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Expression Changes in Programmed Death Ligand 1 from Precancerous Lesions to Invasive Adenocarcinoma in Subcentimeter Pulmonary Nodules: A Large Study of 2022 Cases in China

Xiongwen Yang, MD, PhD^{1,2}, Yi Xiao, MD³, Hao Hu, MD⁴, Zhen-bin Qiu, MD^{1,2}, Yi-fan Qi, MD^{1,2}, Meng-min Wang, MD², Yi-Long Wu, MD, PhD², and Wen-Zhao Zhong, MD, PhD^{1,2}

¹School of Medicine, South China University of Technology, Guangzhou, China; ²Guangdong Lung Cancer Institute, Guangdong Provincial Key Laboratory of Translational Medicine in Lung Cancer, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Southern Medical University, Guangzhou, China; ³Department of Cardio-Thoracic Surgery, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China; ⁴Department of Radiation Therapy, General Hospital of Southern Theater Command, Guangzhou, China

ABSTRACT

Purpose. This large-scale, multicenter, retrospective observational study aimed to evaluate the clinicopathological and molecular profiles associated with programmed death-ligand 1 (PD-L1) expression in precancerous lesions and invasive adenocarcinoma in subcentimeter pulmonary nodules.

Patients and Methods. Patients with histologically confirmed atypical adenomatous hyperplasia (AAH), adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), and invasive adenocarcinoma (ADC) were included. PD-L1 expression was evaluated at each center using a PD-L1 immunohistochemistry 22C3 pharmDx kit (Agilent, Santa Clara, CA, USA). The tumor proportion score (TPS) cutoff values were set at $\geq 1\%$ and $\geq 50\%$.

Results. A total of 2022 nodules from 1844 patients were analyzed. Of these, 9 (0.45%) nodules had PD-L1 TPS $\geq 50\%$, 187 (9.25%) had PD-L1 TPS 1–49%, and 1826 (90.30%) had PD-L1 TPS $< 1\%$. A total of 378 (18.69%), 1016 (50.25%), and 628 (31.06%) nodules were diagnosed as AAH/AIS, MIA, and ADC, respectively, by pathology. A total of 1377 (68.10%), 591 (25.67%), and 54 (2.67%) nodules were diagnosed as pure ground-glass opacity (GGO),

mixed GGO, and solid nodules, respectively, by computed tomography. There was a significant difference between PD-L1 expression and anaplastic lymphoma kinase (ALK) mutation status ($P < 0.001$). PD-L1 expression levels were significantly different from those determined using the International Association for the Study of Lung Cancer (IASLC) grading system ($P < 0.001$).

Conclusions. PD-L1 expression was significantly associated with radiological and pathological invasiveness and driver mutation status in subcentimeter pulmonary nodules. The significance of PD-L1 expression in the evolution of early-stage lung adenocarcinoma requires further investigation.

Keywords PD-L1 · Precancerous lesions · Invasive lung adenocarcinoma · Prevalence

Lung cancer is a malignant tumor with the highest morbidity and mortality worldwide.¹ Early diagnosis and treatment of lung cancer can significantly improve long-term survival and reduce medical costs.² Furthermore, with the popularization of lung cancer screening, an increasing number of people are being diagnosed with pulmonary nodules.³ The treatment of small pulmonary nodules is mainly surgical; however, the clinicopathological features, especially those of subcentimeter pulmonary nodules, remain to be elucidated.⁴

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W.-Z. Zhong, MD, PhD

e-mail: syzhongwenzhao@scut.edu.cn

Lung tumor cells frequently express high levels of programmed death ligand 1 (PD-L1), a ligand of the programmed death-1 (PD-1) receptor on T cells (Fig. 1), allowing tumors to directly inhibit the host immune response by inhibiting the proliferation and function of T cells.^{5–9} Moreover, the treatment landscape of lung cancer has dramatically changed owing to immunotherapy; a very notable factor is the use of PD-L1/PD-1 checkpoint inhibitors.¹⁰ Several large-scale clinical trials have shown a significant improvement in overall survival (OS) concomitant with significantly reduced toxicity following the use of PD-L1/PD-1 checkpoint inhibitors; serious or life-threatening adverse events occurred in 7–15% of the checkpoint inhibitor group and 35–55% of the docetaxel group.^{11–14}

Many studies have reported the expression level of PD-L1 and its clinicopathological characteristics in resectable non-small cell lung cancer (NSCLC); however, the selection of PD-L1 antibodies has been inconsistent.^{15–19} The expression levels of PD-L1 in early lung cancer and their relationship with common driver genes are controversial,^{18–20} and its expression in minimally invasive adenocarcinoma (MIA) and adenocarcinoma in situ (AIS) has been rarely reported.²¹ Miyazawa et al. included only 11 patients with AIS and 12 patients with MIA, all of whom did not express PD-L1.²¹ Limited by the sample size, no statistical difference was observed in the expression levels of PD-L1 between precancerous lesions and invasive adenocarcinomas in our previous study.²² Recently, Zhou et al.²³ evaluated PD-L1 expression in 1123 resected lung specimens of AIS, MIA, and stage

IA1–IA3 invasive adenocarcinomas (ADCs). However, only 11 AISs and 200 ADCs were included, making it difficult to fully evaluate PD-L1 expression in the very early stages of lung cancer.²³

