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Original Article

Histology and oncogenic driver alterations of lung adenocarcinoma in Chinese

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Abstract: Little is known about association of mucin abundancy with oncogenic-driver alterations, immunohistochemical and clinicopathologic features in lung adenocarcinomas among Chinese. We here retrospectively examined the clinicopathologic and molecular characteristics of pulmonary mucin-producing adenocarcinoma (PMPA) and previously-reported non-mucinous lung adenocarcinomas collected at our institution. Among the 897 non-mucinous adenocarcinomas, 61 PMPA with ≤90% mucin and 39 PMPA with >90% mucin, ALK rearrangements were found in 47 (5.2%) non-mucinous adenocarcinomas, 9 (14.8%) PMPA with ≤90% mucin and 12 (30.8%) PMPA with >90% mucin, respectively, with an ordinal association (coefficient, 95% CI=0.11, 0.06 to 0.17). Similarly, KRAS mutations was found in 53 (5.9%) non-mucinous adenocarcinomas, 7 (11.5%) PMPA with ≤90% mucin and 14 (35.9%) PMPA with >90% mucin (coefficient, 95% CI=0.11, 0.05 to 0.16). However, mucinous abundancy was inversely, ordinally linked to the EGFR mutations (coefficient, 95% CI=-0.28, -0.33 to -0.22). Mucin abundancy seemed not associated with the alterations of HER2, BRAF, ROS1, MET and RET. We divided PMPA with >90% mucin into three histologic types, namely columnar mucinous cell with basal nuclei (type I, n=11), cuboidal cell with goblet cell feature (type II, n=16) and mucinous cribriform pattern (type III, n=12). These histologic subtypes were associated with alterations of ALK, KRAS and MET, and the immunohistochemical reactivity of MUC1, MUC2, MUC5ac, MUC6, TTF-1 and CK20, including high positive rate of MUC6 (90.9%) and CK20 (36.4%) in type I, MUC2 (50%) in type II and MUC1 (100%) in type III. In summary, mucin abundancy is associated with immunohistochemical and oncogenic-driver profiles of lung adenocarcinomas among Chinese.

Keywords: Lung cancer, pathology, survival, oncogenic driver, adenocarcinoma

Introduction

Invasive mucinous adenocarcinoma of the lung has been introduced as a new category in the 2015 World Health Organization (WHO) classification of lung tumors, because of its distinct clinical, radiological, pathological, and genetic characteristics [1, 2]. Near 10.4% of lung adenocarcinomas in Asians are mucinous adenocarcinoma [3]. Histologically, its tumor cells show characteristic goblet-cell and/or columnar cell morphology with abundant intracytoplasmic mucin and small basally oriented nuclei. Surrounding alveolar spaces are often filled with extracellular mucin. According to the 2015 WHO classification [1], the pulmonary adenocarcinomas, that produce extracellular mucin but lack the characteristic morphology of goblet cells or columnar cells, must be distinguished from invasive mucinous adenocarcinoma. Therefore, proper classification of pulmonary mucin-producing adenocarcinomas (PMPA) is complicated, and has been described as "difficult and somewhat arbitrary" and "controversial" by the WHO classification [1].

PMPA show various cytological and histological features. The cytological spectrum of PMPA is

broad, including intracytoplasmic mucin within the different types of tumor cells (e.g. goblet cells, columnar cells, cuboidal cells and signet ring cells) and extracellular mucin. It also exhibits several histological patterns, including lepidic, acinar, papillary, micropapillary, solid, and mucinous cribriform patterns [1, 4], Signet-ring cell features are regarded as cytological features rather than primary histological subtypes. They occur most commonly in the solid component of lung adenocarcinomas, but also seen in other patterns. It is noteworthy that PMPA with >90% invasive mucin (>90% invasive mucinous pattern) was termed as pure mucinous, while PMPA with 10-90% invasive mucinous pattern termed as mixed mucinous/nonmucinous pattern [2, 4]. Several studies have investigated the oncogenic-driver alterations, immunohistochemical characteristics and clinicopathologic features of PMPA [2, 4-6]. However, the molecular and immunohistochemical characteristics of PMPA by mucin percentage are poorly understood.

Studies have shown that KRAS mutation was the most frequent genetic alteration seen in invasive mucinous adenocarcinomas (40-76%), while EGFR mutations are relatively uncommon in these cases [4, 7-11]. ALK rearrangements are common (8-40%) in PMPA with signet-ring cell features [4, 10, 12]. However, the association of histology with the alterations of other oncogenic drivers is still unclear in PMPA.

Therefore, we retrospectively characterized the clinicopathological, oncogenic-driver and immunohistochemical profiles of lung adenocarcinomas by their mucin abunancy, which were reclassified according to the 2015 WHO classification of lung adenocarcinomas [1, 2]. We also analyzed histologic, immunohistochemical and oncogenic-driver patterns of the proposed 3 histological subtypes of PMPA with >90% extracellular mucin.

Materials and methods

Patients

We consecutively collected the PMPA resected at Department of Thoracic Surgery, Fudan University Shanghai Cancer Center, Shanghai, China from November 2010 to May 2013. The study has been approved by the Ethical Review Committee of the Fudan University Shanghai Cancer Center. The inclusion criteria for this study were: (1) patients underwent curative resection (lobectomy resection) with mediastinal lymph node dissection; (2) diagnosis of pulmonary adenocarcinoma with an extracellular mucinous component (at least 10%); (3) No concurrent inflammatory/infectious lung diseases or multiple tumors. We also extracted and computed the data of non-mucinous adenocarcinomas from a prior study of ours [13].

Histologic analysis

All available hematoxylin and eosin (H&E)-stained slides with tumor tissue were independently reviewed by three thoracic pathologists (GGS, YL, and QZ). Tumors were re-classified using the terminology and criteria of the 2015 IASLC/ATS/ERS classification of lung adenocarcinoma [1, 14]. The predominant and minor histological patterns as well as cell-types were recorded, including any identifiable histological types. Presence of mucin production was assessed using diastase-resistant PAS staining in all samples with a 5% increment. A tumor was considered as “mucin producing” if there was more than 10% extracellular mucin in any histological pattern of tumor (lepidic, papillary, acinar, micropapillary, or solid). It is worth mentioning that some special histological features were recorded such as STAS, cribriform and psammoma body. Colloid adenocarcinomas were excluded based on their unique histologic feature of abundant extracellular mucin pools, that distend and replace alveolar spaces [15]. In addition, imaging studies of all included patients had excluded the metastasis and other site of origin such as colonic or pancreatic adenocarcinoma.

