Observational Study

Medicine®

OPEN

Clinicopathological, radiographic, and oncogenic features of primary pulmonary enteric adenocarcinoma in comparison with invasive adenocarcinoma in resection specimens

Long Zhao, MD, Shanshan Huang, MD, Jinjun Liu, MD, Juan Zhao, MD, Qiang Li, MD, Huo-Qiang Wang, MD*

Abstract

Primary pulmonary enteric adenocarcinoma (PEAC) is a rare subtype of primary lung adenocarcinoma. However, it is not known whether there are any distinctive clinical or molecular features.

PEACs were retrospectively identified in 28 patients from July 2014 to June 2016. We compared the clinicopathological, radiographic, and oncogenic characteristics of PEAC and primary pulmonary invasive adenocarcinoma (IAC).

A total of 28 PEAC patients and 92 IAC patients were compared. PEAC occurred more frequently in males ($P = .008$), in older patients ($P = .041$), in those with larger lesions ($P = .001$), and in those in a more advanced stage ($P = .011$). Radiologically, PEAC patients had larger lesions ($P = .025$) and more solid ($P = .006$); however, there were no statistically significant differences in lobulation, spiculation, pleural indentation, pleural effusion, and lymphadenopathy between PEAC and IAC. PEAC had higher values of carcinoembryonic antigen ($P = .008$) and carbohydrate antigen 19-9 ($P < .001$) than IAC. PEAC had a higher incidence (40% vs 63%, $P < .001$) of Kristen rat sarcoma viral oncogene homolog (*KRAS*) mutations and a lower incidence (10.71% vs 3.3%, $P < .001$) of epidermal growth factor receptor (*EGFR*) mutations. Villin may be a useful marker in the differential diagnosis of PEAC. *KRAS* mutations occurred more frequently in PEACs, which are cytokeratin 7-negative ($P = .032$). *EGFR* mutation rates were higher in PEACs, which are cytokeratin 20- and caudal type homeobox transcription factor 2-negative ($P = .041$).

PEAC is a rare and heterogeneous nonsmall-cell lung cancer subgroup with distinctive clinicopathological, radiographic, and molecular features. These results need to be further confirmed in future studies.

Abbreviations: CA19-9 = carbohydrate antigen 19-9, CDX-2 = caudal type homeobox transcription factor 2, CEA = carcinoembryonic antigen, CK20 = cytokeratin 20, CK7 = cytokeratin 7, CT = computed tomography, EGFR = epidermal growth factor receptor, GGO = ground-glass opacity, IAC = invasive adenocarcinoma, KRAS = Kristen rat sarcoma viral oncogene homolog, MUC2 = mucin 2, napsin A = novel aspartic proteinase of the pepsin family A, NSCLC = nonsmall-cell lung cancer, PEAC = pulmonary enteric adenocarcinoma, TTF-1 = thyroid transcription factor-1.

Keywords: EGFR, KRAS, primary pulmonary enteric adenocarcinoma, pulmonary carcinosarcoma, pulmonary invasive adenocarcinoma, villin

1. Introduction

During the past several decades, lung cancer has been the most commonly diagnosed cancer and the leading cause of cancer death because of the high smoking prevalence and air pollution in China, especially in men.[1–4] In the recent years, adenocarcinoma histological type has displaced squamous cell carcinoma as the most common form of nonsmall-cell lung cancer (NSCLC) worldwide, accounting for 45% of NSCLCs.[5] Pulmonary adenocarcinoma is morphologically heterogeneous, representing a wide variety of histopathologic patterns. Primary pulmonary enteric adenocarcinoma (PEAC) is a special variant of invasive adenocarcinoma (IAC). PEAC was first described by Tsao and Fraser in 1991.[6] It is a rare subtype of invasive lung adenocarcinoma that has been classified for the first time in the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma (2011)[7] and subsequently proposed in the 2015 World Health Organization classification.[8] At present, no more than 100 cases have been described in the English literature.[6,9–26] Oncogenic driver mutations in PEAC have been reported in case reports, small case series.[17–24] However, it is not known whether there are any distinctive clinical or detailed molecular features.

PEAC is defined as a pulmonary adenocarcinoma with an enteric differentiation component exceeding 50%.[7] Because

Editor: Shan Wang.

This article was approved by the Ethics Committee in Shanghai Pulmonary Hospital.

The authors have no funding and conflicts of interest to disclose.

Department of Nuclear Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China.

* Correspondence: Huo-Qiang Wang, Department of Nuclear Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, 507 Zhengmin Road, Shanghai 200433, China (e-mail: whq2216@sina.cn).

Medicine (2017) 96:39(e8153)

Received: 24 April 2017 / Received in final form: 31 July 2017 / Accepted: 1 September 2017

http://dx.doi.org/10.1097/MD.0000000000008153

its morphological and immunohistochemical features are similar to those of colorectal adenocarcinoma, clinical diagnosis is needed to rule out pulmonary metastasis of gastrointestinal malignancy. PEAC has the pathological characteristics of colorectal and lung adenocarcinomas. Thus, during morphological examination, both the back-to-back angulated acinar structures of colonic adenocarcinoma and the lepidic growth of lung adenocarcinoma may be seen. Accordingly, lung adenocarcinoma markers cytokeratin 7 (CK7), novel aspartic proteinase of the pepsin family A (napsin A), thyroid transcription factor-1 (TTF-1) and enteric differentiation markers cytokeratin 20 (CK20), caudal type homeobox transcription factor 2 (CDX-2), villin, and mucin 2 (MUC2) can be present simultaneously in PEAC. However, the expression levels of these immunohistological markers were not consistent in published English literature.[10,11,18,24,26]

With the advent of precision medicine, where therapeutic decisions are based on the specific histological and molecular characteristics of the patient's tumor, understanding the clinicopathological characteristics of patients with oncogenic driver aberrations is a top priority, and many efforts have been made in this direction. Indeed, an increased understanding of the histogenesis has improved the treatment of lung adenocarcinoma. However, there are rare data that describe the clinical features, gene mutations of PEAC. In this retrospective analysis, we aimed to further clarify PEAC's clinicopathological, radiological, and molecular characteristics, and compare them with those of typical invasive lung adenocarcinoma (IAC), which may be able to increase the diagnostic accuracy for appropriate patient management.

