

Original Article

High expression of E-cadherin in pleural effusion cells predicts better prognosis in lung adenocarcinoma patients

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Abstract: Background: Epithelial-mesenchymal transition (EMT) is of great importance in tumor metastasis. Our previous study demonstrates that epithelial phenotype is related to epidermal growth factor receptor (EGFR) mutation and the sensitivity of EGFR tyrosine kinase inhibitors (TKIs) in advanced non-small cell lung cancer (NSCLC) patients. However, the role of EMT phenotype in malignant pleural effusions in predicting prognosis is unknown in lung adenocarcinoma patients. Method: Pleural effusions of lung adenocarcinoma patients were collected and made into cell block (CB). EGFR mutation was detected using amplification refractory mutation system (ARMS) PCR method and H-score system was applied to evaluate the staining intensity of EMT marker and tumor cell ratio. Results: Forty-three CB samples, including 22 samples before any treatment (baseline, group 1) and 21 with disease progression (group 2) after first-line chemotherapy, were enrolled in this study. The expression of N-cadherin and vimentin were low in the CB tumor cells. There was no significant difference in the tumor cell ratio and E-cadherin expression in the two groups. E-cadherin expression had no association with sex, age and smoking status and also patient response in both the two groups. However, high E-cadherin expression was related to EGFR mutation ($P = 0.032$) and long progression-free survival (PFS) ($P = 0.015$) in group 1 but not group 2 samples. Conclusion: E-cadherin expression in CB samples was associated with EGFR mutation status and patient prognosis in lung adenocarcinoma patients in first-line chemotherapy.

Keywords: EMT, EGFR, non-small cell lung cancer, cell block

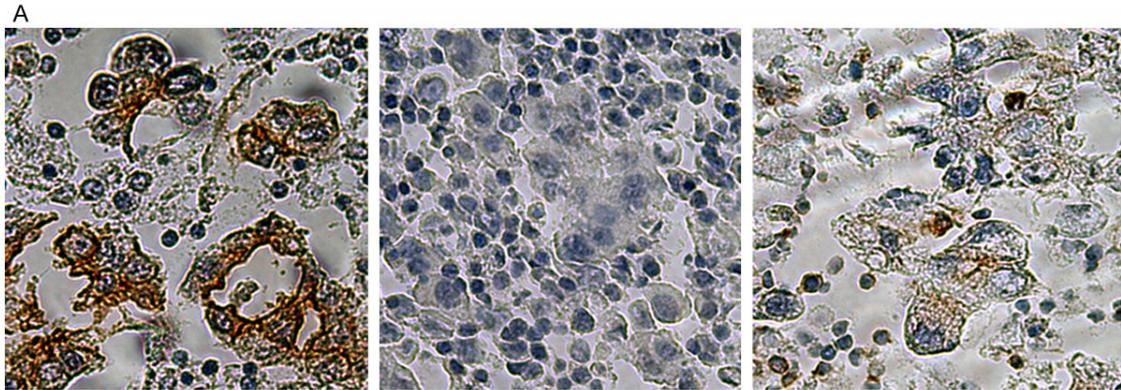
Introduction

Epithelial-mesenchymal transition (EMT) is a process that cells lose their epithelial features, such as E-cadherin, gain mesenchymal properties, such as N-cadherin and vimentin, and become motile and invasive [1-3]. Many studies demonstrated that EMT is associated with the mutant status of epidermal growth factor receptor (EGFR) and the efficacy of EGFR tyrosine kinase inhibitors (TKIs) in NSCLC patients and cell lines [4-7]. The association between EMT and chemotherapy has also been studied widely. The high expression of E-cadherin predicts better outcomes for patients; the reduction of E-cadherin predicts worse overall sur-

vival (OS) and disease-free survival/progression-free survival (DFS/PFS) [8, 9]. When tumors were resistant to chemotherapy drugs, the expression of epithelial phenotype markers decreased; when their expression increased, drug resistance was reversed [10-13].