The immunohistochemistry was carried out on formalin-fixed, paraffin-embedded tissue blocks according to the manufacturers’ instructions. The staining was performed using the Ventana autostainer and its reagents. Thyroid transcription factor-1 (TTF-1, 1:200, Dako, Copenhagen, Denmark), MUC1 (1:200, Novocastra, Newcastle upon Tyne, UK), MUC2 (1:250, Novocastra, Newcastle upon Tyne, UK), MUC5AC (1:500, Novocastra, Newcastle upon Tyne, UK), MUC6 (1:200, Novocastra, Newcastle upon Tyne, UK) and CK20 (cytokeratin 20, 1:50, Dako, Copenhagen, Denmark) were used as primary antibodies. All immunohistochemical

markers were assessed using light microscopy. The immunohistochemical staining intensity was graded as follows: Negative for absent or focal perceptible staining in the membrane, nuclei and cytoplasm in <10%, and Positive for perceptible staining for membranous, nuclei and cytoplasmic staining in >10% of the tumor cells.

Analysis of the oncogenic drivers

The frozen tumor specimens were lysed into TRIzol (Invitrogen, Carlsbad, CA). The genomic DNA or RNA was extracted as per standard protocols (RNeasy Mini Kit, and QiAamp DNA Mini Kit, Qiagen, Hilden, Germany). Total RNA samples were reverse transcribed into single-stranded cDNA using Revert Aid First Strand cDNA Synthesis Kit (Fermentas, St Leon-Rot, Germany). As previously reported, the mutational status of EGFR (exons 18-21), HER2 (exon 20), KRAS (exons 2-3) and BRAF (exons 11-15) was determined using polymerase chain reaction (PCR)-based direct sequencing and verified by DNA sequencing analysis [16-18]. The mutation of MET (exon 13 to 21) was amplified using PCR with cDNA for direct sequencing, and the exon 14 deletion was verified by sequencing of the PCR product of *MET* exon 13 to 15 [19]. A combination of quantitative real-time PCR (qRT-PCR) and reverse transcriptase PCR (RT-PCR) was used to detect ALK, FGFR1/3, ROS1, and RET fusions. The primers were designed to amplify all known fusion variants with the use of cDNA, as previously described [20-23]. All the fusions were further validated using fluorescent in situ hybridization (FISH) [20-22].

Statistical analysis

Pearson's chi-square test or Fisher's exact test was used to investigate the correlations between two categorical variables. The association between one categorical variable and one continuous variable was assessed using independent sample Student *t* test. The recurrence-free survival (RFS) and overall survival (OS) were analyzed using univariate and multivariate Cox proportional hazards regression models. The *P* and P_{trend} was calculated through variance-weighted least squares test and the chi-square statistics for the trend (regression) of frequencies of oncogenic driver alterations on the percentage of mucin (vwls and ptrend syntaxes), respectively. The statistical analyses were conducted using Stata IC version 15 (Stata Corp, College Station, TX, USA). All tests were two-tailed, and a $P<0.05$ was considered as statistically significant.

Results

Clinical characteristics

A total of 901 nonmucinous adenocarcinoma from a prior study [13] and 102 newly identified PMPA were analyzed (**Table 1**). The median age among the patients with PMPA was 59 years (range: 30-80 years), and mean tumor size 3.1 cm (range: 1-11 cm). All patients received pulmonary lobectomy and lymph node dissection. There were no statistical differences of baseline characteristics except that PMPA with >90% mucin vs ≤90% mucin was more likely in never-smokers ($P=0.032$), of early stages ($P=0.016$), and of early N categories ($P=0.025$).

Common oncogenic driver alterations

Of the 897 nonmucinous adenocarcinomas and 100 PMPA with known oncogenic-driver status (the other 2 cases had no complete molecular testing), 161 (16.1%) harbored no known mutation/rearrangements (**Table 2**), with EGFR (n=635, 63.4%) as the most common mutation and KRAS and ALK as the second and third most common alterations, respectively (n=74, 7.1%, and n=68, 6.8%). The frequencies of oncogenic-driver mutations/rearrangements were ordinally associated with the mucin abundancy (**Table 2**, overall $P<0.001$).

Pathological characteristics

We divided the 42 PMPA with >90% of extracellular mucin into three subtypes according to the predominant histological features: I, columnar cells with basal-located nuclei and abundant intracytoplasmic mucin (**Figure 1A**); II, cuboidal cells with goblet cell features (**Figure 1B**) and III, mucinous cribriform pattern (**Figure 1C**).

We also found that the subtype of PMPA with >90% extracellular mucin was associated with the frequencies of ALK ($P<0.001$), KRAS ($P=0.002$) and MET ($P=0.001$) mutations/rear-

Table 1. Baseline characteristics of the pulmonary mucin-producing adenocarcinomas

	Non-mucinous adenocarcinoma#, n (%)	Extracellular mucin ≤90%, n (%)	Extracellular mucin >90%, n (%)	Total, n (%)	*P*^	*P**
Age						
<60 years	441 (48.9)	28 (45.9)	19 (47.5)	488 (48.7)	0.889	0.875
60+ years	460 (51.1)	33 (54.1)	21 (52.5)	514 (51.3)		
Sex						
Female	497 (55.2)	30 (48.4)	24 (60.0)	551 (54.9)	0.471	0.251
Male	404 (44.8)	32 (51.6)	16 (40.0)	452 (45.1)		
Smoker						
Never-smoker	605 (67.1)	34 (56.7)	31 (77.5)	670 (66.9)	0.087	0.032
Smoker	296 (32.9)	26 (43.3)	9 (22.5)	331 (33.1)		
Stage						
I-II	588 (65.3)	43 (69.4)	36 (90.0)	667 (66.6)	0.002	0.016
III-IV	312 (34.6)	19 (30.7)	4 (10.0)	335 (33.4)		
Tumor size						
<1.5 cm	NA	9 (14.5)	6 (15.0)	15 (14.7)		0.95
≥1.5 cm		53 (85.5)	34 (85.0)	87 (85.3)		
N category						
0	NA	43 (71.67)	33 (89.19)	76 (78.35)		0.025
1		4 (6.7)	3 (8.1)	7 (7.2)		
2		13 (21.7)	1 (2.7)	14 (14.4)		
M category						
0	NA	55 (93.2)	34 (91.9)	89 (92.7)		>0.99
1		4 (6.8)	3 (8.1)	7 (7.3)		
Lymphovascular invasion						
Absent	NA	22 (81.5)	14 (93.3)	36 (85.7)		0.395
Present		5 (18.5)	1 (6.7)	6 (14.3)		

Note: SD, standard deviation; NA, not available; #Data extracted and recalculated from Hu et al. (OncoTargets and Therapy, 2014); ^Comparison of the all 3 groups using Chi-square or Fisher exact test; *Comparison of the 2 mucin-producing groups (≤90% versus >90%) using Chi-square or Fisher exact test.

rangements (**Table 3**), but not with the others. ALK rearrangement was not found in any of type-I PMPA with >90% mucin (n=10), while KRAS and MET not mutated in any of type-III PMPA with >90% mucin (n=12). The subgroup analysis showed that the ALK rearrangements linked to the subtypes of PMPA with >90% extracellular mucin in smokers, but KRAS and MET mutations linked to the subtypes in never-smokers (**Table 6**).