2. Materials and methods

2.1. Patients

From July 2014 to June 2016, we retrospectively collected data from 28 patients with PEAC by searched our hospital's database (2 pathologists to confirm the diagnosis), at the Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School of Medicine. Additionally, as a comparative cohort, we analyzed 92 consecutive patients with IACs. Patients with PEAC were included in this retrospective analysis according to the following criteria: histopathological results confirmed by resected tissues, newly diagnosed PEAC, not receiving previous chemotherapy or radiotherapy before surgery, and exclusion of pulmonary metastasis of gastrointestinal malignancy. The staging was performed for all the patients according to the 7th tumor, node, and metastasis classification.

The clinical characteristics of these patients were retrospectively reviewed in terms of clinical presentation, history, and course of disease, including sex, age at diagnosis, presenting symptoms, choice of operation, pathological tumor–lymph node–metastasis stage, and smoking status. Smoking status was divided into 2 categories: nonsmoker and smokers (including current and previous smokers). These clinical data were obtained from the medical records. This study was approved by the institutional review board of our hospital.

2.2. Analysis of tumor markers

Anticoagulant blood samples of 3 mL were collected from all patients for analysis of blood tumor markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) by enzyme-linked sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN) on the day of sample collection.

2.3. Radiology methods

At the time of diagnosis, all patients with PEAC underwent chest computed tomography (CT) scans and 92 patients with IAC underwent chest CT scans in our hospital. Visual analysis and measurements of CT were independently performed by 2 experienced chest radiologists and decisions on CT findings were reached in consensus. Chest CT scans were evaluated focusing on the location and morphologic characteristics (lobulated border, pleural indentation, speculation, etc.).

2.4. Immunohistochemistry

We retrospectively analyzed immunohistological findings of PEAC and these results were reviewed by 2 experienced pathologists in consensus. All patients had hematoxylin and eosin slides and immunohistochemical stains at the time of primary diagnosis, which was performed at the Department of Pathology in our hospital. These surgical specimens for immunohistochemical staining against specific differentiation markers, including CK7 and CK20, TTF-1, napsin A, CDX-2, and villin were formalin-fixed, paraffin-embedded, sectioned, and stained according to standard clinical operating procedures. Histopathologic results were evaluated according to the IASLC/ATS/ERS International Multidisciplinary Classification of Lung Adenocarcinoma (2011).[7] The pathological factors identified and recorded included tumor diameter, tumor location, the number of positive lymph nodes, and immunohistochemistry analyses (positive or negative).

2.5. Mutational analysis

We retrospectively reviewed the status of driver gene mutations in all patients. All patients in our study were routinely examined for molecular aberrations at diagnosis, including in epidermal growth factor receptor (*EGFR*) (exons 18–22), and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) (exons 2–3). Tumor samples were obtained from resected lesions. Genomic DNA or RNA was extracted with RNeasy Mini Kit and QiAamp DNA Mini Kit (Qiagen, Hilden, Germany). The reverse-transcriptase polymerase chain reaction assay was performed using Revert Aid First Strand cDNA Synthesis Kit (Fermentas, St Leon-Rot, Germany). *EGFR* and *KRAS* mutations were analyzed using the amplification refractory mutation system. Cycle sequencing of the purified PCR products was performed by commercially available ADx Mutation Detection Kits (Amory, Xiamen, China). Mutational analyses were carried out according to the manufacturer's protocol.

2.6. Statistics

Patient presenting symptoms and radiological findings are descriptively presented. Qualitative variables were summarized by count, overall prevalence, and percentage, whereas quantitative variables were by mean, standard deviation, and range. Fisher exact tests were performed to assess the relationship between mutation status and each of the factors, including age, gender, smoking status, stage, lymph node metastases, radiological characteristics, and immunohistochemical characteristics. A P value of $<.05$ was considered to indicate a significant difference.

All data were statistically analyzed using a software program (SPSS version 21.0; SPSS, Chicago, IL).

3. Results

3.1. Clinicopathological findings

Detailed clinicopathological information is presented in Table 1. A total of 28 patients were identified; the overall prevalence of PEAC in NSCLC (n = 5558) and primary pulmonary adenocarcinoma (n = 4091) was 0.5% and 0.68%, respectively. There was no significant difference between patients with PEAC and IAC in terms of smoking status, lymph node metastases, site of the main tumor, and location type (central and peripheral). In both PEACs and IACs, tumors were more frequently located in the upper lobe and the peripheral zone, and stage I was the most common pathological stage. The proportion of male population was 78.6% in PEACs and 50.0% in IACs, respectively, suggesting that there was a higher prevalence of male than of female PEAC patients ($P = .008$). Compared with IAC, patients with PEAC were older ($P = .041$) and had larger lesions ($P = .001$) with a more advanced stage ($P = .011$). PEAC patients had higher values of CEA ($P = .008$) and CA19-9 ($P < .001$) than IAC patients.

Table 2 summarizes the detailed clinicopathological characteristics and history of PEACs. Cough was the most common initial presenting symptom, which occurred in 12 (42.9%) patients. Seven patients presented with a history of cough for 2 weeks to 2 years; 3 patients presented with cough and hemoptysis for 1 week; 1 patient complained of cough, fever, and chest/back pain; 1 patient presented with cough and fever for 2 weeks; and 1 patient presented persistent chest/back pain for 2 months. Specifically, pulmonary lesions in 15 out of 28 (53.57%) patients were found during health examination, without any symptoms. All tumors were surgically resected and the patients underwent systematic mediastinal lymphadenectomy. One patient underwent unilateral pulmonary resection, 3 patients underwent segmentectomy, and 24 (85.71%) patients underwent lobectomy. Twenty-six (92.86%) patients showed a solitary mass. Two patients had multiple lesions, 1 stage T3 and 1 stage T4. Thirteen (46.43%) patients had lesions in the left lung and 15 (53.57%) patients in the right lung. All PEAC patients were followed-up to June 31, 2017. Five patients died, 19 survived, and 4 not available, with follow-up period of 1 to 30 months.

3.2. Radiology findings

The pulmonary abnormalities observed on the initial CT scans are summarized in Table 3. Masses were observed in 17 (60.71%) of 28 PEACs, whereas 34 (37.0%) out of 92 IACs ($P = .025$) had masses. There was no significant difference between patients with PEAC and IAC in terms of lobulation, spiculation, pleural indentation, pleural effusion, and lymphadenopathy. Radiologically, most (71.7%) of the main

Table 1

Clinicopathological characteristics of PEAC in comparison with IAC.

