



# The clinicopathological study of lung cancer concealed in end-stage of interstitial lung disease

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**Background:** Most of the patients with interstitial lung disease (ILD) complicated with lung cancer (ILD-LC) showed non-specific clinical manifestations. This study is to explore the incidence of lung cancer concealed in the end-stage of interstitial lung disease (LC-CES-ILD).

**Methods:** A total of 154 cases of lung transplantation from March 2017 to December 2018 were studied retrospectively, of which 7 cases were found to be LC-CES-ILD. Serum tumor biomarkers were examined. HE and immunohistochemical staining were performed for the tumor tissue after the operation. Lung cancer (LC) drive gene was detected by quantitative real-time polymerase chain reaction (qRT-PCR).

**Results:** The percentage of male patients with idiopathic pulmonary fibrosis (IPF) was 44.81%. The expression of all tumor biomarkers was significantly increased in 6 patients with LC-CES-ILD. After operation, apparent destruction of lung tissue structure was observed in 7 patients, and honeycomb appearance could be seen in some areas. After HE staining, 4 cases of acinar type and 2 cases of mucinous adenocarcinoma were found. The results of molecular pathology showed that only one case of mucinous adenocarcinoma had KRAS mutation, and no mutation of LC co-driving gene was found in the rest of the cases.

**Conclusions:** It is necessary to detect the lung tissue of patients with end-stage ILD, which were probably correlated with the occurrence of LC concealed, before lung transplantation.

**Keywords:** Interstitial lung disease (ILD); lung cancer (LC); lung cancer concealed in end-stage of interstitial lung disease (LC-CES-ILD); lung transplantation

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## Introduction

Interstitial lung disease (ILD) is also known as diffuse parenchymal lung disease (DPLD). It is a kind of lung disease that involves alveolar wall and alveolar cavity and has different grade of inflammation and fibrosis. At present, the general respiratory disease community adopts the classification of ILD and idiopathic ILD recommended by

the American Thoracic Society (ATS) and the European Respiratory Disease Society (ERS) (1).

The pathological characteristics of patients with lung cancer (LC) are malignant proliferation of tumor cells, but the etiology of ILD is only a few known. However, no matter what the cause of ILD, lung fibroblasts will show abnormal or malignant proliferation, which is similar to the

pathological characteristics of LC patients.

When ILD develops to the end stage, lung transplantation becomes an effective method for the treatment of end-stage lung disease (2). While, the incidence of LC in ILD patients is increasing. Hitherto, the relationship between ILD and the pathogenesis of LC is not clear. Because most of the clinical manifestations of ILD complicated with LC (ILD-LC) are non-specific, when ILD-LC is diagnosed, it often progresses to the advanced stage of LC. Consequently, how to diagnose the disease in the early stage of ILD-LC has become a serious problem in clinical practice.

With the progress of medical technology, the strategy of diagnosis and treatment of LC has changed from cellular level to precise medical treatment based on molecular level (3). Drive genes are essential genes related to tumorigenesis and development, which play a crucial role in the induction and proliferation of LC. *Epidermal growth factor receptor (EGFR)* gene, *kirsten rat sarcoma viral oncogene (KRAS)* gene, *B-type Raf kinase (BRAF)* gene, *human epidermal growth factor receptor-2 (Her-2)* gene, and other genes are the most commonly used LC drive genes. It is beneficial to develop the best possible treatment option LC by detecting the driving genes in these ILD-LC.

After routine diagnostic procedures, the cases of LC do not occur in the lungs removed from lung transplants. However, 154 cases of lung transplantation were completed in Sino-Japanese Friendship Hospital within two years, of which 7 cases were not found or could not be diagnosed before operation. However, in the lung transplanted out of the lung, pathological examination found that it was finally diagnosed as terminal qualitative disease complicated with LC. This kind of case can be called lung cancer concealed in end-stage of interstitial lung disease (LC-CES-ILD). We retrospectively studied these rare cases and discussed the clinicopathological features.

## Methods

### Patients

We reviewed all cases of lung transplantation in China-Japan Friendship Hospital from March 2017 to December 2018. According to the criteria of the ATS (4), patients with severe lung diseases diagnosed by imaging or pathology and recommended for lung transplantation (2). A total of 154 patients were recruited. Inclusion criteria: (I) patients who met the criteria of ATS and ERS for lung transplantation;

(II) cardiac function of the New York Heart Association (NYHA) III or IV; (III) the experimental treatment of intravenous administration of vasocyclin or similar equivalent preparation is ineffective; (IV) cardiac index (CI)  $<2 \text{ L}/(\text{min}\cdot\text{m}^2)$ . Exclusion criteria: (I) the receptor has a history of malignant tumors in the past 2 years, but localized malignant tumors; (II) the receptor has other primary organ dysfunction that can't be treated (such as heart, liver, kidney, and brain); (III) the patient does not agree to a lung transplant, or mental or psychological problems are unable to cooperate with the medical team and do not comply with the doctor's diagnosis and treatment.