We first examined the expression of several immunohistochemical markers in the PMPA with >90% mucin using immunohistochemistry (**Table 4**), including MUC1, MUC2, MUC5ac, MUC6, TTF-1 and CK20. The immunohistochemical markers were all associated with the types of these PMPA. Interestingly, there was an increasing trend in the positive rates of TTF-1 among the 3 types of PMPA with >90% mucin (3/11, 27.3% in type I, 7/16, 43.8% in type II, and 10/12, 83.3% in type III, *P*=0.017). MUC1 was expressed in all type-III PMPA with >90% mucin, while in less than 30% of other types (*P*<0.001) (**Figure 2A**). MUC2 was expressed in 50% of type-II PMPA with >90% mucin, while in less than 10% of other types (*P*=0.003) (**Figure 2B**). MUC6 was expressed in 90% of type-I PMPA with >90% mucin, while in less than 30% of other types (*P*<0.001) (**Figure 2D**). CK20 positivity was found in 36.4% (4/11) of type-I PMPA with >90% mucin, but not in other types of PMPA with >90% mucin. Finally, MUC5ac was expressed in more than 60% of all types of PMPA with >90% mucin (**Figure 2C**).

Table 2. Abundancy of extracellular mucin and the frequency of oncogenic driver alternations in pulmonary adenocarcinomas among Chinese

Driver gene	Non-mucinous adenocarcinoma#, n(%)	Extracellular mucin ≤90%, n (%)	Extracellular mucin >90%, n (%)	Total	Coefficient (5% CI)^	*P*^	*P**
ALK	47 (5.2)	9 (14.8)	12 (30.8)	68 (6.8)	0.11 (0.06 to 0.17)	<0.001	0.078
BRAF	13 (1.4)	0 (0.0)	1 (2.6)	14 (1.4)	0.01 (-0.02 to 0.03)	0.666	0.39
EGFR	609 (67.6)	20 (32.8)	6 (15.4)	635 (63.4)	-0.28 (-0.33 to -0.22)	<0.001	0.064
FGFR1/3	NA	0 (0.0)	0 (0.0)	0 (0.0)	NA		NA
HER2	18 (2.0)	1 (1.6)	1 (2.6)	20 (2.0)	0.00 (-0.02 to 0.02)	0.962	>0.99
KRAS	53 (5.9)	7 (11.5)	14 (35.9)	74 (7.4)	0.11 (0.05 to 0.16)	<0.001	0.005
MET	NA	0 (0.0)	1 (2.6)	1 (1.0)	NA		0.40
Pan-neg	145 (16.1)	14 (23.0)	2 (5.1)	161 (16.1)	-0.04 (-0.08 to -0.01)	0.015	0.024
RET	12 (1.3)	7 (11.5)	1 (2.6)	20 (2.0)	0.01 (-0.01 to 0.04)	0.246	0.116
ROS1	NA	3 (4.9)	1 (2.6)	4 (4.0)	-0.02 (-0.10 to 0.05)	0.535	>0.99
Total	897 (100.0)	61 (100.0)	39 (100.0)	997 (100.0)			0.001

Note: Fisher exact test shows overall differences among the groups (*P*=0.001). #Data extracted and recalculated from Hu et al. (OncoTargets and Therapy, 2014); ^The test of variance-weighted least squares on the association of mucin abundancy (none, ≤90% vs >90%) with a given oncogenic driver alternation (binary variable); *Fisher exact test on the association of and mucin extent (≤90% versus >90%) with a given oncogenic-driver alternation (binary variable). Pan-neg, no alternations in any of the tested oncogenic drivers; NA, not available.

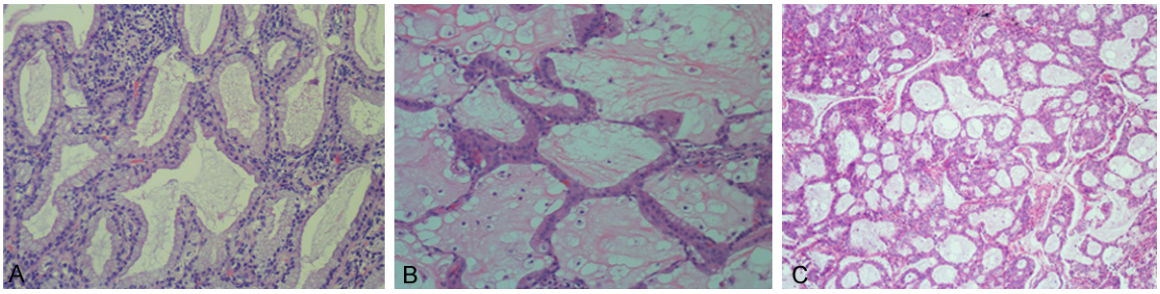

Figure 1. The characteristics of three different morphology in mucin-producing adenocarcinomas with mucin more than 90%: Columnar mucinous cell with basal nuclei (A), Cuboidal cell with goblet cell feature (B), and Mucinous cribriform pattern (C).

Survival analyses

The median follow-up time for the 42 PMPA patients with >90% extracellular mucin was 29.9 months (range, 4.5 to 45.5 months). Our univariate Cox regression analyses showed that sex (*P*=0.038), pathologic stage (*P*=0.005), and lymph node status (*P*=0.034) were associated with RFS, and only stage (*P*=0.012) associated with OS (**Table 5**). Histology subtypes of PMPA with >90% mucin and oncogenic driver mutations were not associated with either RFS or OS. The multivariate Cox regression analysis found that none of the potential factors were associated with RFS (**Table 7**). Our additional univariate Cox regression analyses revealed that MUC1, MUC2, MUC5ac, MUC6, TTF-1 and CK20 did not link to RFS or OS (**Table 8**).

Discussion

This retrospective study on 1,003 lung adenocarcinomas in Chinese showed that mucin abundancy (little, ≤90% versus >90% mucin) was ordinally associated with oncongenic-driver alterations. We also proposed to group the PMPA with >90% extracellular mucin intro three subtypes, namely type I (Columnar cells with basal nuclei and abundant mucin-filled cytoplasm), type II (Cuboidal cells with goblet cell feature) and type III (Mucinous cribriform pattern). The 3 subtypes show unique histological, immunohistochemical and oncogenic driver alterations, although they did not have different RFS or OS.

