Society classification system of lung adenocarcinoma is a stage-independent predictor of survival. J Clin Oncol, 2012, 30: 1438–1446.
6. Nakashima H, Jiang SX, Sato Y, *et al*. Prevalent and up-regulated vimentin expression in micropapillary components of lung adenoeareinomas and its adverse prognostic significance. Pathol Int, 2015, 65: 183–192.
7. Nagano T, Ishii G, Nagai K, *et al*. Structural and biological properties of a papillary component generating a micropapillary component in lung adenocarcinoma. Lung Cancer, 2010, 67: 282–289.
8. Shi Y, Au JS, Thongprasert S, *et al*. A prospective, molecular epidemiology study of *EGFR* mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). J Thorac Oncol, 2014, 9: 154–162.
9. Wu YL, Fukuoka M, Mok TSK, *et al*. Tumor response and health-related quality of life in clinically selected patients from Asia with advanced non-small-cell lung cancer treated with first-line gefitinib: post hoc analyses from the IPASS study. Lung Cancer, 2013, 81: 280–287.
10. Inamura K, Takeuchi K, Togashi Y, *et al*. EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. Mod Pathol, 2009, 22: 508–515.
11. Sakairi Y, Nakajima T, Yasufuku K, *et al*. *EML4-ALK* fusion gene assessment using metastatic lymph node samples obtained by endobronchial ultrasound-guided transbronchial needle aspiration. Clin Cancer Res, 2010, 16: 4938–4945.

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