These cases, who are not diagnosed with a tumor before the operation, but LC was found in the transplanted lung, was screened from 154 lung transplant cases for inclusion in this study. The clinical data, preoperative imaging data, laboratory examination, and pathological data of these patients were collected.

### Preoperative pathological examination

The fasting venous blood of 3 mL was taken and the serum was separated for examination. Microparticle enzyme immunoassay (MEIA) was used. The experimental operation was carried out in strict accordance with the relevant guidelines and instructions, and the changes of serum tumor marker carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA 125), CA-153, neuron-specific enolase (NSE), cytokeratin 19-fragments (CYFRA21-1), CA72-4, CA199 in the samples were analyzed. Positive reference values: CEA ( $<5 \text{ ng/mL}$ ), CA125 ( $<35 \text{ U/mL}$ ), CA-153 ( $<25 \text{ U/mL}$ ), NSE ( $<16.3 \text{ ng/mL}$ ), CYFRA21-1 ( $<3.3 \text{ ng/mL}$ ), CA72-4 ( $<6.9 \text{ U/mL}$ ), CA199 ( $<27 \text{ U/mL}$ ).

### Treatment and observation of lung transplantation samples

The diseased lungs resected after lung transplantation were treated with routine pathological specimens. The specimens were perfused with 10% neutral formalin and fixed overnight. The pathologist scrutinized the specimen according to the standard of lung tissue sampling. Each lung lobe was taken separately, and the number of samples was determined according to the sample situation. The volume of material was at least three tissue blocks/lung lobes. Thus, the suspicious tumor tissue found in naked eye examination, and the number of tissue blocks was increased. Besides, the severed bronchial end, severed vascular end and lymph nodes in the specimen were routinely taken.

### ***Hematoxylin-eosin stain (HE) and immunohistochemical detection***

The tissue was embedded in paraffin and cut into sections of 4  $\mu\text{m}$  thickness. The sections were stained with HE and observed under the microscope. Immunohistochemistry was performed by EnVision method. The primary antibodies (TTF-1, p63, Ki67, and p53) were purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. The second antibody and chromogenic agent were purchased from Roche Diagnostic products (Shanghai) Co., Ltd. The immunohistochemical experiment was carried out according to the reagent instructions, and the corresponding negative and positive controls were established. All pathological sections were read independently by two senior pathologists.

### ***Molecular biological examination***

Ten paraffin specimens of tumor with a thickness of 5  $\mu\text{m}$  were selected for each case. Human LC polygene combined detection kit (Eddard Company) was used to extract DNA, and the fluorescence PCR method was used to detect the LC drive gene. The genes tested included *EGFR*, *reactive oxygen species ROS1*, *BRAF*, *anaplastic lymphoma kinase (ALK)*, *KRAS*, *receptor tyrosine kinase gene (RET)*, *neuroblastoma RAS viral oncogene homolog (NRAS)*, *Her-2*,  *$\alpha$ -isoform of phosphatidylinositol 3-kinase (PIK3Ca)*, *Methoprene-tolerant (Met)*. The detailed information of these genes was shown in *Table S1*.

### ***Statistical analysis***

SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The proportion of patients is represented by n%.

## **Results**

### ***Clinical information of 154 patients undergoing lung transplantation***

As shown in *Table 1*, the male patients accounted for 87% of the 154 lung transplant patients, and 78.57% of the patients were 50–69 years old. The primary type of disease was idiopathic pulmonary fibrosis (IPF), which accounted for 44.81% of the transplanted patients. There was no significant difference in the proportion of bilateral lung sequential transplantation, left single lung transplantation and right single lung transplantation among the three groups.

### ***LC-CES-ILD patient clinical information***

A total of 7 cases of LC were diagnosed pathologically after operation. These ILD patients were not diagnosed as LC before transplantation, but they were found to belong to LC-CES-ILD after transplantation. The clinical information is shown in *Table 2*.

Before transplantation, the clinical diagnosis suspected the five patients of having tumors, but it was not ensured before the operation. However, only No. 2 and No. 7 patients were confirmed to be LC by pathological examination after the surgery. All seven patients were male, with an average age of 57.57 years (46–64 years) and a history of severe smoking (15–90 package years). The primary diseases of lung transplantation were IPF (4 cases), connective tissue disease-associated interstitial lung disease (CTD-ILD) (2 cases), and chronic obstructive pulmonary disease (COPD) (1 case). Two of the patients underwent bilateral sequential lung transplantation, while the others underwent unilateral lung transplantation.

The interval between the last computed tomography (CT) examination and operation was 2–29 days. None of these patients was diagnosed as tumors by CT. Alternatively, positron emission tomography-CT (PET-CT) screening was performed in with No. 3 and No. 6 patients before operation, but the cancer could not be confirmed. Serum tumor biomarkers were screened in 7 patients before the surgery, and CEA, CA125, CA-153, NSE, CYFRA21-1, CA72-4, CA199 was significantly increased in 6 patients. However, the serum biomarkers of No. 2 patients were only slightly elevated in CA125, and the other serum tumor biomarkers were normal. Therefore, this patient was diagnosed as tumor-free before operation.