	PEAC		Invasive adenocarcinoma		
Clinical factors	**N**	**%**	**N**	**%**	***P***
	28	100.0	92	100.0	
Age, mean ± SD (y)	64.8 ± 8.6 (43–82)		60.9 ± 8.9 (40–82)		.041
Sex					.008
Female	6	21.4	46	50.0	
Male	22	78.6	46	50.0	
Smoking history			92		.688
Never	20	71.4	62	67.4	
Former/current	8	28.6	30	32.6	
Size, mean ± SD (cm)	3.43 ± 1.52		2.58 ± 1.01		.001
Pathological stage			92		.011
I	15	53.6	73	79.3	
II	4	14.3	11	12.0	
III	8	28.6	6	6.5	
IV	1	3.6	2	2.2	
Lymph node metastases					.177
N0	21	75.0	79	85.9	
N1/N2	7	25.0	13	14.1	
Site of the main tumor					.906
LUL	8	28.6	33	35.9	
LLL	5	17.9	15	16.3	
RUL	7	25.0	25	27.2	
RML	1	3.6	2	2.2	
RLL	7	25.0	17	18.5	
Location type			92		.072
Central	2	7.1	1	1.1	
Peripheral	26	92.9	91	98.9	
Markers					
CEA, ng/mL	10.40 ± 15.52		4.95 ± 5.55		.008
CA19-9, U/mL	21.34 ± 23.34		5.18 ± 5.12		< .001

CA19-9 = carbohydrate antigen 19-9, CEA = carcinoembryonic antigen, IAC = invasive adenocarcinoma, LLL = left lower lobe, LUL = left upper lobe, PEAC = pulmonary enteric adenocarcinoma, RLL = right lower lobe, RML = right middle lobe, RUL = right upper lobe, SD = standard deviation.

Table 2

Clinicopathological findings of patients diagnosed with primary pulmonary enteric adenocarcinoma.

Case no.	Age	Sex	Smoking	Presenting symptoms	Surgery	Location	Site	Size, cm	TNM	Stage	Driver mutation	Follow-up (Mo)
1	69	F	Never	Right chest/back pain × 2 mo	L	RUL	Peripheral	2.5	T3N2M0	IIIA	None	D (19)
2	45	F	Never	Cough × 3 mo	L	RLL	Central	5.5	T2BN2	IIIA	None	N/A
3	69	M	40-pack years	Right chest/back pain, fever, cough × 6 mo	L	RLL	Peripheral	5.5	T2BN0	IIA	None	D (20)
4	68	M	30-pack years	Cough with hemoptysis × 1 wk	L	RLL	Peripheral	7	T2BN0	IIA	None	A (30)
5	67	M	Never	No symptoms	P	RL	Peripheral	4.5	T4N0	IIIA	None	A (30)
6	70	M	Never	Cough × 2 wk	S	LLL	Peripheral	2.5	T1BN0	IA	EGFR L858R point mutation	A (29)
7	72	M	Never	No symptoms	L	LLL	Peripheral	3.5	T2AN2	IIIA	None	D (13)
8	67	M	Never	No symptoms	L	RLL	Peripheral	3.5	T2AN0	IB	KRAS	N/A
9	66	M	Never	No symptoms	L	LUL	Peripheral	2.2	T1BN0	IA	None	A (24)
10	56	M	Never	Cough × 1 mo	S	LLL	Peripheral	1.8	T1AN0M1a	IV	None	A (21)
11	82	M	Never	Cough × 1 mo	L	RUL	Peripheral	1.6	T1AN0	IA	None	A (21)
12	69	M	Never	No symptoms	L	RUL	Central	3.5	T2AN2	IIIA	KRAS	D (1)
13	43	F	Never	No symptoms	S	RLL	Peripheral	1	T1AN0	IA	None	A (19)
14	70	M	40-pack years	Cough with hemoptysis × 1 wk	L	LUL	Peripheral	2	T2AN0	IB	KRAS	A (13)
15	60	M	Never	No symptoms	L	RML	Central	3.5	T2AN0	IB	KRAS	A (13)
16	66	M	Never	No symptoms	L	LUL	Peripheral	2.2	T1BN0	IA	KRAS	A (15)
17	58	M	Never	Cough with hemoptysis × 1 wk	L	LUL	Peripheral	2	T1AN0	IA	None	A (28)
18	63	M	23-pack years	Cough × 2 y	L	RLL	Peripheral	4.2	T2AN2	IIIA	None	D (16)
19	65	M	Never	No symptoms	L	LUL	Peripheral	3	T1BN0	IA	EGFR exon 19 deletion	A (30)
20	71	M	Never	No symptoms	L	RLL	Peripheral	3	T1BN2	IIIA	None	N/A
21	63	F	Never	Cough × 2 mo	L	LLL	Peripheral	5	T2AN0	IB	KRAS	A (24)
22	55	M	30-pack years	No symptoms	L	LUL	Peripheral	6	T2BN0	IIA	None	A (25)
23	59	M	Never	Cough × 2 wk	L	RUL	Peripheral	2.2	T1BN0	IA	KRAS	N/A
24	80	F	Never	No symptoms	L	RUL	Peripheral	3.2	T2AN0	IB	KRAS	A (31)
25	68	M	40-pack years	No symptoms	L	RUL	Peripheral	2.2	T1BN0	IA	KRAS	A (23)
26	60	F	Never	Cough, fever × 2 wk	L	LUL	Peripheral	3.5	T2AN2	IIIA	EGFR exon 19 deletion	A (29)
27	72	M	50-pack years	No symptoms	L	LUL	Peripheral	6	T2BN0	IIA	KRAS	A (19)
28	62	M	Yes	No symptoms	L	LLL	Peripheral	3.5	T2AN0	IB	None	A (28)

A = alive, D = died, EGFR = epidermal growth factor receptor, KRAS = Kristen rat sarcoma viral oncogene homolog, L = left lung, L = lobectomy, LLL = left lower lobe, LUL = left upper lobe, Mo = month, N/A = not available, P = pneumonectomy, R = right lung, RLL = right lower lobe, RML = right middle lobe, RUL = right upper lobe, S = segmentectomy, TNM = tumor, node, and metastasis.

Table 3

CT findings of PEAC in comparison with IAC.

