Survival analyses and immunohistochemical study of primary signet ring cell carcinoma of the lung adenocarcinoma

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Background: The clinicopathological features, immunohistochemical (IHC) characteristics and survival outcomes of primary lung adenocarcinoma with signet ring cells (LAdSRCs) component were analyzed.

Methods: A retrospective analysis of primary LAdSRCs consecutively collected from 2010 to 2014 was performed and compared with lung adenocarcinoma (ADC). We investigated the survival outcomes and the expression of cytokeratin 7 (CK7), CK20, thyroid transcription factor-1 (TTF-1) and villin in these 35 cases of primary LAdSRCs.

Results: We identified 1,715 patients in total, 35 (2.0%) of whom had signet ring cell (SRC) component. Excepting for 2 cases without IHC information, a CK profile (CK7+/CK20–) were identified in 69.7% (23/33) of LAdSRC; 22 (66.7%) of 33 LAdSRCs exhibited positive TTF-1 expression, but no other TTF-1 positive was found in signet ring cell carcinoma (SRCC) of other organs, and villin was not identified in these 33 cases. Age, sex, tumor size, surgery resection, smoking history, tumor stage, nodal stage, predominant histology subtype, pathology and adjuvant therapy were all significant predictors of relapse-free survival (RFS) and overall survival (OS) in univariable analysis. Compared to lung ADC, the lung ADCs with SRC component had an inferior prognosis. In multivariable analysis, sex, tumor size, tumor stage, nodal stage, predominant histology subtype and adjuvant therapy were still significant predictors for both RFS and OS while the pathology [RFS: hazard ratio (HR), 0.918; 95% confidence interval (CI), 0.581–1.492; P=0.712; OS: HR, 1.392; 95% CI, 0.820–2.364; P=0.221, respectively] was not.

Conclusions: Univariable analysis revealed primary LAdSRC is a rare ADC subtype with a worse prognosis compared with ADC. The high percentage expression of TTF-1 and immunostaining profiles CK7+/CK20- in primary LAdSRC is significant to identify the source of SRCCs.

Keywords: Signet-ring carcinoma of the lung; prognosis; immunohistochemistry

Introduction

Lung cancer is the third most common malignancy in the world, leading to significant mortality and morbidity (1). Adenocarcinomas (ADCs) account for the majority of their pathological types and the incidence of them is now still rising (2,3). Primary lung ADC with signet ring cell (SRC) is a rare subtype of lung ADC, which was originally described by Kish *et al.* in 1989 (4) and lung ADC with SRC was considered to be a unique subtype of lung cancer in the 2004 WHO lung ADC classification, which was designated as SRC ADC (5). However, in the 2015 WHO lung cancer classification, lung ADC with signet ring cells (LAdSRCs) are viewed as a cytologic change that may occur in association with various histological patterns rather than as a separate subtype, as they do not have a prognostic significance (2,6). The poor prognosis that had been associated with LAdSRCs can be attributed to its strong correlation with solid subtype (7-9).

Signet ring cell carcinoma (SRCC) can occur in a variety of organs, but the incidence of SRCCs as a primary lung tumor was lower, ranging from 0.14% to 1.9% in the reported series (2,10). Primary LAdSRCs and metastatic LAdSRCs have different management and treatment, and have different prognostic features. How to distinguish them is especially important. Several recent immunohistochemical (IHC) studies have shown that thyroid transcription factor-1 (TTF-1) was a tissue-specific transcription factor expressed almost exclusively in thyroid tissue and different tumors of the lung (11,12). Simultaneously, immunostaining profiles of cytokeratin 7 (CK7) and CK20 have recently been used to identify SRC from different organ original such as breast, stomach and colon (13,14). Therefore, CK7 and CK20 are important for the identification of primary LAdSRCs and metastatic LAdSRCs.

To date, only a few series of cases of primary SRC in the lung have been reported in the literature (7,15-17). Compared with lung ADC, the clinicopathological features and survival outcome of primary lung SRC were still undetermined. We investigated lung ADC with SRC components and compared the clinicopathological and IHC features of these 35 LAdSRCs and 1,680 lung ADCs.