### ***Detection of tumor tissue in postoperative LC-CES-ILD patients***

The tumor tissue of 5 patients with LC-CES-ILD involved the upper lobe, while two patients involved two lobes of the lung. The lung tissue structure of 7 patients was destroyed, the texture was medium to tight, and honeycomb appearance could be seen in some areas. In some cases, the presence of mucus is prominent, and the boundary of the surrounding lung tissue is unclear (for example, No. 5 patients, *Figure 1A*).

Three cases (patient: No. 1, No. 3, No. 4) with tumor symptoms were found by accident during experiential sampling. Moreover, it is still impossible to accurately

**Table 1** The basic clinical information of 154 lung transplant patients

Item	Number of cases (n)	Proportion
Male/female	134/20	87%/13%
Age (years)		
20–29	4	2.60%
30–39	11	7.14%
40–49	11	7.14%
50–59	40	25.97%
60–69	81	52.60%
70–79	7	4.55%
The type of disease		
IPF	69	44.81%
CTD-ILD	19	12.34%
NSIP	12	7.79%
CHP	8	5.19%
CPFE	6	3.90%
Vasculitis	4	2.60%
Pneumoconiosis	4	2.60%
PPFE	3	1.95%
BOS	1	0.65%
Pulmonary hypertension.	5	3.25%
Bronchiectasis	9	5.84%
COPD	13	8.44%
Unclassified type	1	0.65%
Lung transplantation indication.		
Bilateral lung sequential transplantation	53	34.42%
Left one lung transplantation	44	28.57%
Right one lung transplantation	57	37.01%

IPF, idiopathic pulmonary fibrosis; CTD-ILD, connective tissue disease-associated interstitial lung disease; NSIP, non-specific interstitial pneumonia; CHP, chronic hypersensitivity pneumonia; CPFE, combined pulmonary fibrosis and emphysema; PPFE, pleuroparenchymal fibroelastosis; COPD, chronic obstructive pulmonary disease; BOS, obliterative bronchiolitis syndrome.

determine the location and size of the tumor with the naked eye at the time of re-examination of the specimen (for example, patient No. 4, *Figure 1B*). Four cases were single tumor lesions and three cases where two tumor lesions (*Table 3*).

HE staining showed that the alveolar structure of lung tissue in LC-CES-ILD patients almost disappeared. The interstitial fibrosis was high, and honeycomb lung and fibroblast hyperplasia lesions were formed in some areas of the

lung. Multifocal alveolar epithelial hyperplasia, squamous or mucinous epithelialization, and atypical hyperplasia occurred. Infiltration and aggregation of lymphocytes and thickening of pleura could be seen in some areas of the lung. Histologically, most of the patients were lung adenocarcinoma (6/7), 4 cases were acinar type, and 2 cases were mucinous adenocarcinoma (*Figure 2*). Patients with No. 6 have a small number of pleomorphic cancer components. Microscopically, the tumor

**Table 2** The preoperative clinical information of 7 patients with LC-CES-ILD

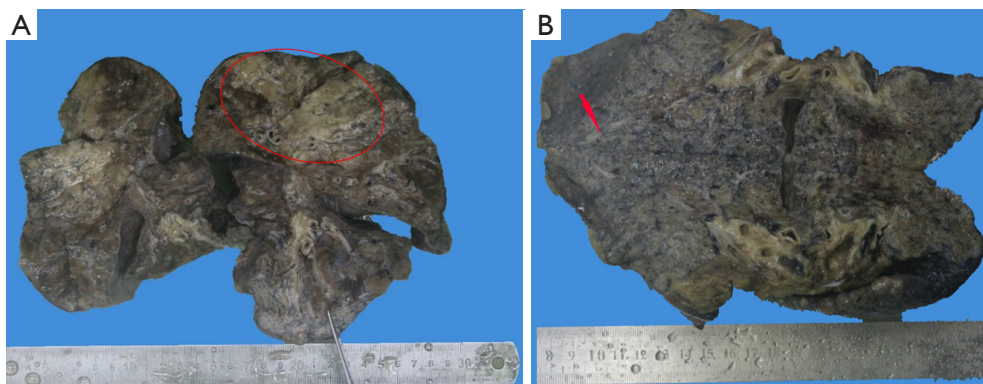
Item	Case number (No.)						
	1	2	3	4	5	6	7
Gender	Male	Male	Male	Male	Male	Male	Male
Age	59	46	63	64	52	61	58
Smoking index (bag/year)	20	90	70	20	30	40	15
Lung transplantation indication							
IPF	+		+		+		+
COPD		+					
CTD-ILD				+		+	
Serum tumor biomarkers detected before operation (nit of marker, range of normal value)							
CEA (ng/mL, <5)	21.49	1.56	5.09	19.59	–	6.07	8.6
CA125 (U/mL, <35)	127	48.6	82.45	948.2	79.19	74.88	127.2
CA-153 (U/mL, <25)	193.2	6.44	34.26	221.3	58.26	74.61	38.73
NSE (ng/mL, <16.3)	18.27	13.37	–	12.94	16.95	–	–
CYFRA21-1 (ng/mL, <3.3)	7.3	2.87	4.73	6.16	6.42	3.41	5.22
CA72-4 (U/mL, <6.9)	9.94	2.45	10.01	17.03	17.57	–	–
CA199 (U/mL, <27)	–	–	51.11	583.4	28.93	525.8	284.1
The results of the last CT examination before operation							
Interstitial pneumonia	+	+	+	+			+
Mediastinal lymphadenopathy	+						
Pulmonary infection					+		
Interstitial fibrosis				+	+	+	
The time between last CT examination and operation (days)	2	12	29	6	7	16	15
Preoperative diagnosis of tumor							
Suspicious	+		+	+	+	+	
No tumor		+					+
Transplantation type							
Bilateral lung sequential		+		+			
Right single lung	+				+		+
Left single lung			+			+	