	PEAC		Invasive adenocarcinoma		
Characteristics	**n = 28**	**%**	**n = 92**	**%**	***P***
Type					.025
Nodule (<30 mm)	11	39.3	58	63.0	
Mass (≥30 mm)	17	60.7	34	37.0	
Characters					.006
Solid	28	100.0	66	71.7	
Part-solid	0	0.0	21	22.8	
GGO	0	0.0	5	5.4	
Marginal characteristics					
Lobulated border	25	89.3	78	84.8	.55
Spiculated margin	12	42.9	38	41.3	.252
Pleural characteristics					
Pleural indentation	15	53.6	60	65.2	.265
Pleural effusion	3	10.7	4	4.3	.208
Pleural attachment	3	10.7	11	12.0	.858
Lymphadenopathy	9	32.1	16	17.4	.092

CT = computed tomography, GGO = ground-glass opacity, IAC = invasive adenocarcinoma, PEAC = pulmonary enteric adenocarcinoma.

tumors appeared as solid nodules on images in the IAC group, whereas all 28 (100%) patients with PEAC presented solid nodules and had no ground-glass opacity (GGO) ($P = .006$).

3.3. Immunohistochemistry results in PEAC

Detailed immunohistochemical characteristics are shown in Table 4 and Fig. 1. TTF-1 expression was detected in all 28 patients, and 10 cases (35.71%) were positive. Napsin A and CK7 expressions were detected in 27 and 26 patients, respectively; 18 (66.67%) and 6 (23.08%) patients were positive. All 10 cases positive for TTF-1 and/or napsin A were also positive for CK7. Both TTF-1 and napsin A were negative in 16 patients, of which 7 patients were positive for CK7. All cases negative for CK7 were also negative for TTF-1 and napsin A. It can be observed that CK7 is a more valuable lung cancer molecular marker for the differential diagnosis of PEAC. Nine patients were tested for the lung adenocarcinoma markers. The expressions of CK7, TTF-1, and napsin A were found to be negative, whereas the expressions of the enteric carcinoma immune markers villin and CK20 and/or CDX-2 were positive. These results indicated that the expression of lung adenocarcinoma immune markers in PEAC patients can be negative.

Table 4

Immunohistochemistry results of primary pulmonary enteric adenocarcinoma.

Case no.	CK7	TTF-1	Napsin A	CK20	CDX-2	Villin
1	+	+	+	−	−	−
2	+	−	−	−	+	−
3	+	−	−	−	+	p+
4	−	−	−	−	+	+
5	+	+	−	−	−	p+
6	+	+	p+	−	−	+
7	+	p+	+	−	+	+
8	+	+	+	−	−	−
9	+	+	−	N/A	+	+
10	+	+	−	−	−	+
11	−	−	−	+	−	+
12	−	−	−	+	+	+
13	+	−	−	−	−	+
14	−	−	−	+	+	+
15	−	−	−	+	+	+
16	−	−	−	−	+	+
17	+	−	−	−	+	+
18	+	−	−	p+	p+	+
19	+	+	+	−	−	p+
20	−	−	−	+	+	+
21	+	−	N/A	−	−	+
22	N/A	−	−	N/A	−	p+
23	−	−	−	+	p+	+
24	+	−	−	+	+	+
25	−	−	−	p+	+	+
26	+	+	+	−	−	+
27	+	+	N/A	−	−	+
28	+	−	−	−	+	+

CDX-2 = caudal type homeobox transcription factor 2, CK = cytokeratin, napsin A = novel aspartic proteinase of the pepsin family A, N/A = not available, p = partially, TTF-1 = thyroid transcription factor-1.

A total of 9 out of 26 (34.62%) patients were positive for CK20. All 28 patients were tested for CDX-2 and villin expressions, and 16 (57.14%) and 25 (89.28%) were positive, respectively. There were 2 patients who had negative CK20, CDX-2, and villin expressions, but their CK7, TTF-1, and napsin A expressions were all positive. Eight patients were positive for both CK20 and CDX-2, which were positive for villin. Both CK20 and CDX-2 expressions were negative in 10 out of 27 (37.04%) patients, of which 8 were positive for the marker villin. A total of 17 out of 27 (62.96%) patients who were tested for the immunohistological markers of enteric differentiation CK20 and CDX-2 were positive for the expression of one or both proteins, of which 16 patients (94.12%) were positive for villin. It is suggested that villin expression detection can aid in the differential diagnosis of PEAC.

3.4. Mutational status differences between IAC and PEAC patients

The mutation analysis results are illustrated in Fig. 2. *EGFR* mutations were examined in 28 patients with PEAC and in 92 patients with IAC, respectively. On the other hand, *KRAS* mutations were examined in 25 patients with PEAC and in 92 patients with IAC, respectively. The *EGFR* mutation rate was 3 out of 28 (10.7%) in PEACs; exon 19 deletion and exon 21 L858R point mutation were detected in 2 and 1 cases, respectively. The frequency of *EGFR* mutations was higher in the IAC group (63.0%, $P < .001$), whereas the frequency of *KRAS* mutations was higher in the PEAC group (40%) than in the IAC group (3.3%, $P < .001$). In PEAC patients, *KRAS* mutations have a higher incidence than *EGFR* mutations, whereas the opposite can be observed in the IAC group.