Methods

The institutional review board of Shanghai Chest Hospital supported this study [KS(Y)1668]. Patients with lung ADC underwent surgical resection between 2010 and 2014 were all identified. Patients with history of malignancies within 5 years (including metastatic tumors in lung), and distant metastases were ineligible for the analysis; atypical adenomatous hyperplasia (AAH), adenocarcinoma *in situ* (AIS) and minimally invasive adenocarcinoma (MIA) were non-invasive lesions and free from lymph node (LN) metastasis. Thus, patients with these lesions were also excluded. All the patients received preoperative assessments including chest CT scanning, abdominal CT or ultrasound, brain CT scanning or MRI and radionuclide bone scanning, to confirm the diagnosis of primary lung tumor. Positron emission tomography (PET)-CT was an optimal substitution if necessary. The postoperative pathological staging of all patients was based on the 8th edition of tumor-node-metastasis (TNM) staging system.

The resected lung specimens and LNs were fixed in 10% formalin and then embedded in paraffin. Subsequently, the hematoxylin and eosin (HE)-stained sections were evaluated microscopically by experienced pulmonary pathologists in our hospital. Diagnosis of SRC was determined by histological evaluation. SRC was a mucin-secreting ADC that was rich in cytoplasmic mucin and pushes the nucleus to the side of the cell. IHC studies were performed on formalin-fixed, paraffin-embedded tissue sections using the avidin-biotin-peroxidase complex method in a Dako Autostainer (Dako, Carpinteria, CA, USA). The pathological results and immunohistochemistry results of the specimens were provided by the Pathology Department of Shanghai Chest Hospital.

After the exclusion, a total of 1,823 patients were all primary lung ADC, including 35 LAdSRCs and 1,788 ADCs. Of the 1,823 lung ADCs, 35 patients were excluded for receiving neoadjuvant chemotherapy and 73 patients were excluded because they were lost to follow-up. The remaining 1,715 patients including 35 LAdSRCs and 1,680 ADCs were included in the study. All the patients were followed up through out-patient clinic or telephone every 3 months for the first year after surgery, every 6 months for the next 3 years and then annually.

Statistical analysis

All the clinicopathologic data and distributions of survival were analyzed by SPSS 23.0 software package (SPSS Inc., Chicago, IL, USA) and Prism 5 (Graphpad Software Inc., La Jolla, CA, USA). The curves of relapse-free survival (RFS) and overall survival (OS), as well as the comparisons, were calculated by Kaplan-Meier survival curves and

the log-rank test. Two-sided P<0.05 was set as statistical significance in this study.

Results

Clinicopathologic features

The characteristics are all listed in *Table 1*. Of those 1,715 patients included in the study, there were 718 males (41.9%) and 997 females (58.1%), with an average age of 59.5 (range from 22 to 84) years. We found that several clinicopathologic characteristics significantly differed between LAdSRCs and ADCs. The LAdSRCs show more male (P=0.011), more advanced disease stage (P<0.001), larger tumor size (P=0.032) and higher nodal stage (P<0.001).

The clinical details of 35 LAdSRCs are listed in *Table 2*. As for the tumor stage, 18 patients had pathologic stage (p-stage) III tumors, 13 patients had p-stage I tumors and 4 patients had p-stage IV tumors. In terms of predominant pathological subtypes, LAdSRCs was more common in solid subtype when compared with ADCs (37.1% *vs.* 9.3%, P<0.001) and 45.7% (16/35) of them contained solid components. There were no significant differences in the age and smoking history.

Survival analysis

The median follow-up time and the median survival time of these patients were 62.4 and 40.8 months, respectively. Univariable analysis (*Table 3*) revealed that age, sex, tumor stage, tumor size, nodal stage, surgery resection, predominant histology subtype, adjuvant therapy, smoking history and SRC component were all significant predictors of RFS and OS while the sex, tumor stage, tumor size, nodal stage, predominant histology subtype and adjuvant therapy were still significant predictors in multivariable analysis (*Table 4*).

During the follow-up, 22 (62.9%) LAdSRCs and 435 (25.9%) lung ADCs experienced a relapse, and finally 17 (48.6%) and 235 (14.0%) patients died, respectively. There were significant differences in RFS and OS for lung ADCs with SRC component compared with those lung ADCs (*Figures 1,2*).

Immunohistochemistry study

The *Table 2* summarize the results of immunostaining.

Except for two cases (No. 31 and No. 34) of missing IHC information, 22 of the 33 LAdSRCs (66.7%) were TTF-1 positive; 23 of 33 LAdSRCs (69.7%) expressed a CK profile of CK7+ or CK20–, and 2 (6%) were positive for both CK7 and CK20. However, villin was not identified in these 33 cases.