IPF, idiopathic pulmonary fibrosis; CTD-ILD, connective tissue disease-associated interstitial lung disease; COPD, chronic obstructive pulmonary disease.

cells were fusiform, which has obvious heteromorphism and more mitosis. Only the tumor cells in No. 4 patients were keratinizing squamous cell carcinoma (*Figure 3*).

Therefore, we can find that the boundaries of tissue between LC and ILD is not clear, as the tumor center or

edge is often visible with the radical lesions of the base cells, squamous cell carcinoma surrounding the phenomenon of epidermal squamous, mucus adenocarcinoma surrounding visible dermalbiosis phenomenon, which suggests that the occurrence of tumor stoma has a certain relationship with



**Figure 1** Specimen of the lung lobe of No. 5 patient. (A) Right lobe, visible honeycomb lung changes (red circle); (B) left lobe, most of the lung tissue consolidation (red arrow).

the metastasis and proliferation of ILD.

Three of the patients were involved the visceral pleura, and 3 patients detected lymph node metastasis. Postoperative pathology stages show 1 case in stage Ia1, 3 cases in stage IIb, and 3 cases in stage IIIa. Immunohistochemistry showed that TTF-1 was positive (*Figure 4A*) and p63 was negative (*Figure 4B*) in adenocarcinoma, a few cells were TTF-1 positive and did not express p63 in mucinous adenocarcinoma, p63 was diffusely positive, and TTF-1 was negative in squamous cell carcinoma. The expression of Ki-67 (*Figure 4C*) was more than 10% in all tumor tissues and p53 positive (*Figure 4D*) in some tumor tissues (*Table 3*).

The results of the molecular pathological examination showed that only one case of mucinous adenocarcinoma had KRAS mutation, and no mutation of LC drive gene was detected in the rest of the patients (*Table S2*). Follow-up indicated that two patients died of infection after transplantation; the rest of the patients were followed up for 68–347 days, and three patients had tumor metastasis (*Table 4*).

## Discussion

LC and ILD also have many similarities in the pathogenesis, in addition, the two diseases have many similar risk factors, including smoking, advanced age, and so on (5). When ILD occurs, cytokines, chemokines, and growth factors promote the fibrosis process, and then lead to the destruction of the lung tissue structure, thus resulting in a series of reactions, and eventually develop into neoplastic lesions (6,7).

It is also different for the incidence of LC induced by different types of ILD. The incidences of LC were 1.43% in ILD-only groups (8), however, the incidence of LC was

4.4% to 48% in patients with IPF (9), while the prevalence of LC was as high as 8.9% in patients with pulmonary fibrosis complicated with emphysema (CPFE) (10). Therefore, it is confirmed that the incidence of ILD-LC may be underestimated because it is not detected from the LC-CES-ILD patients in our study, and the actual prevalence of ILD-LC may be higher than the data reported in the literature. Therefore, it is challenging to discover LC based on ILD, which is also a difficult point in the diagnosis and treatment of interstitial diseases.

It has been reported that the probability of accidental detection of LC in transplanted resected lungs is 0.8–2.2% (11), compared with a slightly higher proportion. Seven patients performed high-resolution CT screening before transplantation. The interval was only 2–29 days, which was significantly lower than the range in reference (12), but still failed to reduce the removal of ILD-LC from the lungs effectively. It is suggested that more effective methods are needed for the preoperative diagnosis of ILD-LC.

It is the key to confirming ILC-CES-LC by careful naked eye observation of the diseased lungs resected in end-stage ILD transplantation. Adenocarcinoma was the most common histological type of ILD-LC, accounting for 43% (11), followed by squamous cell carcinoma. Most of our cases were adenocarcinoma. The differentiation of mucinous adenocarcinoma was found in several cases, and a rare pleomorphic cancer component was found in one case (12). Hata *et al.* (13) summarized the surgical specimens and found that ILD-LC had a higher proportion of pleural invasion than LC alone. Three/seven cases in this study also confirmed the destruction of visceral pleura, for the prognostic significance of this phenomenon, more cases are needed to accumulate summary further.