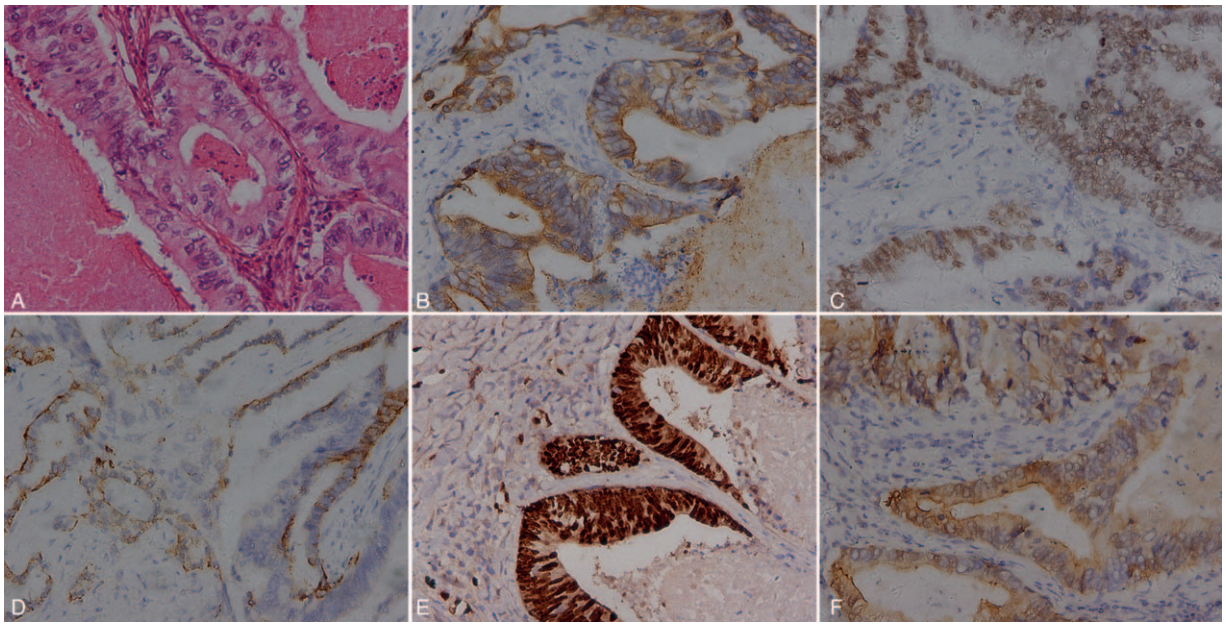

Figure 1. Immunohistochemical staining of pulmonary enteric adenocarcinoma is here shown. The neoplastic cells are cuboidal to tall columnar in hematoxylin and eosin (A). The immunohistochemical staining revealed simultaneously positivity for CK7 (B), TTF-1 (C), napsin A (D), CDX-2 (E), and Villin (F). Original magnification ×400 (A–F). CDX-2 = caudal type homeobox transcription factor 2, CK = cytokeratin, napsin A = novel aspartic proteinase of the pepsin family A, TTF-1 = thyroid transcription factor-1.

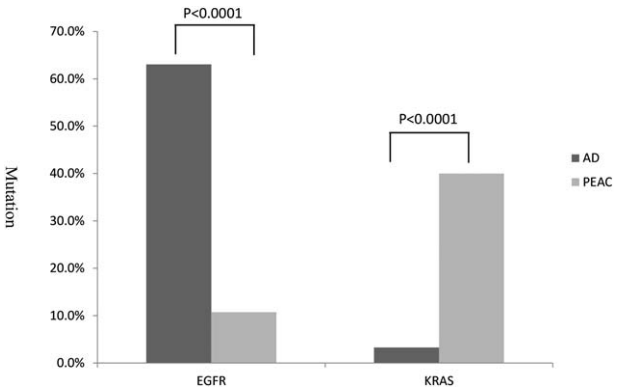

Figure 2. Percentages for the mutational statuses in IAC and PEAC patients. There were a higher percentage of EGFR mutants versus wild-type tumors in invasive adenocarcinoma. The KRAS mutations in PEAC patients are 12 times that of IAC. EGFR = epidermal growth factor receptor, IAC = invasive adenocarcinoma, KRAS = Kristen rat sarcoma viral oncogene homolog, PEAC = pulmonary enteric adenocarcinoma.

3.5. Gene mutation and immunohistochemical characteristics in PEAC

In the immunohistochemistry aspect, the *KRAS* mutation frequencies were as follows: 75% (6/8) for patients CK7-negative, 25% (4/16) for CK7-positive, 30% (3/8) for CK20- and CDX-2-negative, and 50% (7/14) for CK20- and/or CDX-2-positive. It is suggested that *KRAS* mutation was more frequently found in PEACs CK7-negative ($P = .032$). *KRAS* mutation was more frequently found in patients with CK20- or CDX-2-positive expression ($P = .421$); however, no significant differences were established during statistical analysis. The *EGFR* mutation frequencies were 0% (0/9) for patients with CK7-negative, 16.67% (3/18) for CK7-positive, 30% (3/10) for CK20- and CDX-2-negative, and 0% (0/17) for CK20- and/or CDX-2-positive expressions. It is suggested that *EGFR* mutation rates were higher in patients with CK20- and CDX-2-negative expressions ($P = .041$). *EGFR* mutation was more frequently found in patients with CK7-positive expression ($P = .529$); however, no significant differences were found during statistical analysis (Table 5).

4. Discussion

PEAC has emerged as a subtype of lung adenocarcinoma with distinct pathological features for more than 20 years. So far, we still know little about the clinical, pathological, and molecular features of PEAC because the limited cases were published. The present study aims to compare the clinicopathological, radiological, and molecular characteristics of PEAC and IAC; in addition, we offered comprehensive data of PEAC patients.

PEAC is an exceptionally rare subtype of invasive lung adenocarcinoma based on our data. The mean age of diagnosis for IACs is 60.9 years, as compared with 64.8 years for PEACs in this study. Compared with IAC patients, patients with PEAC had larger lesions and a more advanced stage. We presumed that PEAC was intrinsically more aggressive and had a relatively more delayed onset of clinical symptoms than IAC. There were more females with IAC, but more males in this cohort of PEAC patients, which may be comparable to the characteristics of colorectal carcinoma in which there are more males affected.[5] PEAC had higher values of CEA ($P = .008$) and CA19-9 ($P < .001$) than IAC. This result is consistent with PEACs with enteric differentiation component, and also consistent with the performance of patients with colon cancer.[27] Among patients with colon cancer, CEA and CA19-9 levels can predict the prognosis, metastasis, and the efficacy of ramucirumab-targeted therapy.[28,29] We hypothesized that they may also be valuable in assessing the prognosis and treatment response of PEAC, but further studies are needed to confirm this.

In addition, patients with PEAC had no special symptoms and only had general symptoms of common respiratory diseases, such as cough, bloody sputum, fever, and chest pain, which can easily be ignored. The majority (53.57%) of patients with PEAC who had no obvious symptoms were diagnosed incidentally during chest imaging in our study. These presentations may be due to the fact that tumors of both IAC and PEAC are mostly located in the peripheral zone, which may be because the adenocarcinoma originated in the smaller bronchial branch and had a relatively indolent growth pattern. In the cohort of PEAC patients, the follow-up period was 1 to 30 months, larger studies with longer follow-ups are necessary to accurately determine the course of PEACs.

At CT, patients with PEAC had larger lesions than patients with IAC, which is consistent with pathological results in our study. There was no significant difference between patients with PEAC and IAC in terms of lobulation, spiculation, pleural indentation, and pleural effusion. These radiological image traits are useful in predicting malignancy in lung nodules in the literature.[30,31] Besides, all 28 patients with PEAC did not present any GGO, whereas GGO accounted for 28.2% in the cohort of IAC patients. It was observed that tumor lesions of PEAC are more likely to have a solid growth pattern. Again, these CT presentations indicated that PEAC has a more aggressive nature than IAC.