The proposed 3 subtypes of PMPA with >90% mucin exhibit unique immunophenotypic fea-

Table 3. The histopathologic characteristics of different oncogenic driver mutations in mucin-producing adenocarcinoma with more than 90% of extracellular mucin

	I. Columnar cell with basal nuclei and abundant mucin-filled cytoplasm, n (%)	II. Cuboidal cell with goblet cell feature, n (%)	III. Mucinous cribriform pattern, n (%)	Total	*P*
ALK					
Absent	**10 (100.0)**	15 (88.2)	2 (16.7)	27 (69.2)	<0.001
Present	0 (0.0)	2 (11.8)	**10 (83.3)**	12 (30.8)	
BRAF					
Absent	9 (90.0)	17 (100.0)	12 (100.0)	38 (97.4)	0.256
Present	1 (10.0)	0 (0.0)	0 (0.0)	1 (2.6)	
EGFR					
Absent	8 (80.0)	13 (76.5)	12 (100.0)	33 (84.6)	0.20
Present	2 (20.0)	4 (23.5)	0 (0.0)	6 (15.4)	
FGFR1/3					
Absent	10 (100.0)	17 (100.0)	12 (100.0)	39 (100.0)	NA
KRAS					
Absent	6 (60.0)	7 (41.2)	**12 (100.0)**	25 (64.1)	0.002
Present	**4 (40.0)**	**10 (58.8)**	0 (0.0)	14 (35.9)	
HER2/ERBB2					
Absent	9 (90.0)	17 (100.0)	12 (100.0)	38 (97.4)	0.256
Present	1 (10.0)	0 (0.0)	0 (0.0)	1 (2.6)	
MET					
Absent	6 (60.0)	6 (35.3)	**12 (100.0)**	24 (61.5)	0.001
Present	**4 (40.0)**	**11 (64.7)**	0 (0.0)	15 (38.5)	
RET					
Absent	6 (60.0)	7 (41.2)	11 (91.7)	24 (61.5)	0.18
Present	4 (40.0)	10 (58.8)	1 (8.3)	15 (38.5)	
ROS1					
Absent	10 (100.0)	17 (100.0)	11 (91.7)	38 (97.4)	0.564
Present	0 (0.0)	0 (0.0)	1 (8.3)	1 (2.6)	
Pan-negative					
Absent	8 (80.0)	**17 (100.0)**	**12 (100.0)**	37 (94.9)	0.061
Present	2 (20.0)	0 (0.0)	0 (0.0)	2 (5.1)	

Note: Pan-negative, no mutations/rearrangements of the 9 oncogenic driver genes were found. Bolded cells indicate characteristic pattern of the subtype of pulmonary mucin-producing adenocarcinoma.

tures. MUC1 was expressed in all of the type III tumors, while <30% in other types. MUC1 thus may be used as a positive marker for type III tumors, although it was not found differentially expressed in mucinous and nonmucinious lung adencarcinomas [24]. On the other hand, due to the higher expression rate in type I (91% vs <25% in other types), MUC6 may be a positive/sensitive marker for type I tumors. It is interesting that higher expression of MUC6 was found in less mucin-secreting cuboidal cells of lung adenocarcinomas [24]. Finally, MUC5ac protein expression was found in >80% of all subtypes (100% in types I and II) and may be useful to highlight or confirm the mucinous component. Indeed, recent reports show that MUC5ac protein and mRNA were highly expressed in mucin-secretion components of lung carcinomas [24-27].

The proposed 3 subtypes of PMPA with >90% mucin are associated with the alterations of ALK, KRAS and MET, but not those of BRAF, EGFR, FGFR1/3, HER2/ERBB2, RET and ROS1. A higher frequency (83%) of ALK rearrangements in type III (vs types I and II) PMPA with >90% mucin suggests they may be helpful in treating this type of PMPA. This finding is con-

Table 4. Expression of MUC1, MUC2, MUC5AC, MUC6, CK20 and TTF1 proteins in pulmonary mucin-producing adenocarcinomas with more than 90% of extracellular mucin

Marker		I. Columnar cell with basal nuclei and abundant mucin-filled cytoplasm, n (%)	II. Cuboidal cell with goblet cell feature, n (%)	III. Mucinous cribriform pattern, n (%)	Total	*P*
MUC1	Negative	8 (72.7)	13 (81.3)	0 (00.0)	21	<0.001
	Positive	3 (27.3)	3 (18.8)	**12 (100.0)**	18	
MUC2	Negative	10 (90.9)	8 (50.0)	12 (100.0)	30	0.003
	Positive	1 (9.1)	**8 (50.0)**	0 (0.0)	9	
MUC5ac	Negative	0 (0.0)	0 (00.0)	**4 (33.3)**	4	0.01
	Positive	**11 (100.0)**	**16 (100.0)**	8 (66.7)	35	
MUC6	Negative	1 (9.1)	12 (75.0)	**11 (91.7)**	24	<0.001
	Positive	**10 (90.9)**	4 (25.0)	1 (8.3)	15	
TTF1	Negative	8 (72.7)	9 (56.3)	2 (16.7)	19	0.017
	Positive	3 (27.3)	7 (43.8)	10 (83.3)	20	
CK20	Negative	7 (63.6)	**16 (100.0)**	**12 (100.0)**	35	0.004
	Positive	**4 (36.4)**	0 (0.0)	0 (0.0)	4	
Tota1		11 (100)	16 (100)	12 (100)	39	

Note: Bolded cells indicate likely useful immunohistochemical patterns.

Table 5. Univariate analysis on the factors associated with survivals of pulmonary mucin-producing adenocarcinoma with more than 90% of extracellular mucin

Factors	Recurrence-free survival		Overall survival	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Sex (male vs female)	4.2 (1.1-16)	0.038	2.7 (0.4-19.3)	0.332
Age (65+ vs <65 years)	0.3 (0-2.2)	0.227	2.9 (0.4-20.5)	0.294
Age (60+ vs <60 years)	0.51 (0.12-2.07)	0.348	1.18 (0.14-8.45)	0.871
Stage (III-IV vs I-II)	9.9 (2-49.6)	0.005	21.9 (2-243.4)	0.012
Size (1.5+ vs <1.5 cm)	1.9 (0.2-15.7)	0.535	NA	
Grade (high vs low)	0.9 (0.4-2.3)	0.827	2.7 (0.4-19.5)	0.313
Lymphovascular invasion (+ vs -)	12.5 (0.8-199.8)	0.074	13 (0.8-207.6)	0.07
N category (2, 1 vs -)	3.2 (1.1-9.4)	0.034	4.1 (1-17.5)	0.054
M category (+ vs -)	NA			
Psammoma body (+ vs -)	0.7 (0.2-3.6)	0.711	0.9 (0.1-9.2)	0.954
Lepidic spread (+ vs -)	0.8 (0.2-3.1)	0.705		
STAS spread (+ vs -)	1.9 (0.5-6.9)	0.356	1.6 (0.2-11.2)	0.65
Histology subtypes				
Histology (columnar, cuboidal vs mucinous cribriform subtypes)	1.1 (0.4-2.5)	0.899	1.4 (0.4-5.4)	0.633
I. Columnar cell with basal nuclei and abundant mucin-filled cytoplasm	0.4 (0-2.9)	0.342	NA	
II. Cuboidal cell with goblet cell feature	2.9 (0.7-11.6)	0.136	3.9 (0.4-37.5)	0.239
III. Mucinous cribriform pattern	0.6 (0.1-2.7)	0.481	0.7 (0.1-6.9)	0.771
Oncogenic driver genes				
Gene (all)	0.9 (0.6-1.2)	0.385	1 (0.6-1.5)	0.955
ALK (+ vs -)	1.7 (0.5-6.4)	0.422	0.7 (0.1-6.4)	0.721
EGFR (+ vs -)	0.6 (0.1-5)	0.65	1.6 (0.2-15.2)	0.693
KRAS (+ vs -)	1.7 (0.5-6.4)	0.424	2.1 (0.3-14.8)	0.464