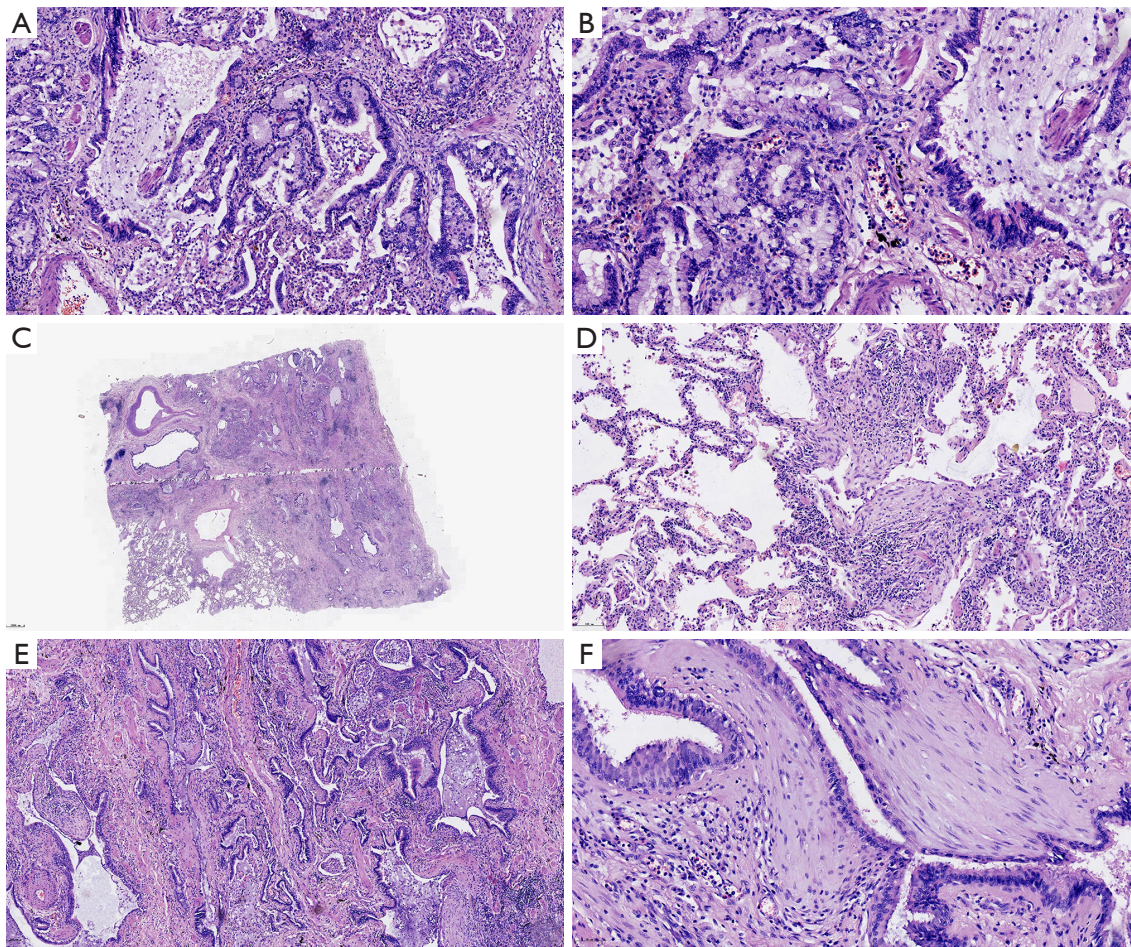
**Table 3** The detection of tumor tissues in 7 patients with LC-CES-ILD

Item	Case number (No.)						
	1	2	3	4	5	6	7
<b>Histological types</b>							
Invasive adenocarcinoma	+	+	+				+
Squamous cell carcinoma				+			
Invasive mucinous adenocarcinoma					+	+	
<b>Histological subtype</b>							
Acinar type	+ (80%)	+	+ (70%)		+	+ (60%)	+ (80%)
Solid type	+ (20%)						+ (20%)
Keratinized type				+			
Lepidic type						+ (15%)	
Papillary type			+ (25%)			+ (20%)	
Number of tumor lesions	1	2	2	1	1	1	2
Tumor size (cm)	1.5	0.8/0.15	1.2/0.3	0.7	7.5	10	0.8/1.5
Location of tumor in lung	Upper left	Upper left	Upper left	Lower left	Upper right and middle right	Upper left	Lower right
<b>Identification of tumor by naked eye</b>							
Unrecognizable	+	+	+	+			
Visible					+	+	+
<b>Pleural involvement</b>							
Affected	+		+		+		
Unaffected		+		+		+	+
Lymph node involvement	7/19	0/7	0/6	0/16	1/5	0/10	1/11
Stage of pTNM	T2aN1M0 IIb	T3N0M0 IIb	T3N0M0 IIb	T1aN0M0 Ia1	T4N1M0 IIla	T4N0M0 IIla	T3N1M0 IIla
<b>Immunohistochemical results</b>							
TTF-1	+	+	+	-	+ Partial	-	+
P63	-	-	-	+	-	-	-
Ki-67	+ (10%)	+ (50%)	+ (20%)	+ (15%)	+ (50%)	+ (20%)	+ (40%)
P53	+ Partial	+	-	+ Partial	-	+ Partial	+ Partial

The pathological diagnosis of LC from ILD, especially invasive tumors, is not difficult, but squamous epithelial metaplasia and hyperplasia of squamous epithelium or mucinous epithelium often occur at the same time of interstitial fibrosis, which is similar to the carcinomatous interstitial reaction of tumor. This needs to be differentiated from invasive tumors. The deletion of p63 positive basal cells can assist in the diagnosis

of invasive adenocarcinoma. The expression of p53 and Ki-67 was significantly increased in tumors (14,15), which has a particular significance in differential diagnosis.