Table 5

Gene mutation and immunohistochemical characters in PEAC.

Characteristics	PEAC					PEAC				
	KRAS mutation		Wild type			EGFR mutation		Wild type		
	N	%	N	%	*P*	N	%	N	%	*P*
	10		14			3		24		
CK7 expression					.032					.529
Negative	6	75	2	25		0	0	9	100	
Positive	4	25	12	75		3	16.7	15	83	
CK20 and CDX-2 expression					.421					.041
Negative	3	30	7	70		3	30	7	70	
Positive	7	50	7	50		0	0	17	100	

CDX-2 = caudal type homeobox transcription factor 2, CK = cytokeratin, EGFR = epidermal growth factor receptor, KRAS = Kristen rat sarcoma viral oncogene homolog, PEAC = pulmonary enteric adenocarcinoma.

The cytomorphological and immunohistochemical characteristics of PEAC have been described many times from gross morphology to microstructure in the literature,[6,9,10,12,24] and PEACs are morphologically very heterogeneous.[10,26] The results of immunohistochemistry analyses in our series are similar to those of previous studies in PEAC patients.[10,11,18] The order of positive results for lung adenocarcinoma markers from the highest to the lowest rate was CK7, TTF-1, and napsin A. Even Nottegar et al[26] reported that all PEACs exhibited CK7 expression. In addition, in our study, patients negative for CK7 were also both TTF-1- and napsin A-negative, whereas some patients who are TTF-1- and napsin A-negative were CK7-positive. Therefore, we presumed that CK7 is a more valuable and reliable lung adenocarcinoma marker in the differential diagnosis of PEAC than metastatic colorectal adenocarcinoma, which is in accord with previous reports.[10,11,16,24,26] However, the existence of false-negative results should be noted.[14,15,19–21,24] The order of positive results for colorectal cancer markers from the highest to the lowest rate was villin, CDX-2, and CK20 in our series, which is consistent with the results of Lin et al[24] and Inamura et al.[10] By contrast, Nottegar et al[26] reported that all PEAC cases present a certain positivity for CDX-2 in the area with intestinal morphology and 76.1% (35/46) of PEAC cases present positivity for villin. Inamura et al[10] reported that CK20 may be a useful marker for the distinction of PEACs from MCRs in spite of CDX-2 with higher positive rates, and Wang et al[18] found that PEACs showed positive staining for CK20, CDX-2, MUC2, and villin in 22.2%, 66.7%, 44.4%, and 66.7% of 9 cases, respectively. In our study, 89.28% (25/28) of PEAC cases presented positivity for villin, and 80% of cases with both CK20- and CDX-2-negative expressions were villin-positive, which has a much higher positive rate. Lin et al[24] also concluded that 36.36% of patients with both CDX-2- and CK20-negative expressions were MUC2-positive. There are few cases of PEAC immunoreactive for villin described in the literature.[16–19,24,26] Villin is a very sensitive and relatively specific marker of gastrointestinal adenocarcinomas. The expression of villin is largely restricted to the brush border of the epithelium in the gastrointestinal and urogenital tracts and is localized in the microvilli of the brush border.[32] However, Nambu et al[33] reported that villin was not detected in nontumorous lung tissues of pulmonary adenocarcinomas, but was expressed in 31.6% of the pulmonary adenocarcinomas. Tan et al[34] reported that villin was expressed in 67% of the pulmonary adenocarcinomas with rootlets. Tsao and Fraser[6] and Weidner[9] reported that tumor cells of PEAC had numerous microvilli with well-developed microfilamentous cores and apical rootlets that showed long extensions into the apical cytoplasm in ultrastructural appearance, as revealed by electron microscopy. These findings help us understand why villin has a higher positive expression in PEAC cases. Villin proved to be a very sensitive and helpful enteric marker in the differential diagnosis between PEAC and other lung adenocarcinomas.

The 2 gene mutations, namely, EGFR and KRAS, have been widely studied in Asian NSCLC.[35–37] In our study, PEAC patients had a higher incidence (40%) of KRAS mutations than IAC patients but a lower incidence (10.71%) of EGFR mutations. This is consistent with the results of Nottegar et al[26] and the results reported in our review (18 cases detected for oncogenic drivers, reviewed in Table 6).[17–24] Nottegar et al[26] found that

Table 6

Review of all literature for PEAC detected for gene mutation (18 cases).

First author (Ref.)	Age	Sex	Smoking	Site	Size, cm	EGFR	KRAS	BRAF	EML4-ALK	CK7	TTF-1	Napsin A	CK20	CDX-2	Villin	MUC2
Lin et al[24]	53	F	Never	Multiple bilateral	5	W	W	W	W	–	–	N/A	+	+	+	N/A
Handa et al[22]	70	M	Yes	RML	2.8	L858R point mutation	N/A	N/A	N/A	+	+	N/A	–	–	N/A	N/A
Garajová et al[21]	68	M	Yes	RLL	3.5	W	Exon 12 mutation	N/A	W	+	–	–	–	+	N/A	–
	71	F	Yes	RLL	3.5	W	Exon 12 mutation	N/A	W	–	–	N/A	+	+	N/A	N/A
Metro et al[23]	74	M	Yes	N/A	N/A	W	Q22K	N/A	N/A	+	–	N/A	–	+	N/A	N/A
	65	M	N/A	LUL	1	W	Exon 12 mutation	N/A	N/A	–	–	N/A	+	+	N/A	N/A
Wang et al[18]	65	M	Never	RLL	2.3	W	W	N/A	N/A	+	p+	+	–	+	–	–
	56	F	Never	RUL	3	W	W	N/A	N/A	+	+	–	–	+	–	+
	60	M	Yes	RUL	3.5	W	W	N/A	N/A	+	+	–	–	+	+	–
	63	F	Yes	RUL	2.7	W	W	N/A	N/A	+	–	+	–	–	+	p+
	65	F	Yes	RUL	2	W	W	N/A	N/A	+	+	–	–	–	+	–
	74	M	Yes	LLL	1.5	W	W	N/A	N/A	+	–	+	–	–	–	+
	61	M	Yes	RUL	6	W	W	N/A	N/A	+	–	–	p+	+	+	–
	34	F	Never	RUL	4.8	W	W	N/A	N/A	+	–	–	–	+	+	p+
	63	F	Yes	RUL	3.3	W	W	N/A	N/A	+	–	–	+	+	p+	–
Stojsic et al[19]	26	F	N/A	LLL	8	W	W	N/A	N/A	–	–	–	+	+	+	–
	24	M	N/A	LLL	6	W	Exon 12 mutation	N/A	N/A	–	–	–	+	+	+	–
Qureshi et al[17]	61	F	N/A	Multiple bilateral	1.7	W	N/A	N/A	N/A	+	–	N/A	+	+	–	N/A