Note: HR, hazard ratio; CI, confidence interval; STAS, spread through alveolar spaces; +, present; -, absent or undetectable; vs, versus.

sistent with that in Asian studies [3, 28-32], but contradictory to that of an American study showing no association [4]. We also show that such a high frequency of ALK rearrangement

Table 6. The histopathologic characteristics of different oncogenic driver mutations in mucin-producing adenocarcinoma with more than 90% of extracellular mucin, by smoker status

	Never-smoker					Smoker				
	Type I, n (%)	Type II, n (%)	Type III, n (%)	Total	*P*	Type I, n (%)	Type II, n (%)	Type III, n (%)	Total	*P*
ALK										
Absent	2 (100.0)	4 (100.0)	0 (0.0)	6 (75.0)	0.071	8 (100.0)	11 (84.6)	2 (20.0)	21 (67.7)	<0.001
Present	0 (0.0)	0 (0.0)	2 (100.0)	2 (25.0)		0 (0.0)	2 (15.4)	**8 (80.0)**	10 (32.3)	
BRAF										
Absent	7 (87.5)	13 (100.0)	10 (100.0)	30 (96.8)	0.258	2 (100.0)	4 (100.0)	2 (100.0)	8 (100.0)	NA
Present	1 (12.5)	0 (0.0)	0 (0.0)	1 (3.2)						
EGFR										
Absent	6 (75.0)	9 (69.2)	10 (100.0)	25 (80.7)	0.155	2 (100.0)	4 (100.0)	2 (100.0)	8 (100.0)	NA
Present	2 (25.0)	4 (30.8)	0 (0.0)	6 (19.4)						
FGFR1/3										
Absent	8 (100.0)	13 (100.0)	10 (100.0)	31 (100.0)	NA	2 (100.0)	4 (100.0)	2 (100.0)	8 (100.0)	NA
KRAS										
Absent	6 (75.0)	7 (53.9)	10 (100.0)	23 (74.2)	0.033	0 (0.0)	0 (0.0)	2 (100.0)	2 (25.0)	0.071
Present	**2 (25.0)**	**6 (46.2)**	0 (0.0)	8 (25.8)		2 (100.0)	4 (100.0)	0 (0.0)	6 (75.0)	
HER2/ERBB2										
Absent	7 (87.5)	13 (100.0)	10 (100.0)	30 (96.8)	0.258	2 (100.0)	4 (100.0)	2 (100.0)	8 (100.0)	NA
Present	1 (12.5)	0 (0.0)	0 (0.0)	1 (3.2)						
MET										
Absent	6 (75.0)	6 (46.2)	10 (100.0)	22 (71.0)	0.011	0 (0.0)	0 (0.0)	2 (100.0)	2 (25.0)	0.071
Present	**2 (25.0)**	**7 (53.9)**	0 (0.0)	9 (29.0)		2 (100.0)	4 (100.0)	0 (0.0)	6 (75.0)	
RET										
Absent	6 (75.0)	7 (53.9)	9 (90.0)	22 (71.0)	0.142	0 (0.0)	0 (0.0)	2 (100.0)	2 (25.0)	0.071
Present	2 (25.0)	6 (46.2)	1 (10.0)	9 (29.0)		2 (100.0)	4 (100.0)	0 (0.0)	6 (75.0)	
ROS1										
Absent	8 (100.0)	13 (100.0)	9 (90.0)	30 (96.8)	0.581	2 (100.0)	4 (100.0)	2 (100.0)	8 (100.0)	NA
Present	0 (0.0)	0 (0.0)	1 (10.0)	1 (3.2)						
Pan-negative										
Absent	6 (75.0)	13 (100.0)	10 (100.0)	29 (93.6)	0.06	2 (100.0)	4 (100.0)	2 (100.0)	8 (100.0)	NA
Present	2 (25.0)	0 (0.0)	0 (0.0)	2 (6.5)						

Note: The proposed subtypes of pulmonary mucin-producing adenocarcinoma with >90% extracellular mucin: type I, Columnar cell with basal nuclei and abundant mucin-filled cytoplasm; type II, Cuboidal cell with goblet cell feature; type III, Mucinous cribriform pattern; Pan-negative, no mutations/rearrangements of the 9 oncogenic driver genes were found. Bolded cells indicate characteristic pattern of the subtype of pulmonary mucin-producing adenocarcinoma.

in smokers, but not never-smokers. ALK rearrangement thus may link to the pathogenesis of this type of PMPA in smokers.

We observe KRAS and MET mutations in the types I and II of PMPA with >90% mucin, but not in type III, which was also valid in both smokers and never-smokers. This observation is important because an "assumed" type I or II PMPA with >90% mucin showing a KRAS or MET mutation, is more likely a metastasis than a pulmonary primary. Further investigation may be warranted in these cases. Previously, the frequency of KRAS mutations is found higher in lung adenocarcinomas with higher percentages of mucin [4], which appears to contradict to the lack of KRAS mutations in type III PMPA with >90% mucin in our study. The possible reason of the discrepancy or contradiction may be the racial difference between the prior study and ours, and the unique molecular profile of type III PMPA with >90% mucin. In fact, the cohort in Kadota et al. had 34 cases with cribriform patterns (type III), but did not separate the cases with predominantly cuboidal- or goblet-cells patterns (the proposed types I and II) [4]. In contrast to the KRAS mutations in never-smokers as reported here, KRAS has been reported to be associated with smoker status, mucinous features and signet-ring