Discussion

Since Kish *et al.* (4) originally reported 5 cases of 5,500 lung ADC patients with LAdSRC (0.14%), there were sporadic reports on it (4,5,10,16,18-21). Although the 2015 WHO lung tumor classification discontinued the separate subtype of LAdSRCs and defined SRC as a type of cytologic characteristics (6), there has been no reduction in the focus on LAdSRCs.

In previously reported cases, the incidence of primary SRCC in the lung was 0.14% to 1.9% (2,10). In our study, 35 cases (2.0%) of primary LAdSRCs were seen among 1,715 cases of surgically resected primary lung ADCs, which was basically consistent with previous research. In the case described by Kish *et al.*, the proportion of SRCC components of primary LAdSRCs was defined as 10% to 50% and we defined LAdSRCs as tumor with SRC component in at least 5% of the tumor by light microscope according to the 2015 WHO classification.

The 35 cases of primary LAdSRCs with an average age of 57.1 (range from 35 to 75) years. Compared with lung ADC, lung ADC with SRC component has worse RFS (P<0.001) and OS (P<0.001) (*Figures 1,2*). This finding was in agreement with Tsuta *et al.* (7) who found 39 primary LAdSRCs and 20 cases (51.3%) having died by the end of the study. A population-based epidemiologic study of 262 Cases conducted by Ou and colleagues (8) also indicated that primary LAdSRCs carry a worse prognosis when compared to ADCs. However, some previous reports indicated that the poor prognosis associated with LAdSRCs can be attributed to its strong association with solid subtypes (7-9). Our study also revealed that LAdSRCs are more common in solid subtype when compared with ADCs (37.1% *vs.* 9.3%, P<0.001). Furthermore, we divided LAdSRCs into two groups based on whether they contained solid components, and performed a survival analysis with ADCs and the results showed that the RFS and OS of the two groups were significantly worse than the ADCs (P=0.0026 and P=0.0002, respectively) (*Figures S1,S2*). This result suggested that perhaps SRC itself was a poor prognostic factor, but not the strong

Table 1 Clinical characteristics of patients with LAdSRCs and ADC

Characteristics	LAdSRCs (N=35)		ADCs (N=1,680)		P
	No.	%	No.	%	
Age, years					0.582
<60	19	54.3	833	49.6	
≥60	16	45.7	847	50.4	
Sex					0.011
Male	22	62.9	696	41.4	
Female	13	37.1	984	58.6	
Stage					<0.001
I–II	13	37.1	1,426	84.9	
III–IV	22	62.9	254	15.1	
T-size, cm					0.032
≤3	25	71.4	1,453	86.5	
>3, ≤7	9	25.7	212	12.6	
>7	1	2.9	15	0.9	
N stage					<0.001
N0	16	45.7	1,380	82.1	
N1	1	2.9	91	5.4	
N2	18	51.4	209	12.4	
Surgery resection					0.452
Sublobectomy	7	20.0	258	15.4	
Lobectomy	28	80.0	1,422	84.6	
Predominant histology subtype					<0.001
L	0	0	210	12.5	
A or P	14	40.0	1,221	72.7	
M or S	13	37.1	157	9.3	
Variant	8	22.9	92	5.5	
Adjuvant therapy					<0.001
Ever	27	77.1	303	18.0	
Never	8	22.9	1,377	82.0	
Smoking history					0.696
Ever	14	40.0	618	36.8	
Never	21	60.0	1,062	63.2	

LAdSRC, lung adenocarcinoma with signet ring cell; ADC, adenocarcinoma; L, lepidic; A, acinar; P, papillary, M, micropapillary; S, solid.