However, the expression and prognostic role of EMT markers in pleural effusion samples has not been fully studied. In the previous study [14], we showed that pleural effusion cell block (CB) samples were qualified to detect ALK, ROS1 and RET fusion genes. Here, we determined to use CB samples to detect the expression of EMT marker (E-cadherin, N-cadherin and vimentin) using immunohistochemistry to

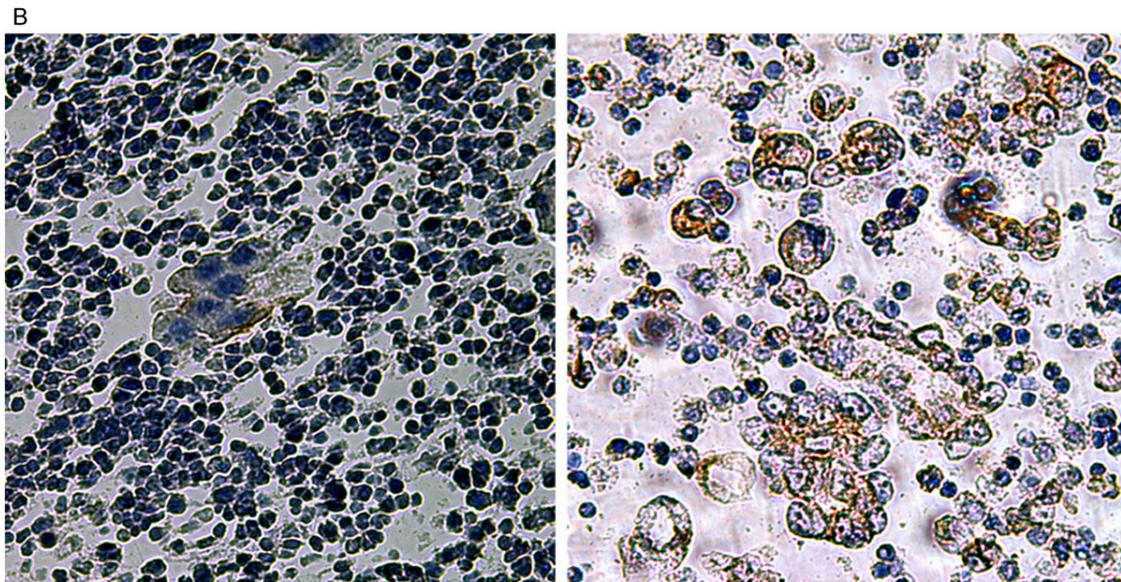
E-cadherin expression in lung adenocarcinoma pleural effusion