Detecting the ILD-LC drive gene, we discovered the mutation of KRAS in only one case of mucinous adenocarcinoma, and the results were consistent with those reported in the literature (16,17). The mutation



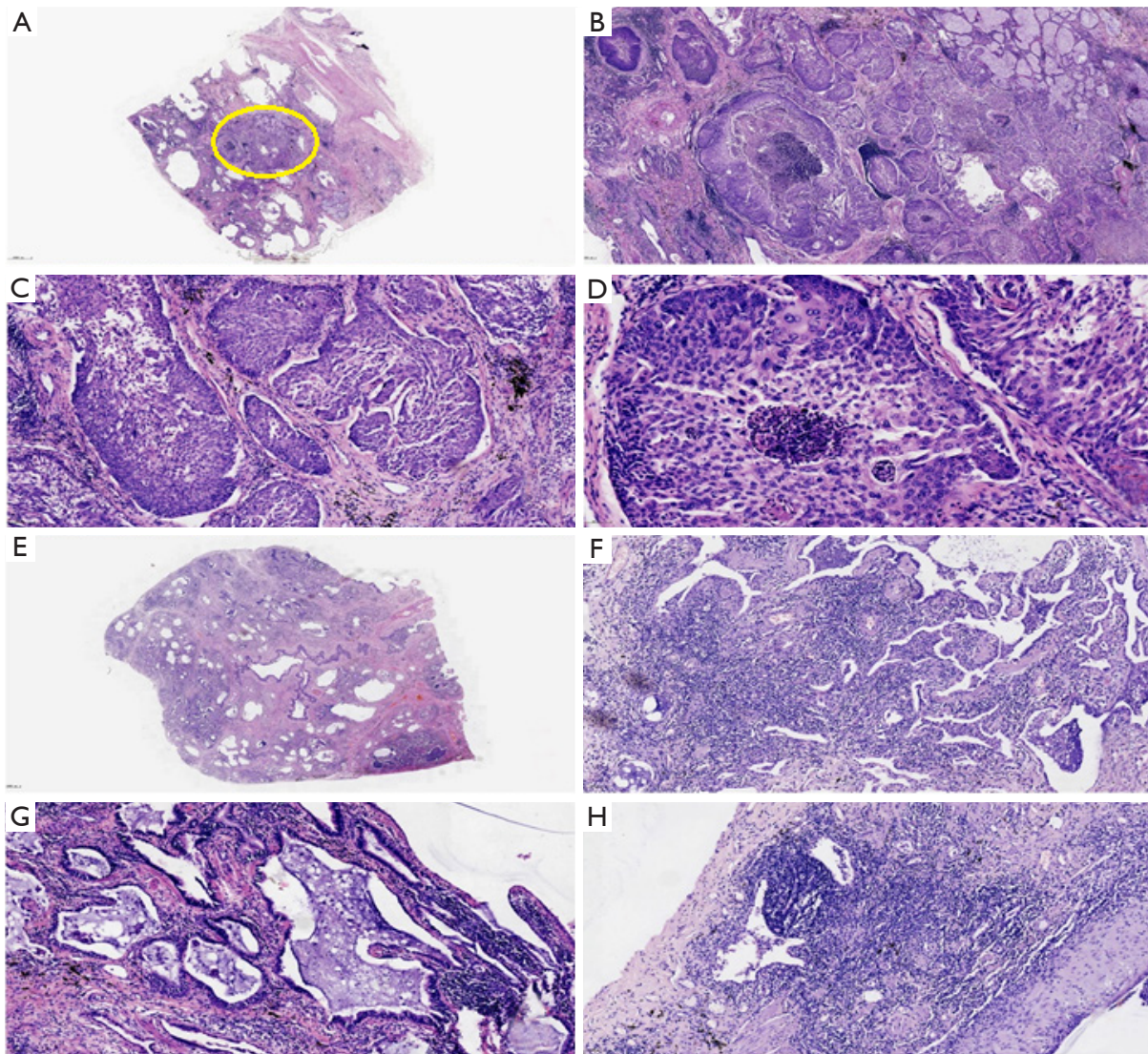
**Figure 2** HE of lung tissue in No.5 patient. (A) Abnormal mucous glands were stained with HE in the infiltrating growth area,  $\times 20$  times. Interstitial fibrous tissue hyperplasia, local lesions can be seen residual metaplastic mucous epithelium (shown by the blue arrow). (B) HE staining of abnormal mucous glands,  $\times 40$  times. The nucleus of the tumor was obviously irregular, the nucleus was located at the bottom of the base, the mucous vesicles could be seen in the cytoplasm, and mucus retention could be seen in the glandular cavity. (C) Ultra-low-power scanning slice image HE staining. The pathological changes in the surrounding lung tissue were diffusely distributed. Fibrous tissue proliferated, showing patchy distribution,  $\times 1$  time. (D) HE staining,  $\times 20$  times, in the areas with mild lesions of peripheral interstitial disease. A slightly basophilic focus of fresh fibroblast hyperplasia can be seen. (E) HE staining,  $\times 10$  times. The surrounding lung tissue showed fibrosis, the lung structure was obviously destroyed, forming clusters of different sizes of a cystic cavity, and showed honeycomb changes. The wall of the capsule is fibrous tissue, covered by bronchiole epithelium, and contains mucus and inflammatory cells in the cavity. (F) HE staining around the fibrous tissue,  $\times 40$  times. The proliferation of fibroblasts and myofibroblasts could be observed. The focus of fibroblastic hyperplasia composed of mucinous blue staining matrix is located around the dense and hyperplastic fibrous tissue.