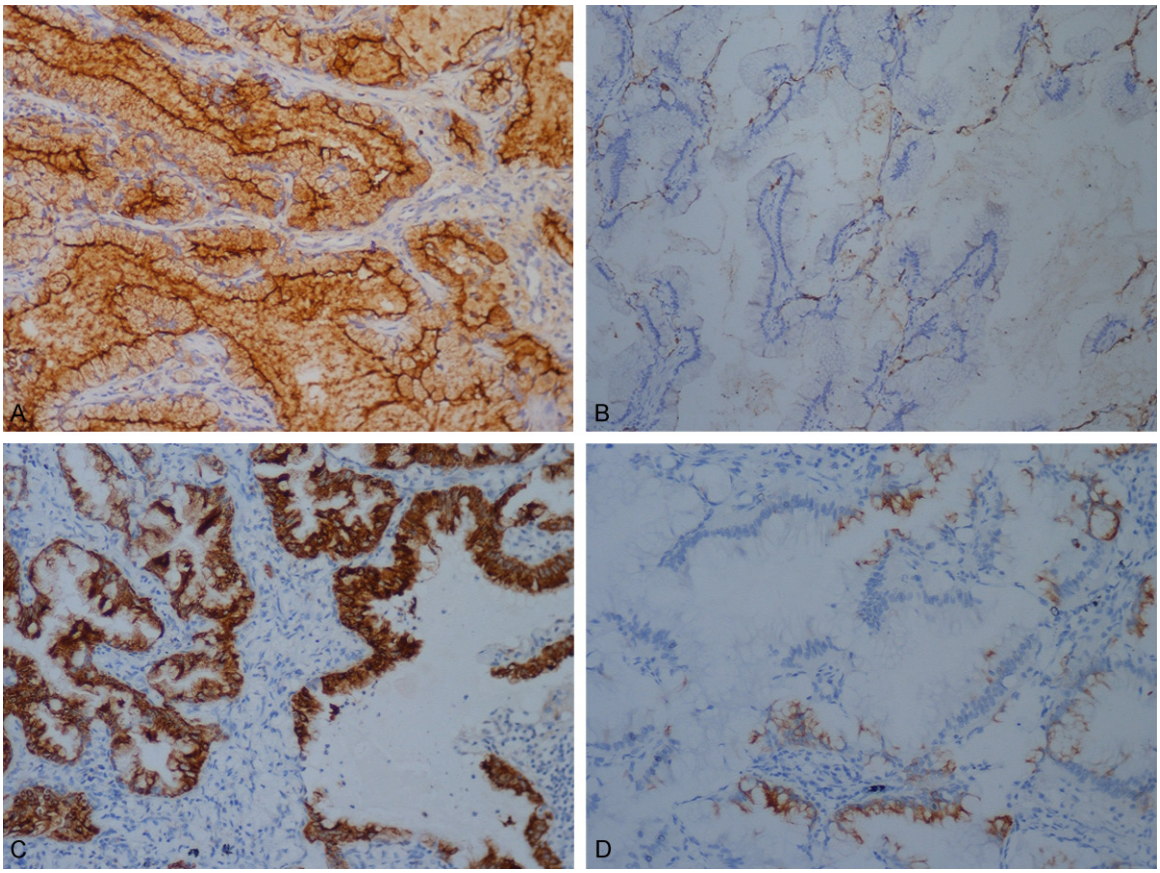

Figure 2. Representative photomicrographs of pulmonary mucin-producing adenocarcinomas positive for MUC proteins, including MUC1 (A), MUC2 (B), MUC5AC (C) and MUC6 (focally positive, D).

Table 7. Multivariate analysis on the factors associated with the survivals of pulmonary mucin-producing adenocarcinoma with more than 90% of mucin

Factors	Recurrence-free survival		Overall survival	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Sex (male vs female)	5 (0.7-37.8)	0.118		
Stage (III-IV vs I-II)	6 (0.1-630.2)	0.453	NA	
Lymphovascular invasion (+ vs -)	NA*		NA	
N category (2, 1 vs -)	3.9 (0.5-29.1)	0.178		
Cuboidal cell with goblet cell feature	6.9 (0.7-64.5)	0.091		

Note: HR, hazard ratio; CI, confidence interval; STAS, spread through alveolar spaces; +, present; -, absent or undetectable; vs, versus. *, for recurrence-free survival, inclusion of lymphovascular invasion as a factor led to no valid Cox regression models; therefore, it was excluded in the final multivariate survival model.

cell morphology in studies on lung adenocarcinomas [6, 33-35]. The mucin content (>90%) in our cases may contribute to the different associations of KRAS mutations with smoker status.

MET mutations were found in 1.7 to 6.5% of lung adenocarcinomas [19, 36-38], although as many as 65% of lung adenocarcinomas may have immunohistochemically detectable MET protein [36]. Given the increasingly important role of MET amplification and mutation in treating lung adenocarcinomas, the presence of MET mutation in types I and II of PMPA with >90% mucin provides one more possible therapeutic target for those tumors, but not for type III PMPA with >90% mucin due to their lack of MET mutation. Therefore, subtyping PMPA with >90% mucin may have additional clinical usefulness.

Table 8. Univariate analysis on the immunohistochemical markers associated with the survivals of pulmonary mucin-producing adenocarcinoma with more than 90% of mucin

Factors	Recurrence-free survival		Overall survival	
	HR (95% CI)	*P*	HR (95% CI)	*P*
MUC1	0.6 (0.1-2.5)	0.486	1 (0.1-7.2)	0.989
MUC2	1.6 (0.4-6.9)	0.501	NA	
MUC5ac	1 (0.1-8.5)	0.972	0.5 (0-4.4)	0.501
MUC6	0.6 (0.1-2.9)	0.499	1.8 (0.3-13)	0.548
TTF-1	0.5 (0.1-2.2)	0.372	1 (0.1-6.8)	0.961
CK20	NA		NA	

Note: HR, hazard ratio; CI, confidence interval; STAS, spread through alveolar spaces; +, present; -, absent or undetectable; vs, versus.

Several strengths of our study are noteworthy. First, we provided early evidence that mucin abundancy was ordinally associated with oncogenic-driver alterations. Near all of the prior works were focused on presence versus absence of mucin, while we categorized mucin abundancy into 3 tiers (little, ≤90% and >90%). Second the expression of mucin proteins and genes has been compared in mucinous and nonmucinous adenocarcinomas of the lung [24-26, 39], but to our knowledge none of them used the 2015 WHO classification of lung tumors. Because all of our cases were re-classified using the 2015 WHO classification of lung tumors, our findings on the PMPA with >90% extracellular mucin will be much more relevant to our current practice than prior reports. Third, EGFR, ALK, KRAS and ROS1 are the molecular targets for analysis recommended by the National Comprehensive Cancer Network guidelines. We reported the frequencies of some oncogenic driver alterations in lung adenocarcinomas that could serve as additional therapeutic targets and help clinical management. Finally, we reported distinct histologic, immunohistochemical and oncogenic-driver features in 3 subtypes of PMPA with >90 mucin. These findings may shed lights on future classification of PMPA and improving the histology-molecular correlation of PMPA, particularly on small or limited samples. Future validation studies are needed.