E-cadherin

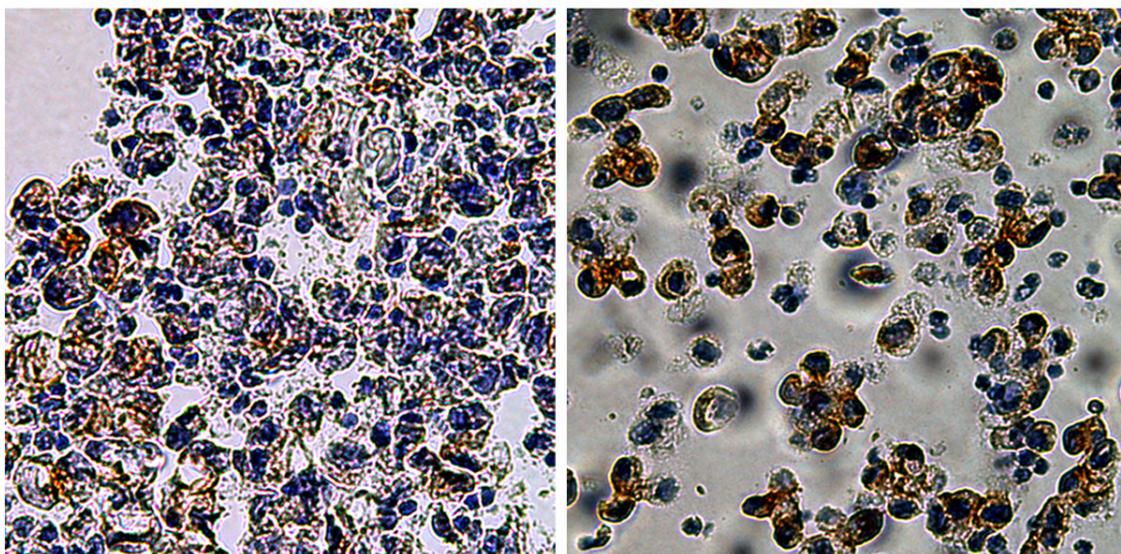
N-cadherin

Vimentin



H-score: 5

H-score: 75



H-score: 150

H-score: 210

E-cadherin expression in lung adenocarcinoma pleural effusion

Figure 1. EMT marker expression in CB samples (400 ×). A. Three markers were stained by immunohistochemistry. B. H-score system was used to assess the staining intensity and tumor cell ratio of E-cadherin. Four different levels of staining were shown in the figure: H-score 5 indicated E-cadherin negative expression in this study; H-score 75, 150 and 210 indicated positive.

Table 1. Clinicopathologic characteristics of lung adenocarcinoma patients

		Group 1 patients (n = 22)			Group 2 patients (n = 21)		
		E-cadherin negative (%) n = 7	E-cadherin positive (%) n = 15	p value	E-cadherin negative (%) n = 7	E-cadherin positive (%) n = 14	P value
Sex	Male	5 (22.7)	7 (31.8)	0.268	4 (19.0)	5 (23.8)	0.319
	Female	2 (9.1)	8 (36.4)		3 (14.3)	9 (42.9)	
Age	< 65	5 (22.7)	11 (50.0)	0.384	6 (28.6)	7 (33.3)	0.118
	≥ 65	2 (9.1)	4 (18.2)		1 (4.8)	7 (33.3)	
Smoking status	Never/light smoker	4 (18.2)	11 (50.0)	0.387	2 (9.5)	10 (47.6)	0.080
	Smoker	3 (13.6)	4 (18.2)		5 (23.8)	4 (19.0)	
EGFR	Wild type	6 (27.3)	5 (22.7)	0.032	5 (23.8)	7 (33.3)	0.245
	Mutation	1 (4.5)	10 (45.5)		2 (9.5)	7 (33.3)	

Table 2. Response to first-line chemotherapy in lung adenocarcinoma patients

		Group 1 patients (n = 22)			Group 2 patients (n = 21)		
		E-cadherin negative (%) n = 7	E-cadherin positive (%) n = 15	p value	E-cadherin negative (%) n = 7	E-cadherin positive (%) n = 14	P value
ORR	PR	0 (0.0)	1 (4.5)	0.682	1 (4.8)	2 (9.5)	0.479
	SD + PD	7 (31.8)	14 (63.6)		6 (28.6)	12 (57.1)	
DCR	PR + SD	3 (13.6)	12 (54.5)	0.107	3 (14.3)	9 (42.9)	0.238
	PD	4 (18.2)	3 (13.6)		4 (19.0)	5 (23.8)	

investigate the association between their expression and clinical characteristics as well as outcome.

Materials and methods

Patients

The pleural effusions of 43 patients were collected in Shanghai Pulmonary Hospital from Oct. 1, 2012 to Oct. 1, 2013. All samples were confirmed to contain lung adenocarcinoma cells by pathologists. Patients were aged ≥ 18 years-old, and performance status score was 0-1. All of them had signed an informed consent. They received single drug or platinum-contained double drugs treatment for the first-line therapy and tumor response was assessed using response evaluation criteria in solid tumors (RECIST Version 1.1). This study was approved by the Ethics Committee of Shanghai pulmonary Hospital.