rate of EGFR in ILD-LC was significantly lower than that in LC patients without ILD, but the mutation of KRAS was relatively common, indicating that ILD-LC may be different from LC without ILD in pathogenesis and molecular phenotype. At the same time, the effect of targeted drugs on ILD-LC is minimal, therefore, the difference between the two should be studied, and the effective treatment for ILD-LC should be studied further.

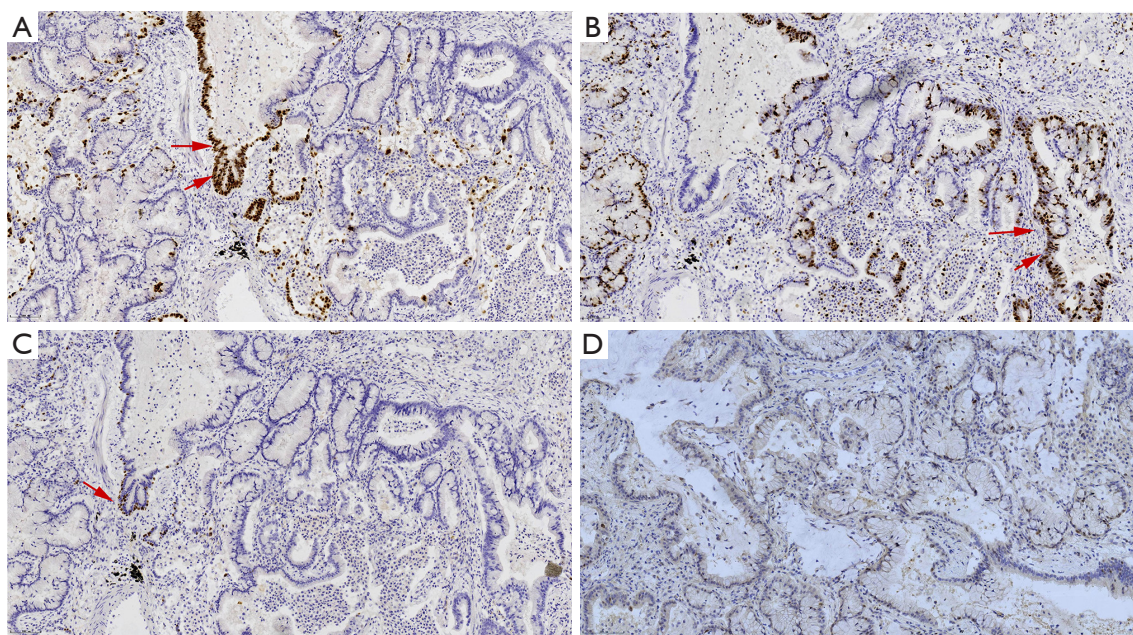
## Conclusions

The incidence of ILD-LC should not be ignored, and be more aware of the development of this disease and follow-up of ILD patients in clinical practice. Before lung transplantation in ILD end-stage patients, a variety of methods are needed to find the hidden LC. This study reveals that ILD combined LC differs from LC in clinical manifestations, pathological characteristics





**Figure 3** HE staining of lung tissue in No. 4 patients. (A) HE staining of the tumor site in the pulmonary parenchyma near the hilum of the lung, ultra-low power scanning section (yellow circle). The size is about 0.7 cm, and the boundary for the surrounding lung tissue is apparent. (B) HE staining map of tumor cell mass,  $\times 5$  times. The tumor showed infiltration growth, the size of the nests was different, and necrosis could be seen in the center of some nests. (C) HE staining map of tumor cell mass,  $\times 20$  times. It showed invasive growth, cancerous interstitial hyperplasia, carbon deposition in the stroma, accompanied by lymphocyte infiltration and aggregation. (D) HE staining map of tumor cell mass,  $\times 40$  times. The heteromorphism of tumor cells was obvious, keratosis could be seen in the nest, and tumor necrosis was found in the center of the nest. (E) HE staining of ultra-low power scanning sections. The structure of lung tissue was destroyed, interstitial fibrous tissue proliferated and honeycomb formed,  $\times 1$  time. (F) HE staining of fibrous tissue,  $\times 20$  times. There are a large number of lymphocytes infiltration and aggregation in the stroma of fibrous tissue hyperplasia. (G) HE staining of alveolar tissue,  $\times 20$  times. The honeycomb structure was formed, a large number of inflammatory cells infiltrated in the stroma, the cyst cavity was covered with ciliated columnar epithelium, and the mucus and inflammatory exudation and retention in the cavity. (H) Pleural HE staining,  $\times 20$  times. Pleural thickening, a large number of inflammatory cells infiltration and aggregation.