In conclusion, there was an ordinal association of mucin abundancy with oncogenic driver alterations in lung adenocarcinomas among Chinese. The three newly proposed subtypes of PMAP with >90% mucin show unique immunohistochemical patterns in MUC1, MUC2, MUC6 and CK20 and distinct frequencies of alterations in ALK, KRAS and MET, which are different from those of the PMPA in earlier reports. Those markers and oncogenic-driver alterations may help improve the diagnosis, prognostication and treatment of PMPA. This study thus sheds lights on the oncogenic driver alterations associated with histologic characteristics of lung adenocarcinomas, which will be very useful for selecting molecular tests on small samples.

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Disclosure of conflict of interest

None.

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References

[1] Travis WD, Brambilla E, Burke AP, Marx A and Nicholson AG. WHO classification of tumours of the lung, pleura, thymus and heart. Lyon: IARC; 2015.

[2] Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, Van Schil PE, Garg K, Austin JH, Asamura H, Rusch VW, Hirsch FR, Scagliotti G, Mitsudomi T, Huber RM, Ishikawa Y, Jett J, Sanchez-Cespedes M, Sculier JP, Takahashi T, Tsuboi M, Vansteenkiste J, Wistuba I, Yang PC, Aberle D, Brambilla C, Flieder D, Franklin W, Gazdar A, Gould M, Hasleton P, Henderson D, Johnson B, Johnson D, Kerr K, Kuriyama K, Lee JS, Miller VA, Petersen I, Roggli V, Rosell R, Saijo N, Thunnissen E, Tsao M and Yankelewitz D. International association for the study of lung cancer/american thoracic society/european respiratory society international multidis-

ciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011; 6: 244-285.
[3] Lee B, Lee T, Lee SH, Choi YL and Han J. Clinicopathologic characteristics of EGFR, KRAS, and ALK alterations in 6,595 lung cancers. Oncotarget 2016; 7: 23874-23884.
[4] Kadota K, Yeh YC, D'Angelo SP, Moreira AL, Kuk D, Sima CS, Riely GJ, Arcila ME, Kris MG, Rusch VW, Adusumilli PS and Travis WD. Associations between mutations and histologic patterns of mucin in lung adenocarcinoma: invasive mucinous pattern and extracellular mucin are associated with KRAS mutation. Am J Surg Pathol 2014; 38: 1118-1127.
[5] Rossi G, Cavazza A, Righi L, Sartori G, Bisagni A, Longo L, Pelosi G and Papotti M. Napsin-A, TTF-1, EGFR, and ALK status determination in lung primary and metastatic mucin-producing adenocarcinomas. Int J Surg Pathol 2014; 22: 401-407.
[6] Li H, Pan Y, Li Y, Li C, Wang R, Hu H, Zhang Y, Ye T, Wang L, Shen L, Sun Y and Chen H. Frequency of well-identified oncogenic driver mutations in lung adenocarcinoma of smokers varies with histological subtypes and graduated smoking dose. Lung Cancer 2013; 79: 8-13.
[7] Sun F, Wang P, Zheng Y, Jia W, Liu F, Xiao W, Bao J, Wang S and Lu K. Diagnosis, clinicopathological characteristics and prognosis of pulmonary mucinous adenocarcinoma. Oncol Lett 2018; 15: 489-494.
[8] Boland JM, Maleszewski JJ, Wampfler JA, Voss JS, Kipp BR, Yang P and Yi ES. Pulmonary invasive mucinous adenocarcinoma and mixed invasive mucinous/nonmucinous adenocarcinoma-a clinicopathological and molecular genetic study with survival analysis. Hum Pathol 2018; 71: 8-19.
[9] Lu F, Li S, Dong B, Zhang S, Lv C and Yang Y. Identification of lung adenocarcinoma mutation status based on histologic subtype: retrospective analysis of 269 patients. Thorac Cancer 2016; 7: 17-23.
[10] Dong YJ, Cai YR, Zhou LJ, Su D, Mu J, Chen XJ and Zhang LI. Association between the histological subtype of lung adenocarcinoma, EGFR/KRAS mutation status and the ALK rearrangement according to the novel IASLC/ATS/ERS classification. Oncol Lett 2016; 11: 2552-2558.
[11] Cai D, Li H, Wang R, Li Y, Pan Y, Hu H, Zhang Y, Gong R, Pan B, Sun Y and Chen H. Comparison of clinical features, molecular alterations, and prognosis in morphological subgroups of lung invasive mucinous adenocarcinoma. Onco Targets Ther 2014; 7: 2127-2132.
[12] Possidente L, Landriscina M, Patitucci G, Borgia L, Lalinga V and Vita G. ALK rearrangement in specific subtypes of lung adenocarcinoma: immunophenotypic and morphological features. Med Oncol 2017; 34: 76.
[13] Hu H, Pan Y, Li Y, Wang L, Wang R, Zhang Y, Li H, Ye T, Zhang Y, Luo X, Shao L, Sun Z, Cai D, Xu J, Lu Q, Deng Y, Shen L, Ji H, Sun Y and Chen H. Oncogenic mutations are associated with histological subtypes but do not have an independent prognostic value in lung adenocarcinoma. Onco Targets Ther 2014; 7: 1423-1437.
[14] Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, Chirieac LR, Dacic S, Duhig E, Flieder DB, Geisinger K, Hirsch FR, Ishikawa Y, Kerr KM, Noguchi M, Pelosi G, Powell CA, Tsao MS, Wistuba I; WHO Panel. 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.
[15] Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger K, Yatabe Y, Ishikawa Y, Wistuba I, Flieder DB, Franklin W, Gazdar A, Hasleton PS, Henderson DW, Kerr KM, Nakatani Y, Petersen I, Roggli V, Thunnissen E and Tsao M. Diagnosis of lung adenocarcinoma in resected specimens: implications of the 2011 international association for the study of lung cancer/American thoracic society/European respiratory society classification. Arch Pathol Lab Med 2013; 137: 685-705.
[16] Bu S, Wang R, Pan Y, Yu S, Shen X, Li Y, Sun Y and Chen H. Clinicopathologic characteristics of patients with HER2 insertions in non-small cell lung cancer. Ann Surg Oncol 2017; 24: 291-297.
[17] Wang R, Pan Y, Li C, Zhang H, Garfield D, Li Y, Ye T, Hu H, Luo X, Li H, Zhang Y, Zhang J, Zhou X, Shen L, Pao W, Sun Y and Chen H. Analysis of major known driver mutations and prognosis in resected adenosquamous lung carcinomas. J Thorac Oncol 2014; 9: 760-768.
[18] Sun Y, Ren Y, Fang Z, Li C, Fang R, Gao B, Han X, Tian W, Pao W, Chen H and Ji H. Lung adenocarcinoma from East Asian never-smokers is a disease largely defined by targetable oncogenic mutant kinases. J Clin Oncol 2010; 28: 4616-4620.
[19] Zheng D, Wang R, Ye T, Yu S, Hu H, Shen X, Li Y, Ji H, Sun Y and Chen H. MET exon 14 skipping defines a unique molecular class of non-small cell lung cancer. Oncotarget 2016; 7: 41691-41702.
[20] Pan Y, Zhang Y, Li Y, Hu H, Wang L, Li H, Wang R, Ye T, Luo X, Zhang Y, Li B, Cai D, Shen L, Sun Y and Chen H. ALK, ROS1 and RET fusions in 1139 lung adenocarcinomas: a comprehensive study of common and fusion pattern-specific clinicopathologic, histologic and cytologic features. Lung Cancer 2014; 84: 121-126.