Immunohistochemistry staining of E-cadherin

Five-μm thick sections were used for E-cadherin, N-cadherin and vimentin immunohistochemistry staining. The staining procedure and intensity calculating were as we reported before [4]. Of the 43 CB samples, 22 were collected before treatment (base line, group 1) and 21 were collected at disease progression (group 2). We used a cut-off H-score value 15 because under this score the tumor cell ratio was limited (namely below 5% or around 10% but had low staining intensity). Besides, our data showed that it can divide the patients into two significantly different groups in studying the association of EGFR mutation status and patient outcomes. The staining was classified into four levels: -(negative, H-score below 15), 1+ (weak positive, H-score between 16 and 100), 2+ (positive, H-score between 101 and 200) and 3+ (strong positive, H-score over 200). We

E-cadherin expression in lung adenocarcinoma pleural effusion

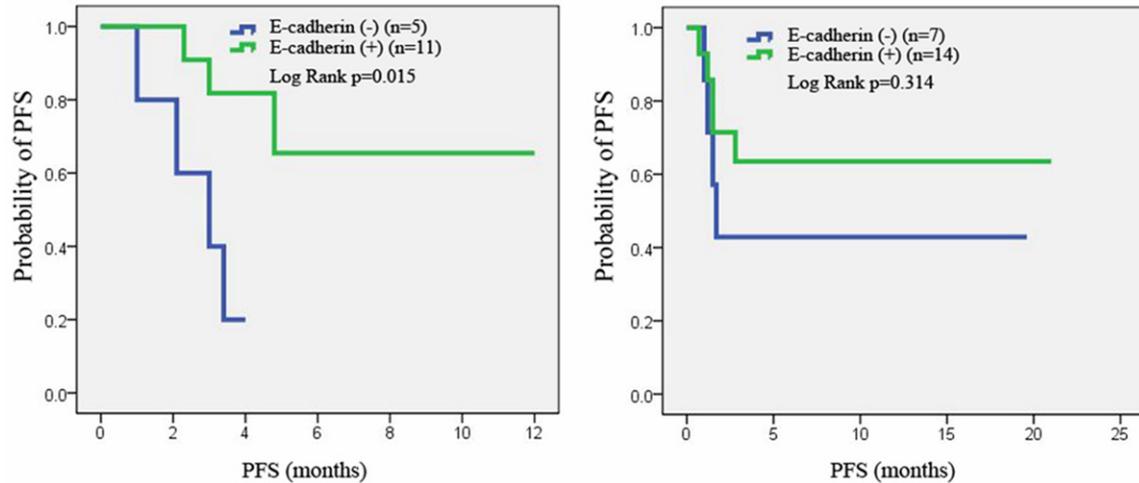


Figure 2. PFS of the baseline patients (group 1) and disease progression patients (group 2). (Left) PFS of the group 1 patients: the E-cadherin positive patients had longer PFS than the negative patients. (Right) PFS of the group 2 patients: no significant difference was found between the E-cadherin positive and negative patients.

Table 3. Cox regression analysis of 22 base-line patients

	<i>P</i>	RR	RR 95% CI
EGFR mutation	.506	.654	(.186, 2.292)
Sex	.480	1.763	(.365, 8.504)
Age	.939	.941	(.199, 4.442)
Smoking status	.445	.528	(.103, 2.722)
E-cadherin staining	.099	.291	(.067, 1.261)

define E-cadherin (-) when H-score is below 15; E-cadherin (+) when H-score is higher than 15.

EGFR mutation detection

EGFR was detected using amplification refractory mutation system (ARMS) PCR method. The procedure was conducted according to the manufacturer's instruction. Briefly, a total of 80 ng DNA was used in 8 PCR reactions, and then (1) initial denaturation at 95°C for 5 min, (2) 15 cycles of 95°C 25 s, 64°C 20 s, and 72°C 20 s, (3) 31 cycles of 93°C 25 s, 60°C 35 s, and 72°C 20 s before analyzing the results.

Statistical analysis

The association between H-score of E-cadherin and the clinical characteristics were analyzed by Chi-square test or Fisher exact test. Patient survival was analyzed using Kaplan-Meier method and compared between different groups using the log-rank test or Cox regres-

sion analysis. All statistical tests were two-sided, and significant difference was defined as $P < 0.05$ (SPSS Inc., Chicago, Ill).