**Figure 4** The immunohistochemical staining of lung tissue in No. 5 patients. (A) Immunohistochemical staining of TTF-1, ×20 times. The bronchiole epithelium was TTF1 positive (shown by red arrow). Most of the other adenocarcinoma cells did not express TTF1, and only a few tumor cells were positive. (B) Immunohistochemical staining of Ki-67, ×20 times. The positive expression of Ki-67 was found in the nucleus of the tumor, and about 50% of the tumor cells were positive. In contrast to neoplastic glands, the residual metaplastic mucous epithelium was Ki-67 negative (shown by the red arrow). (C) Immunohistochemical staining of p63, ×20 times. The basal nuclei were positive and the neoplastic glands were negative. The highly abnormal glands grew infiltratively and the basal cells disappeared. Metaplastic mucous epithelium of residual basal cells can be seen in the local lesions (shown by the red arrow). (D) Immunohistochemical staining of P53, ×20 times. Most tumor cells do not express P53.

**Table 4** The follow-up of 7 patients with LC-CES-ILD

Item	Case number (No.)						
	1	2	3	4	5	6	7
Follow-up (days)	68	9	90	54	182	300	347
Follow-up results							
No recurrence of tumor	+		+				
Tumor metastasis					+	+	+
Death		+		+			
Causes of death		Infected		Infected			

and molecular phenotypes, and this finding has a guiding effect on future clinical practice.

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**Footnote**

*Conflicts of Interest:* All authors have completed the ICMJE

uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2019.11.36>). 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). This work was approved by the ethics committee at China-Japan Friendship Hospital (No. 2012-60). Informed consent was waived due to the retrospective nature of the study.

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**Supplementary**

**Table S1** Gene locus information in this study

Detection gene	Exon/codon	Mutation type
<i>EGFR</i> gene	Exon-19	19-del
	Exon-21	L858R
	Exon-20	T790M
	Exon-18	G719X
	Exon-20	S768I
	Exon-21	L861Q
<i>KRAS</i> gene	Exon-2	G12D/S, G12A/V/R/C, G13C
<i>BRAF</i> gene	Exon-15	V600E/K/R/D
<i>NRAS</i> gene	Exon-2	G12C/A/V, G13R/C
	Exon-3	Q51R/K/L/H
<i>HER2</i> gene	Exon-20	20-ins/ G776>VC (1)
<i>PIK3CA</i> gene	Exon-20	H1047R
	Exon-9	E545K
<i>ALK</i> Fusion gene	ALK-Exon-20	E13; A20, E6ins33; A20, E20; A20, E18; A20, E2; A20, E17; ins68A20, E2; ins117A20, E13; ins69A20, E6; A20, E6; A19, E6; ins18A20, E20; ins18A20, E17del58; ins39A20, E17ins65; A20, E17; ins30A20, E17ins61; ins34A20, E3; ins53A20, KI24; A20, KI17; A20, KL9; A20, T4; A20
<i>ROS1</i> Fusion gene	ROS1-Exon-32/34/35	SLC34A2 Exon-4/14
		CD74 Exon-6, SDC4 Exon-2/4
		SLC34A2 Exon-4/14, EZR Exon-10
		CD74 Exon-6, SDC4 Exon-4
		TPM3 Exon-8
		LRIG3 Exon-16, GOPC Exon-8
<i>RET</i> Fusion gene	RET-Exon-12	CCDC6 exon 1; RET exon 12
		NCOA4 exon6; RET exon 12
		KIF5B exon 15; RET exon 12
		KIF5B exon 16; RET exon 12
		KIF5B exon 23; RET exon 12
		KIF5B exon 22; RET exon 12

**Table S2** The results of molecular pathological examination

Item	Case number						
	1	2	3	4	5	6	7
<i>NRAS</i>	-	-	-	-	-	-	-
<i>EGFR</i>	-	-	-	-	-	-	-
<i>ALK</i>	-	-	-	-	-	-	-
<i>ROS1</i>	-	-	-	-	-	-	-
<i>Braf</i>	-	-	-	-	-	-	-
<i>Her-2</i>	-	-	-	-	-	-	-
<i>PI3KCA</i>	-	-	-	-	-	-	-
<i>RET</i>	-	-	-	-	-	-	-
<i>KRAS</i>	-	-	-	-	-	+	-

- means that the results of examination are negative and + means that the results of examination are positive. The test results in *KRAS* in No. 6 patients were exon2 G12A 35G>C, exon2 G12V 35G>T, exon2 G12R 34G>C, exon2 G12C 34G>T, exon2 G13C 37G>T.