Table 2 Clinical details of 35 lung ADCs with clear cell component

Cases	Age/sex	Stage	Subtype	Smoking	Immunohistochemistry
1	44/M	IIIa	S + SRC	Never	CK+, CK7+, TTF-1+
2	60/F	IIIb	A + SRC	Never	CK+, CK7+, TTF-1+
3	69/M	IIIa	A + L + SRC	Ever	CK+, CK7+, TTF-1–
4	57/F	IVb	S + SRC	Never	CK+, CK7+, CK20+, TTF-1+, villin–
5	47/F	Ib	S + SRC	Never	CK+, CK7+, CK20–, TTF-1+, villin–
6	37/M	IIIa	S + SRC	Never	CK+, CK7+, CK20–, TTF-1–, villin–
7	58/F	IVa	A + P + SRC	Never	CK+, CK7+, CK20–, TTF-1+, villin–
8	46/F	IVa	IMA + SRC	Never	CK+, CK7+, CK20–, TTF-1–, villin–
9	66/M	Ib	S + SRC	Ever	CK+, CK7+, CK20–, TTF-1+, villin–
10	46/M	Ib	S + SRC	Ever	CK+, CK7+, TTF-1+, villin–
11	46/M	IIIa	A + P + SRC	Never	CK+, CK7+, CK20–, TTF-1+, villin–
12	64/M	IIIa	IMA + S + SRC	Ever	CK+, CK7+, CK20–, TTF-1+, villin–
13	66/F	Ia	P + SRC	Never	CK+, CK7+, CK20–, TTF-1+, villin–
14	57/F	Ia	S + IMA + SRC	Never	CK+, CK7+, CK20–, TTF-1+, villin–
15	75/F	Ia	S + SRC	Never	CK+, CK7+, CK20–, TTF-1+, villin–
16	71/F	Ia	IMA + SRC	Never	CK+, CK7+, CK20–, TTF-1+
17	68/M	IIIa	A + SRC	Never	CK+, CK7+, CK20–, TTF-1+, villin–
18	45/F	IIIa	IMA + SRC	Never	CK+, CK7+, TTF-1+
19	69/M	Ia	A + SRC	Never	CK+, CK7+, CK20–, TTF-1+, villin–
20	66/M	Ib	A + SRC	Ever	CK+, CK7+, CK20–, TTF-1+, villin–
21	50/M	Ib	A + IMA + SRC	Ever	CK+, CK7+, CK20–, TTF-1–, villin–
22	59/M	IIIa	S + SRC	Ever	CK+, CK7+, CK20–, TTF-1–, villin–
23	59/M	Ia	IMA + SRC	Ever	CK+, CK7+, CK20–, TTF-1+, villin–
24	36/F	Ib	IMA + SRC	Never	CK+, CK7+, CK20–, TTF-1+, villin–
25	51/M	IIIa	A + S + SRC	Ever	CK+, CK7+, CK20+, TTF-1–, villin–
26	48/M	IIIa	S + IMA + SRC	Ever	CK+, CK7+, TTF-1–, villin–
27	52/F	IVa	P + A + SRC	Never	CK+, CK7+, TTF-1+
28	72/M	IIIa	S + SRC	Ever	CK+, CK7+, CK20–, TTF-1+, villin–
29	59/M	Ib	A + SRC	Never	CK+, CK7+, CK20–, TTF-1+, villin–
30	35/M	IIIa	S + A + SRC	Never	CK+, CK7+, CK20–, TTF-1–, villin–
31	68/M	IIIa	Pure SRC	Never	Unknown
32	63/M	IIIa	A + S + SRC	Ever	CK+, CK7+, CK20–, TTF-1–, villin–
33	60/M	IIIb	IMA + SRC	Ever	CK+, CK7+, CK20–, TTF-1–, villin–
34	66/M	IIIb	S + SRC	Ever	Unknown
35	63/F	IIIa	A + P + SRC	Never	CK+, CK7+, TTF-1–, villin-

ADC, adenocarcinoma; M, male; F, female; A, acinar; P, papillary; S, solid; L, lepidic; IMA, invasion mucinous adenocarcinoma; SRC, signet ring cell; CK, cytokeratin; TTF-1, thyroid transcription factor-1.

Table 3 Univariable analyses for RFS and OS in patients with resected lung ADC

Characteristics	RFS			OS		
	HR	95% CI	P	HR	95% CI	P
Age, years	0.598	0.489–0.736	<0.001	0.697	0.586–0.921	<0.001
Sex	1.401	1.166–1.684	<0.001	1.531	1.195–1.961	0.001
Stage	2.895	2.635–3.181	<0.001	2.960	2.613–3.353	<0.001
T-size, cm	1.458	1.403–1.515	<0.001	1.487	1.413–1.564	<0.001
N stage	2.898	2.629–3.196	<0.001	2.994	2.631–3.406	<0.001
Surgery resection	1.694	1.245–2.305	0.001	1.549	1.016–2.362	0.042
Predominant histology subtype	2.210	1.565–3.123	<0.001	2.531	1.648–3.888	<0.001
Adjuvant therapy	1.244	1.007–1.538	0.043	1.449	1.218–1.723	<0.001
Smoking history	1.506	1.253–1.812	<0.001	1.546	1.207–1.982	0.001
Pathology	2.376	1.548–3.647	<0.001	2.738	1.670–4.490	<0.001

RFS, relapse-free survival; OS, overall survival; ADC, adenocarcinoma; HR, hazard ratio; CI, confidence interval.

