

Original Article

Molecular and clinicalpathological features of lung adenocarcinoma with micropapillary pattern

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Abstract: Objective: To evaluate the clinicalpathological features of lung adenocarcinoma with micropapillary pattern and its relationship with EGFR mutation, EML4-ALK and ROS1 fusion. Method: We analyzed the clinicalpathological characteristics of 87 lung adenocarcinoma cases with micropapillary pattern (MP group) and 123 lung adenocarcinoma cases without micropapillary pattern (non-MP group). EGFR mutation and ROS1 fusion were detected by ARMS PCR, EML4-ALK fusion was analyzed using immunohistochemistry. Results: Within MP group, EGFR mutation showed no relevance with smoking behavior. When compared to non-MP group, MP group had significantly higher percentage of female patient, bigger tumor size, higher incidence of lympho node metastasis and pulmonary membrane invasion. The EGFR mutation rate of MP group was higher than the non-MP group (72.4% vs 53.7%) but it was not statistically significant ($P > 0.05$). In addition, EML4-ALK fusion or ROS1 fusion shown no difference in mutation rate between MP and no-MP group. Conclusions: Lung adenocarcinoma with micropapillary pattern showed distinct molecular biological behavior.

Keywords: Micropapillary, lung neoplasms, adenocarcinoma, receptor,epidermal growth factor

Introduction

Lung carcinoma is one of the most prevalent malignancies in the world and is with high mortality rate in both men and women. Micropapillary (MP) pattern as one particular type of tumor, is reported to be with high invasive ability, early lymph node and organ metastasis, and also poor prognosis [1, 2]. In 2011, the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS) and European Respiratory Society (ERS) introduced a new adenocarcinoma classification. Based on this new classification, lung adenocarcinoma with a micropapillary pattern is classified as new independent subtype [3]. In this paper, we studied the clinicalpathological features, EGFR mutation, EML4-ALK fusion and ROS1 fusion of lung adenocarcinoma with micropapillary pattern.

Materials and methods

Surgical specimens and patient characteristics

210 primary lung adenocarcinoma cases were collected from the Department of Pathology, the First Affiliated Hospital, College of Medicine,

Zhejiang University. All specimens were fixed in 10% formalin, embedded in paraffin and cut in 4 μm thickness and went through the usual hematoxylin and eosin staining. The clinical and pathological data was reviewed from impatient medical records. The H&E slides were review by two experienced pathologists. According to the 2011 classification, micropapillary composition $\geq 5\%$ was considered as lung adenocarcinoma with MP pattern. The 210 samples were divided into two groups, 87 lung adenocarcinoma with micropapillary pattern (MP group) and 123 lung adenocarcinoma without micropapillary pattern (non-MP group). In MP group, there were 24 males and 63 females, age ranged from 27-80 (median of 59.4). In non-MP group, there were 57 males and 66 females, age ranged from 34-81 (median of 60.5).

Molecular detection

The formalin fixed and paraffin embedded tissue blocks were cut in 3 or 5 μm thickness. 10 slices of 5 μm were collected for DNA and RNA isolation. All extraction were carried out using DNA/RNA extraction kit (AmoyDx, Xiamen). EGFR mutation and ROS1 fusion were tested

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Table 1. Clinicopathological and molecular features of MP and non-MP group

	non-MP group	MP group	p value
Male	57	24	0.006
Female	66	63	
< 60 years old	51	42	0.328
≥ 60 years old	72	45	
Smoking	39	18	0.077
Non-smoking	84	69	
Peripheral type	109	75	0.601
Central type	14	12	
< 3 cm	89	51	0.038
≥ 3 cm	34	36	
No LN metastasis	95	35	0.000
With LN metastasis	28	52	
No pulmonary membrane invasion	88	48	0.014
With pulmonary membrane invasion	35	39	
EGFR wildtype	47	24	0.109
EGFR mutation	76	63	
No EML4-ALK fusion	118	82	0.744
With EML4-ALK fusion	5	5	
No ROS1 fusion	122	86	1.000
With ROS1 fusion	1	1	

Table 2. Comparison of EGFR mutation types between MP group and non-MP group

	MP group	Non-MP group	P value
19DEL	24	26	0.280
L858R	32	45	0.977
Other types	7	6	0.348
Total	87	123	

using ARMS Detection kit (AmoyDx, Xiamen). 1 slice of 3 μm was tested for EML4-ALK fusion by immunohistochemistry (Roth, Germany, 1:500).

Statistical analysis

Statistical analysis was performed using the SPSS statistics software (Version 17.0, Chicago). The correlation of MP and non-MP group with clinicopathological and molecular features was studied via Chi-square test. A P value < 0.05 was considered as to be statistically significant.

Results

Clinicopathologic characteristics

As summarized in **Table 1**. MP group had significantly higher female patients than non-MP

group (P < 0.05). Additionally, the number of tumor with diameter ≥ 3 cm, lymph node metastasis and pulmonary membrane invasion was all significantly bigger than non-MP group (P < 0.05).