BRAF = B type rapidly accelerated fibrosarcoma kinase, CDX-2 = caudal type homeobox transcription factor 2, CK = cytokeratin, EGFR = epidermal growth factor receptor, EML4-ALK = echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase, KRAS = Kristen rat sarcoma viral oncogene homolog, LLL = left lower lobe, LUL = left upper lobe, MUC2 = mucin 2, N/A = not available, napsin A = novel aspartic proteinase of the pepsin family A, p = partially, PEAC = pulmonary enteric adenocarcinoma, RLL = right lower lobe, RML = right middle lobe, RUL = right upper lobe, Ref. = reference, TTF-1 = thyroid transcription factor-1, W = wild type.

PEACs showed a high frequency of *KRAS* mutations (60.9%) but a low incidence of *EGFR* gene mutations (2.2%), respectively. Table 6 showed a frequency of *KRAS* mutations (31.25%) and incidence of the *EGFR* gene mutations (5.56%), respectively. Although the significant association noted between *KRAS* mutations and lung adenocarcinoma histological subtypes is controversial, at present, most studies have shown that *KRAS* mutations were more commonly associated with the solid predominant subtype and invasive mucinous adenocarcinoma.[36] We speculated that PEAC may be a heterogeneous NSCLC subgroup with distinctive molecular features.

In PEACs previously reported,[17–24] the frequency of *KRAS* mutations is presented in Table 6: 0% (0/3) for CK20- and CDX-2-negative and 35.71% (4/14) for CK20- and/or CDX-2-positive expressions, respectively, as compared with 30% and 50% in our series. We surmised that PEAC patients are more likely to harbor *KRAS* mutations, especially those with CK20- and CDX-2-positive expressions. We also observed that *KRAS* mutation was more frequently found in patients with CK7-negative expression. Table 6 presents the frequency of *KRAS* mutations in PEACs: 15.38% for CK7-negative and 60% for CK7-positive expressions, respectively. These results suggest that mutations in *KRAS* are also more likely to occur in the group of PEACs with pneumocyte marker loss. *KRAS* is one of the most frequently mutated oncogenes in lung adenocarcinomas. However, the prognostic and predictive roles of *KRAS* status in lung cancer remain controversial. Until recently, there have been a few studies on the efficacy of combinatorial therapy in *KRAS*-mutant lung cancers.[38,39] Therefore, we presume that these results may provide some information in the studies of targeted therapy in *KRAS*-mutated PEAC. Table 6 also presents the frequency of *EGFR* mutations in PEACs: 0% (0/5) for patients with CK7-negative, 7.69% (1/13) for CK7-positive, 25% (1/4) for CK20- and CDX-2-negative, and 0% (0/14) for CK20- and/or CDX-2-positive expressions. These results are similar to those of our study. We speculate that mutations in *EGFR* are more likely to occur in the group of PEACs enteric marker loss. Targeted therapy in *EGFR*-mutated lung adenocarcinoma has been proven to be effective.[40] Thus, we could consider that targeted therapy may also be effective in *EGFR*-mutated PEAC. From the above results in our series, we hope to have a more comprehensive understanding of the molecular features of PEAC and expect these data will be properly utilized in clinical practice in the future. These data indicate that immunohistochemistry results of PEAC may predict genetic abnormalities and provide information for the selection of a targeted therapy, especially when a genetic test is not feasible. There are some limitations to this study: small sample size and single-center study. Therefore, our results need to be confirmed by more rigorous and comprehensive studies in the future.

In conclusion, PEAC is an exceptionally rare subtype of invasive lung adenocarcinoma. Compared with IAC, PEAC may be more likely to occur in males, in older patients, and in those with larger lesions. PEAC had higher values of CEA and CA19-9 than IAC. Villin may be a very sensitive and reliable marker in the differential diagnosis of PEAC. In this study, PEAC showed a high frequency of *KRAS* mutations but a low incidence of the *EGFR* mutations, but mutation status vary with the different expression of immunohistochemical markers that indicate the usefulness in predicting genetic abnormalities. Thus, patients with PEAC may benefit from targeted therapy as well as conventional chemotherapy and surgery.

Acknowledgments

The authors thank Chun Yan Wu and Likun Hou of Department of Pathology, Shanghai Pulmonary Hospital, for expert assistance with histology diagnosis.