Table 4 Multivariable analyses of RFS and OS in patients with resected lung ADC

Characteristics	RFS			OS		
	HR	95% CI	P	HR	95% CI	P
Age, years	1.010	1.000–1.019	0.047	1.011	0.998–1.024	0.097
Sex	1.273	1.049–1.546	0.015	1.432	1.097–1.870	0.008
Stage	2.557	2.035–3.212	<0.001	2.255	1.648–3.085	<0.001
T-size, cm	1.227	1.164–1.293	<0.001	1.264	1.176–1.359	<0.001
N stage	1.356	1.086–1.693	0.007	1.529	1.131–2.067	0.006
Surgery resection	0.868	0.626–1.202	0.393	0.638	0.406–1.002	0.051
Predominant histology subtype	0.506	0.350–0.733	<0.001	1.429	1.049–1.945	0.024
Adjuvant therapy	0.458	0.359–0.585	<0.001	0.338	0.243–0.471	<0.001
Smoking history	1.198	0.987–1.455	0.068	1.309	1.002–1.710	0.049
Pathology	0.918	0.581–1.492	0.712	1.392	0.820–2.364	0.221

RFS, relapse-free survival; OS, overall survival; ADC, adenocarcinoma; HR, hazard ratio; CI, confidence interval.

association with solid subtype. SRC was only acted as a significant predictor of survival in univariate analysis (*Table 3*), but not in multivariate analysis (*Table 4*). The reason may be the fact that those lung ADCs with SRC component were associated with advanced disease stage (P<0.001) (stage III/IV, 62.9%, *Table 1*).

By analyzing the IHC results of these 35 cases, we found some common features. Except for two cases without IHC information (*Table 2*), 22 of the 33 LAdSRCs (66.7%) are

TTF-1 positive; 23 of 33 LAdSRCs (69.7%) show a CK profile of CK7+ or CK20–, and 2 (6%) expressed both CK7+ and CK20+. However, villin was not identified in these 33 cases. TTF-1 is a 38 kDa nuclear protein member of the homologous gene transcription factor NKX2 family which was encoded by a single locus (22). Several recent IHC studies revealed that TTF-1 was a tissue-specific transcription factor expressed almost exclusively in thyroid tissue and different tumors of the lung, and it shown that it
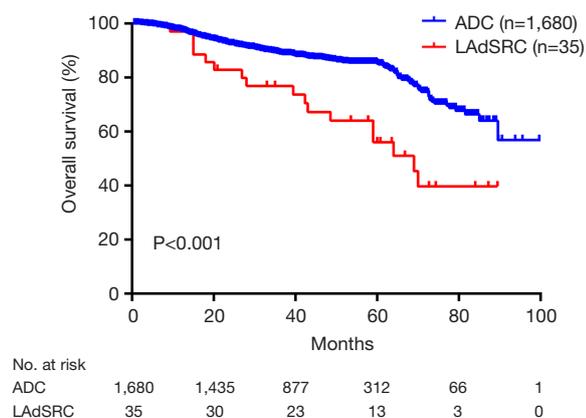
Figure 1 Kaplan-Meier survival curves for RFS according to LAdSRCs and lung ADCs. RFS, relapse-free survival; LAdSRC, lung adenocarcinoma with signet ring cell; ADC, adenocarcinoma.
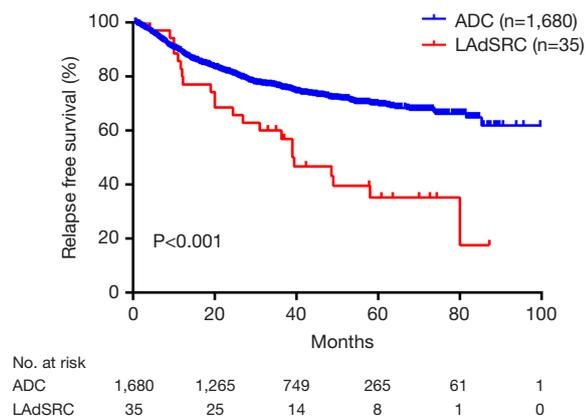