Results

EMT markers staining in all the CB samples

E-cadherin, N-cadherin and vimentin were stained respectively, but the expression of N-cadherin and vimentin were in a very low level in CB tumor cells (**Figure 1**). So in the following research, we only studied the association between E-cadherin expression and clinical characteristics as well as patient outcomes. H-score values of different E-cadherin staining were shown in **Figure 1**. We compared the tumor cell ratio and E-cadherin expression in two groups, but they both had no significant difference. There was also no significant difference between clinical characteristics and E-cadherin expression (**Table 1**).

Association between EGFR mutation and E-cadherin expression

In this study, all the patients performed EGFR mutation detection. In the base line samples (group 1), high E-cadherin expression was related to EGFR mutation ($P = 0.032$), but this result was not found in group 2 ($P = 0.245$) (**Table 1**). It suggested that lung adenocarcinoma epithelial phenotype was more likely to be driven by EGFR mutation.

E-cadherin expression in lung adenocarcinoma pleural effusion

E-cadherin expression and survival in advanced lung adenocarcinoma patients

We examined the objective response rate (ORR) and disease control rate (DCR) between E-cadherin (-) and E-cadherin (+) expression in the two groups, but found no significant difference (**Table 2**). In group 1, sixteen patients met disease progression. In these patients, the positive expression of E-cadherin had longer PFS than the negative expression ($P = 0.015$, 95% CI, 1.068-4.932), with a median PFS 4.5 months versus 3.0 months. However, the same result was not found in the group 2 patients (**Figure 2**). Cox regression analysis of the group 1 patients did not support E-cadherin expression as an independent prognostic factor ($P = 0.099$) (**Table 3**).

Discussion

The relationship between EMT phenotype and chemotherapy effect in advanced-stage lung adenocarcinoma patients with pleural effusions was studied by the authors. We found that tumor cells in the pleural effusions mainly expressed epithelial phenotype marker E-cadherin. The high expression of E-cadherin was related with EGFR mutation, which is consistent with the previous report [4]. There was no significant difference in E-cadherin expression between the baseline and disease progression groups, but higher E-cadherin expression in baseline was associated with longer PFS when treated with chemotherapy.

It was reported that pleural effusion CB samples [15, 16] and cell cultures derived from malignant pleural effusions [17] could be used to detect E-cadherin, N-cadherin and vimentin expression. Here, our results showed that E-cadherin expression had variability in different samples but N-cadherin and vimentin expression were in a low level in all the samples. This difference may be generated from the fact that the epithelial markers expression always has negative correlation with that of mesenchymal markers. The low expression of N-cadherin and vimentin could be related to high expression of E-cadherin in this research. So we only evaluated E-cadherin expression in the following data analysis. Meta analysis studies showed that in lung cancer patients, E-cadherin down-regulation indicated worse OS as well as DFS/PFS [8, 18]. In chemotherapy

drug-resistant patients and cell lines, E-cadherin expression decreased [10-13]; if the expression was increased, it could enhance the sensitivity of the cell line to the drug [10]. Our result is consistent with the above in that E-cadherin high expression predicts better outcome in chemotherapy. It was reported that the expression of E-cadherin could reduce the active form of Rho family protein, which might explain the negative association of E-cadherin expression and cell proliferation/migration in NSCLC [19]. In this study, we did not observe the change of E-cadherin expression between group 1 and group 2 patients. One of the reasons could be that sample type is different from the above studies. The pleural effusion lung adenocarcinoma cells are epithelial originated, no matter what stage the patients are in. But we do not exclude the possibility that the expression of E-cadherin between the two groups is different when sample size is enlarged and the samples are in pairs.

There are limitations that we should consider in this study. The first is that the sample size is small. We could obtain more information if we increase the sample amount. The second is that the samples are not in pairs. If samples of different time points between base line and disease progression were collected, the expression change of EMT markers during therapy and their function in predicting prognosis would be deciphered more clearly.

In conclusion, CB samples can be used to detect the expression of epithelial marker E-cadherin; high expression of E-cadherin is associated with EGFR mutation as well as longer PFS.

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Disclosure of conflict of interest

None.

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