[21] Wang R, Pan Y, Li C, Hu H, Zhang Y, Li H, Luo X, Zhang J, Fang Z, Li Y, Shen L, Ji H, Garfield D, Sun Y and Chen H. The use of quantitative real-time reverse transcriptase PCR for 5' and 3' portions of ALK transcripts to detect ALK rearrangements in lung cancers. Clin Cancer Res 2012; 18: 4725-4732.

[22] Wang R, Hu H, Pan Y, Li Y, Ye T, Li C, Luo X, Wang L, Li H, Zhang Y, Li F, Lu Y, Lu Q, Xu J, Garfield D, Shen L, Ji H, Pao W, Sun Y and Chen H. RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. J Clin Oncol 2012; 30: 4352-4359.

[23] Wang R, Wang L, Li Y, Hu H, Shen L, Shen X, Pan Y, Ye T, Zhang Y, Luo X, Zhang Y, Pan B, Li B, Li H, Zhang J, Pao W, Ji H, Sun Y and Chen H. FGFR1/3 tyrosine kinase fusions define a unique molecular subtype of non-small cell lung cancer. Clin Cancer Res 2014; 20: 4107-4114.

[24] Copin MC, Buisine MP, Leteurtre E, Marquette CH, Porte H, Aubert JP, Gosselin B and Porchet N. Mucinous bronchioloalveolar carcinomas display a specific pattern of mucin gene expression among primary lung adenocarcinomas. Hum Pathol 2001; 32: 274-281.

[25] Kim YK, Shin DH, Kim KB, Shin N, Park WY, Lee JH, Choi KU, Kim JY, Lee CH, Sol MY and Kim MH. MUC5AC and MUC5B enhance the characterization of mucinous adenocarcinomas of the lung and predict poor prognosis. Histopathology 2015; 67: 520-528.

[26] Tsuta K, Ishii G, Nitadori J, Murata Y, Kodama T, Nagai K and Ochiai A. Comparison of the immunophenotypes of signet-ring cell carcinoma, solid adenocarcinoma with mucin production, and mucinous bronchioloalveolar carcinoma of the lung characterized by the presence of cytoplasmic mucin. J Pathol 2006; 209: 78-87.

[27] Duruisseaux M, Antoine M, Rabbe N, Rodenas A, Mc Leer-Florin A, Lacave R, Poulot V, Duchene B, Van Seuningen I, Cadranel J and Wislez M. Lepidic predominant adenocarcinoma and invasive mucinous adenocarcinoma of the lung exhibit specific mucin expression in relation with oncogenic drivers. Lung Cancer 2017; 109: 92-100.

[28] Yoshida A, Tsuta K, Nakamura H, Kohno T, Takahashi F, Asamura H, Sekine I, Fukayama M, Shibata T, Furuta K and Tsuda H. Comprehensive histologic analysis of ALK-rearranged lung carcinomas. Am J Surg Pathol 2011; 35: 1226-1234.

[29] Jokoji R, Yamasaki T, Minami S, Komuta K, Sakamaki Y, Takeuchi K and Tsujimoto M. Combination of morphological feature analysis and immunohistochemistry is useful for screening of EML4-ALK-positive lung adenocarcinoma. J Clin Pathol 2010; 63: 1066-1070.

[30] Wang H, Zhang W, Wang K and Li X. Correlation between EML4-ALK, EGFR and clinicopathological features based on IASLC/ATS/ERS classification of lung adenocarcinoma. Medicine (Baltimore) 2018; 97: e11116.

[31] Kuroda N, Ohara M, Wada Y, Yasuoka K, Mizuno K, Yorita K, Obayashi C and Takeuchi K. Cytological features in eight patients with ALK-rearranged lung cancer. Diagn Cytopathol 2018; 46: 516-519.

[32] Miyata K, Morita S, Dejima H, Seki N, Matsutani N, Mieno M, Kondo F, Soejima Y, Tanaka F and Sawabe M. Cytological markers for predicting ALK-positive pulmonary adenocarcinoma. Diagn Cytopathol 2017; 45: 963-970.

[33] Zheng D, Wang R, Zhang Y, Pan Y, Cheng X, Cheng C, Zheng S, Li H, Gong R, Li Y, Shen X, Sun Y and Chen H. The prevalence and prognostic significance of KRAS mutation subtypes in lung adenocarcinomas from Chinese populations. Onco Targets Ther 2016; 9: 833-843.

[34] Wang WY, Liang DN, Yao WQ, Wu WL, Li JN, Chen M, Liao DY, Zhang M and Li GD. Immunohistochemical screening and fluorescence in situ hybridization confirmation of ALK translocation in lung adenocarcinoma and its clinicopathological significance: a single-center large-scale investigation of Chinese patients. Hum Pathol 2014; 45: 1414-1422.

[35] Sun PL, Seol H, Lee HJ, Yoo SB, Kim H, Xu X, Jheon S, Lee CT, Lee JS and Chung JH. High incidence of EGFR mutations in Korean men smokers with no intratumoral heterogeneity of lung adenocarcinomas: correlation with histologic subtypes, EGFR/TTF-1 expressions, and clinical features. J Thorac Oncol 2012; 7: 323-330.

[36] Yeung SF, Tong JHM, Law PPW, Chung LY, Lung RWM, Tong CYK, Chow C, Chan AWH, Wan IYP, Mok TSK and To KF. Profiling of oncogenic driver events in lung adenocarcinoma revealed MET mutation as independent prognostic factor. J Thorac Oncol 2015; 10: 1292-1300.

[37] Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. Nature 2014; 511: 543-550.

[38] Xia N, An J, Jiang QQ, Li M, Tan J and Hu CP. Analysis of EGFR, EML4-ALK, KRAS, and c-MET mutations in Chinese lung adenocarcinoma patients. Exp Lung Res 2013; 39: 328-335.

[39] Guo M, Tomoshige K, Meister M, Muley T, Fukazawa T, Tsuchiya T, Karns R, Warth A, Fink-Baldauf IM, Nagayasu T, Naomoto Y, Xu Y, Mall MA and Maeda Y. Gene signature driving invasive mucinous adenocarcinoma of the lung. EMBO Mol Med 2017; 9: 462-481.