References

[1] Chen W, Zheng R, Zeng H, et al. The incidence and mortality of major cancers in China, 2012. Chin J Cancer 2016;35:73.
[2] Fajersztajn L, Veras M, Barrozo LV, et al. Air pollution: a potentially modifiable risk factor for lung cancer. Nat Rev Cancer 2013;13:674–8.
[3] Chen ZM, Peto R, Iona A, et al. Emerging tobacco-related cancer risks in China: a nationwide, prospective study of 0.5 million adults. Cancer 2015;121(suppl 17):3097–106.
[4] Kim C, Gao YT, Xiang YB, et al. Home kitchen ventilation, cooking fuels, and lung cancer risk in a prospective cohort of never smoking women in Shanghai, China. Int J Cancer 2015;136:632–8.
[5] Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975–2013. Bethesda, MD: National Cancer Institute; 2016. Available from: http://seer.cancer.gov/csr/1975_2013/.
[6] Tsao MS, Fraser RS. Primary pulmonary adenocarcinoma with enteric differentiation. Cancer 1991;68:1754–7.
[7] Travis WD, Brambilla E, Noguchi M, et al. International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma. J Thorac Oncol 2011;6:244–85.
[8] Travis WD, Brambilla E, Burke AP, et al. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. 4th edIARC Press, Lyon:2015.
[9] Weidner N. Pulmonary adenocarcinoma with intestinal-type differentiation. Ultrastruct Pathol 1992;16:7–10.
[10] Inamura K, Satoh Y, Okumura S, et al. Pulmonary adenocarcinomas with enteric differentiation: histologic and immunohistochemical characteristics compared with metastatic colorectal cancers and usual pulmonary adenocarcinomas. Am J Surg Pathol 2005;29:660–5.
[11] Yousem SA. Pulmonary intestinal-type adenocarcinoma does not show enteric differentiation by immunohistochemical study. Mod Pathol 2005;18:816–21.
[12] Satoh Y, Hoshi R, Tsuzuku M, et al. Cytology of pulmonary cytology of pulmonary adenocarcinomas showing enteric differentiation. Acta Cytol 2006;50:250–6.
[13] Maeda R, Isowa N, Onuma H, et al. Pulmonary intestinal-type adenocarcinoma. Interact Cardiovasc Thorac Surg 2008;7:349–51.
[14] Li HC, Schmidt L, Greenson JK, et al. Primary pulmonary adenocarcinoma with intestinal differentiation mimicking metastatic colorectal carcinoma: case report and review of literature. Am J Clin Pathol 2009;131:129–33.
[15] Hatanaka K, Tsuta K, Watanabe K, et al. Primary pulmonary adenocarcinoma with enteric differentiation resembling metastatic colorectal carcinoma: a report of the second case negative for cytokeratin 7. Pathol Res Pract 2011;207:188–91.
[16] Lin D, Zhao Y, Li H, et al. Pulmonary enteric adenocarcinoma with villin brush border immunoreactivity: a case report and literature review. J Thorac Dis 2013;5:E17–20.
[17] Qureshi A, Furrukh M. Enteric adenocarcinoma lung: a rare presentation in an Omani woman. BMJ Case Rep 2013;2013:bcr2012007667.
[18] Wang CX, Liu B, Wang YF, et al. Pulmonary enteric adenocarcinoma: a study of the clinicopathologic and molecular status of nine cases. Int J Clin Exp Pathol 2014;7:1266–74.
[19] Stojsic J, Kontic M, Subotic D, et al. Intestinal type of lung adenocarcinoma in younger adults. Case Rep Pulmonol 2014;2014:282196.
[20] László T, Lacza A, Tóth D, et al. Pulmonary enteric adenocarcinoma indistinguishable morphologically and immunohistologically from metastatic colorectal carcinoma. Histopathology 2014;65:283–7.
[21] Garajová I, Funel N, Fiorentino M, et al. MicroRNA profiling of primary pulmonary enteric adenocarcinoma in members from the same family reveals some similarities to pancreatic adenocarcinoma—a step towards personalized therapy. Clin Epigenetics 2015;7:129.
[22] Handa Y, Kai Y, Ikeda T, et al. Pulmonary enteric adenocarcinoma. Gen Thorac Cardiovasc Surg 2016;64:749–51.
[23] Metro G, Valtorta E, Siggillino A, et al. Enteric-type adenocarcinoma of enteric enteric the lung harbouring a novel KRAS Q22K mutation with concomitant KRAS polysomy: a case report. Ecancermedicalscience 2015;9:559.

[24] Lin LI, Xu CW, Zhang BO, et al. Clinicopathological observation of primary lung enteric adenocarcinoma and its response to chemotherapy: a case report and review of the literature. Exp Ther Med 2016;11:201–7.
[25] El Hammoumi MM, El Ochi R, Kabiriel H. Primary lung adenocarcinoma with enteric morphology associated with primary colon adenocarcinoma. Arch Bronconeumol 2016;52:221.
[26] Nottegar A, Tabbò F, Luchini C, et al. Pulmonary adenocarcinoma with enteric differentiation: immunohistochemistry and molecular morphology. Appl Immunohistochem Mol Morphol 2016;DOI: 10.1097/PAI.0000000000000440. [Epub ahead of print].
[27] Dolscheid-Pommerich RC, Manekeller S, Walgenbach-Brünagel G, et al. Clinical performance of CEA, CA19-9, CA15-3, CA125 and AFP in gastrointestinal cancer using LOCI^{TM}-based assays. Anticancer Res 2017;37:353–9.
[28] Stojkovic Lalosevic M, Stankovic S, Stojkovic M, et al. Can preoperative CEA and CA19-9 serum concentrations suggest metastatic disease in colorectal cancer patients? Hell J Nucl Med 2017;20:41–5.
[29] Yoshino T, Obermannová R, Bodoky G, et al. Baseline carcinoembryonic antigen as a predictive factor of ramucirumab efficacy in RAISE, a second-line metastatic colorectal carcinoma phase III trial. Eur J Cancer 2017;78:61–9.
[30] Liu Y, Balagurunathan Y, Atwater T, et al. Radiological image traits predictive of cancer status in pulmonary nodules. Clin Cancer Res 2017;23:1442–9.
[31] McWilliams A, Tammemagi MC, Mayo JR. Probability of cancer in pulmonary nodules detected on first screening CT. N Engl J Med 2013;369:910–9.
[32] Bacchi CE, Gown AM. Distribution and pattern of expression of villin, a gastrointestinal-associated cytoskeletal protein, in human carcinomas: a study employing paraffin-embedded tissue. Lab Invest 1991;64:418–24.
[33] Nambu Y, Iannettoni MD, Orringer MB, et al. Unique expression patterns and alterations in the intestinal protein villin in primary and metastaticpulmonary adenocarcinomas. Mol Carcinog 1998;23:234–42.
[34] Tan J, Sidhu G, Greco MA, et al. Villin, cytokeratin 7, and cytokeratin 20 expression in pulmonary adenocarcinoma with ultrastructuralevidence of microvilli with rootlets. Hum Pathol 1998;29:390–6.
[35] Dong YJ, Cai YR, Zhou LJ, et al. 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;11:2552–8.
[36] Yoshizawa A, Sumiyoshi S, Sonobe M, et al. Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. J Thorac Oncol 2013;8:52–61.
[37] Lee B, Lee T, Lee SH, et al. Clinicopathologic characteristics of EGFR, KRAS, and ALK alterations in 6,595 lung cancers. Oncotarget 2016;7:23874–84.
[38] Ambrogio C, Gómez-López G, Falcone M, et al. Combined inhibition of DDR1 and Notch signaling is a therapeutic strategy for KRAS-driven lung adenocarcinoma. Nat Med 2016;22:270–7.
[39] Manchado E, Weissmueller S, Morris JP IV, et al. A combinatorial strategy for treating KRAS-mutant lung cancer. Nature 2016;534:647–51.
[40] de Lima Lopes GJr, Segel JE, Tan DS, et al. Cost-effectiveness of epidermal growth factor receptor mutation testing and first-line treatment with gefitinib for patients with advanced adenocarcinoma of the lung. Cancer 2012;118:1032–9.