Molecular characteristics

In the analyzed 210 lung adenocarcinoma samples, we found 139 cases with EGFR mutation (66.2%). Within the 139 cases, there were 77 samples with L858R, 50 samples with 19DEL, 3 samples with 20INS, two samples with S768I, two samples with L861Q and one sample with G719X. In addition, there were also four samples with double mutation, 19DEL and T790M, G719X and L861Q, G719X and S768I, L858R and S768I respectively. As showed in **Table 1**, MP group had 63 samples with EGFR

mutation (72.4%), 5 with EML4-ALK fusion (5.7%) and 1 with ROS1 fusion (1.1%). Meanwhile the non-MP group had 66 cases with EGFR mutation (53.7%), 5 with EML4-ALK fusion (4.1%) and 1 with ROS1 fusion (0.8%). Statistical analysis indicated that there was no significant difference in EGFR mutation between the MP and non-MP group (P > 0.05). Comparison of EGFR mutation types between MP and no-MP group was summarized in **Table 2**. There was also no significant difference between two groups in EGFR mutation types (P > 0.05). In non-MP group, non-smoking patients showed significantly higher rate of EGFR mutation as compared to the smoking individuals (P < 0.05). In MP group, EGFR mutation had no relevance with gender, age, smoking behavior, tumor position, tumor size, lymph node metastasis or pulmonary membrane invasion (**Table 3**).

Discussion

A micropapillary pattern is defined as a pattern exhibiting micropapillary morphology without a fibrovascular core. Since its first discovery and description in breast cancer [4], this structure had also been addressed in other tumor types.

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Table 3. Relationship of EGFR mutation in MP and non-MP group with clinicalpathological features

	MP group		P value	Non-MP group		P value
	EGFR wildtype	EGFR mutation		EGFR wildtype	EGFR mutation	
Male	26	31		5	19	
Female	21	45	0.116	19	44	0.435
< 60 years old	18	33		15	27	
≥ 60 years old	29	43	0.575	9	36	0.101
Smoking	26	58		3	15	
Non-smoking	21	18	0.015	21	48	0.376
Peripheral type	39	70		11	54	
Central type	8	6	0.121	3	9	1.000
< 3 cm	34	55		14	37	
≥ 3 cm	13	21	0.997	10	26	0.973
No LN metastasis	36	59		10	25	
With LN metastasis	11	17	0.894	14	38	0.866
No pulmonary membrane invasion	34	54		16	32	
With pulmonary membrane invasion	13	22	0.878	8	31	0.183

In addition to its unique structure, tumors with micropapillary structure is associated with general lymph node metastasis, late clinical stage and poor outcomes. Therefore micropapillary is considered as a marker for poorly-differentiation and highly invasive tumor [5, 6].

In the analysis of 87 lung adenocarcinoma samples with micropapillary (MP group) and 123 without micropapillary structure (non-MP group), we found that MP group had higher percentage of female patients, bigger tumor size, higher number of cases with lymph node metastasis and pulmonary membrane invasion. Our findings were in agreement with previous researches which again indicated that lung adenocarcinoma with micropapillary structure was with high aggressive ability [7, 8].

Motoi et al [9] had studied 100 lung adenocarcinoma samples and reported that micropapillary structure was highly correlated with EGFR mutation. Other papers had also mentioned that adenocarcinoma was with higher EGFR mutation rate [10, 11]. In this present study, we found that the mutation rate of EGFR in MP group was 72.4%, which was higher than the 53.7% of non-MP group. However the difference was not significant, we speculated that micropapillary morphology in most of the samples were always mixed with other adenocarcinoma types and only occupied small proportion, therefore these small part of EGFR muta-

tions were not detected. To test this speculation, we re-defined the MP group which micropapillary composition $\geq 20\%$ was considered as lung adenocarcinoma with MP pattern. In the new analysis, the EGFR mutation rate of MP group was 69.0% (20/29). This result was still not significantly different from non-MP group ($P > 0.05$, data not shown), and the result was consistent with our previous study [12]. According to this outcome, our speculation was wrong. We had also hypothesized that micropapillary pattern and other types of lung adenocarcinoma had no difference in EGFR mutation. The reason why EGFR mutation rate in MP group was higher was simply due to higher number of female and non-smoking patient. We roughly conclude that micropapillary pattern was independent factor for EGFR mutation in lung adenocarcinoma, however we did not find papers reporting this conclusion. It's a pity for that our MP and non-MP were separately selected therefore we were not able to carry out the multiple factor variance analysis. We had also compared the types of EGFR mutation between MP and non-MP groups but it was not significantly different. So, this hypothesis is also uncertain. Of course, it's maybe that our analyzed sample number might still too small to reflected the fact of EGFR mutation.

Interestingly, in non-MP group, EGFR mutation was commonly found in non-smoking female patients. Whereas in lung adenocarcinoma

with micropapillary pattern, EGFR mutation had no correlation with gender or smoking behavior, this finding suggested that micropapillary still have its specific molecular mechanism.

EML4-ALK fusion and ROS1 fusion were normally observed in adenocarcinoma with ponsphaera or signet-ring features [13, 14]. Up to date, there had been no papers mentioned about the relationship between micropapillary pattern and EML4-ALK fusion or ROS1 fusion. In this study we found 5 sample of EML4-ALK fusion and 1 sample of ROS1 fusion in both MP and non-MP groups, and there was no significant difference. For a more comprehensive conclusion, we should enlarge our analyzed sample numbers.

Overall, micropapillary pattern in lung adenocarcinoma suggested high invasive ability and unique molecular features and this morphological characteristic could be a powerful marker for disease prognosis. It was recommended for pathologists to describe the micropapillary pattern in the pathologic reports which this description could be useful for clinical follow-up and treatment.

Disclosure of conflict of interest

None.

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