Figure 2 Kaplan-Meier survival curves for OS according to LAdSRCs and lung ADCs. OS, overall survival; LAdSRC, lung adenocarcinoma with signet ring cell; ADC, adenocarcinoma.

can be used as a marker to differentiate these tumors from malignant tumors that appear to be similar to those found in other organs (11,23,24). Merchant *et al.* (16) indicated that TTF-1 was expressed in a high percentage of primary LAdSRCs and was very specific and that TTF-1 would be extremely valuable in distinguishing primary LAdSRCs from those arising in other organs. In that study, 82.4% of the 17 LAdSRCs expressed TTF-1 positivity, but no non-pulmonary SRCCs was positive for TTF-1, which was in line with our research results.

The immunostaining profiles of CK7 and CK20 also played an important role in distinguishing SRC from different organ sources (13,14). From these previous

studies, it was found that the CK profile of almost all breast SRCCs was expressed as CK7+/CK20−. In contrast, almost all colonic SRCCs showed an inverse pattern of immunostaining CK7−/CK20+. In the present study, 35 primary LAdSRCs showed the same high expression rate (69.7%) as breast SRCCs in CK7+/CK20−. These results suggest that the CK7+/CK20− mode favors primary LAdSRCs or breast SRCCs, whereas the CK7−/CK20+ pattern favors colonic origin. Villin is a protein that specifically binds to the actin core bundle of microvilli. Under normal physiological conditions, villus protein is mainly expressed in the digestive tract epithelial cells, renal proximal tubules and hepatobiliary ducts, and was not expressed in the lung (25,26), which was consistent with our results, we didn't identify any villin (+) in these 33 cases. However, Merchant *et al.* (16) have revealed the expression of villin in primary LAdSRCs recently, villin (+) was identified in 5 (29.4%) of the 17 primary LAdSRCs in his study. The role of villin in identifying the source of SRC remain to be further studied.

There are several limitations in this study. First, the sample size of LAdSRCs was relatively small, so there may produce a bias. Second, those clinical and pathological data were retrospective in nature, and they should be confirmed in prospective trials if possible.

In summary, primary LAdSRC is a rare ADC subtype with a poor prognosis compared with ADC. The high percentage expression of TTF-1 and immunostaining profiles CK7+/CK20− in primary LAdSRC was significant to identify the source of SRCCs.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.11.54). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of Shanghai Chest Hospital [KS(Y)1668] and written informed consent was obtained from all patients.

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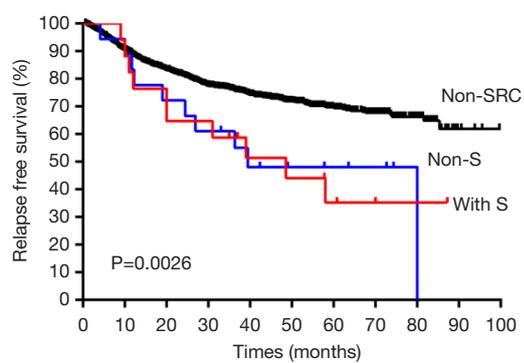
Figure S1 Kaplan-Meier survival curves for RFS based on whether LAdSRCs contained solid components. RFS, relapse-free survival; LAdSRC, lung adenocarcinoma with signet ring cell; S, solid component.
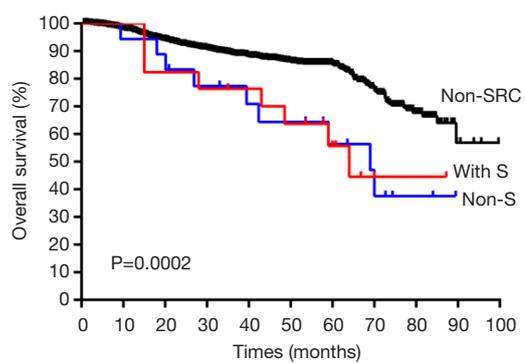
Figure S2 Kaplan-Meier survival curves for OS based on whether LAdSRCs contained solid components. OS, overall survival; LAdSRC, lung adenocarcinoma with signet ring cell; S, solid component.