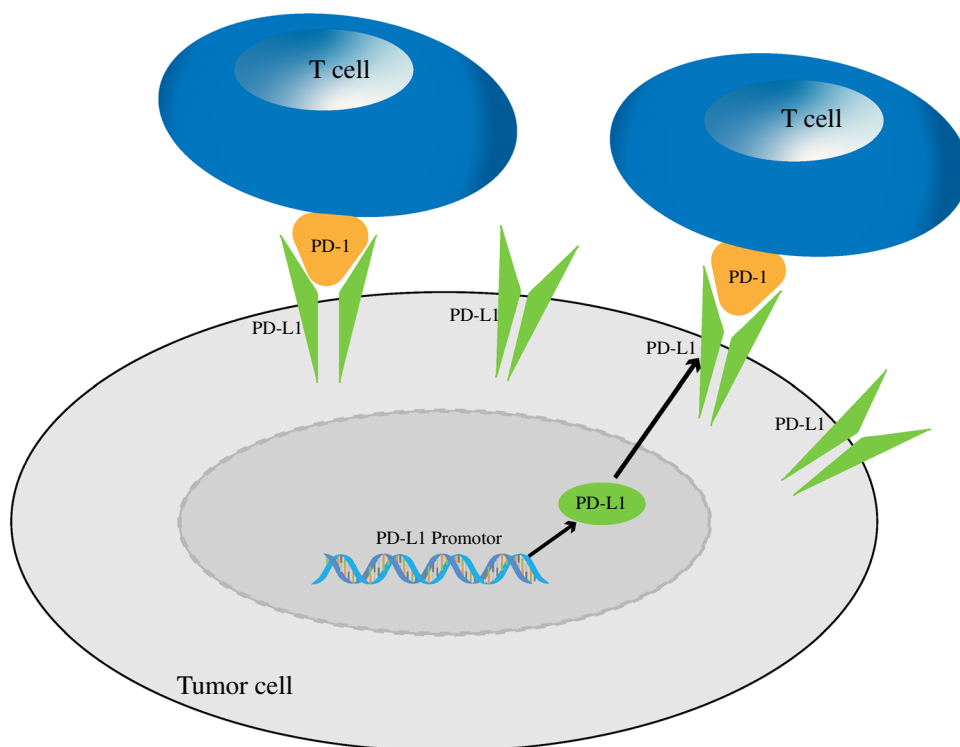
To the best of the authors' knowledge, there have been no studies on the changes in the expression of PD-L1 in subcentimeter pulmonary nodules between preinvasive lesions and invasive ADC or the relationship between PD-L1 and common driver gene mutations. Therefore, this study aimed to conduct a large-scale, retrospective study in a consecutive population from three institutions in China to investigate the prevalence of PD-L1 expression in Chinese subcentimeter lung adenocarcinoma (LUAD), as measured by the PD-L1 immunohistochemistry (IHC) 22C3 assay. We subsequently explored the clinicopathological and molecular characteristics associated with PD-L1 expression in Chinese patients with subcentimeter LUAD.

PATIENTS AND METHODS

Data Collection

From January 2018 to August 2021, the electronic medical records and radiology information systems of the Guangdong Provincial People's Hospital and Guangdong Lung Cancer Institute, General Hospital of Southern Theater Command, and the Third Affiliated Hospital of Sun Yat-sen University were used for patients with pulmonary nodules

FIG. 1. Simple model diagram of PD-1/PD-L1 expression. PD-1 programmed death 1, PD-L1 programmed death ligand 1



identified on chest CT scans. All nodules were measured using a computer to determine whether they were subcentimeter nodules (≤ 1 cm), which were pathologically confirmed postoperatively.

Inclusion and exclusion criteria were as follows: (1) complete resection of the nodule and clear postoperative pathological diagnosis, (2) complete and traceable hospitalization data, providing detailed medical history, (3) postoperative pathological diagnosis was AAH, AIS, MIA, ADC, excluding benign lesions, (4) the measurement of diameter was based on chest CT, that is, the stage was cT1aN0M0, stage IA1, (5) the pathological diagnosis of multiple primary subcentimeter nodules is based on the pathological results of a single subcentimeter nodule, that is, the same patient can be combined with multiple pathological results of AAH, AIS, MIA, and/or ADC, (6) all the included samples were tested by PD-L1, and (7) patients who previously received neoadjuvant chemotherapy, radiotherapy, targeted therapy, or immunotherapy were excluded from the study. The pathologic diagnosis of AAH, AIS, MIA, and ADC is based on the fifth edition of the WHO Classification of Thoracic

Tumors published by the International Agency for Research on Cancer (IARC) in 2021.²⁴

PD-L1 IHC and Driver Gene (EGFR Mutation and Anaplastic Lymphoma Kinase (ALK) Rearrangement) Tests

PD-L1 expression in tumor cells was assessed using a PD-L1 IHC assay (PD-L1 IHC 22C3 pharmDX; Dako, Carpinteria, California, USA) according to the manufacturer's instructions. Two experienced pathologists assessed PD-L1 expression levels in all cases. Inconsistent cases were reviewed by a third pathologist to reach consensus. The expression of PD-L1 on tumor cells was quantified using the tumor proportion score (TPS). PD-L1 expression was quantified as TPS:

$$\text{TPS} = \left(\frac{\text{The number of viable tumor cells positive for PD-L1}}{\text{Total number of viable neoplastic cells}} \right) \times 100\%$$

Staining status was classified by TPS into three groups: $< 1\%$ (negative), $\geq 1\%$ and $\leq 49\%$ (weakly positive), and

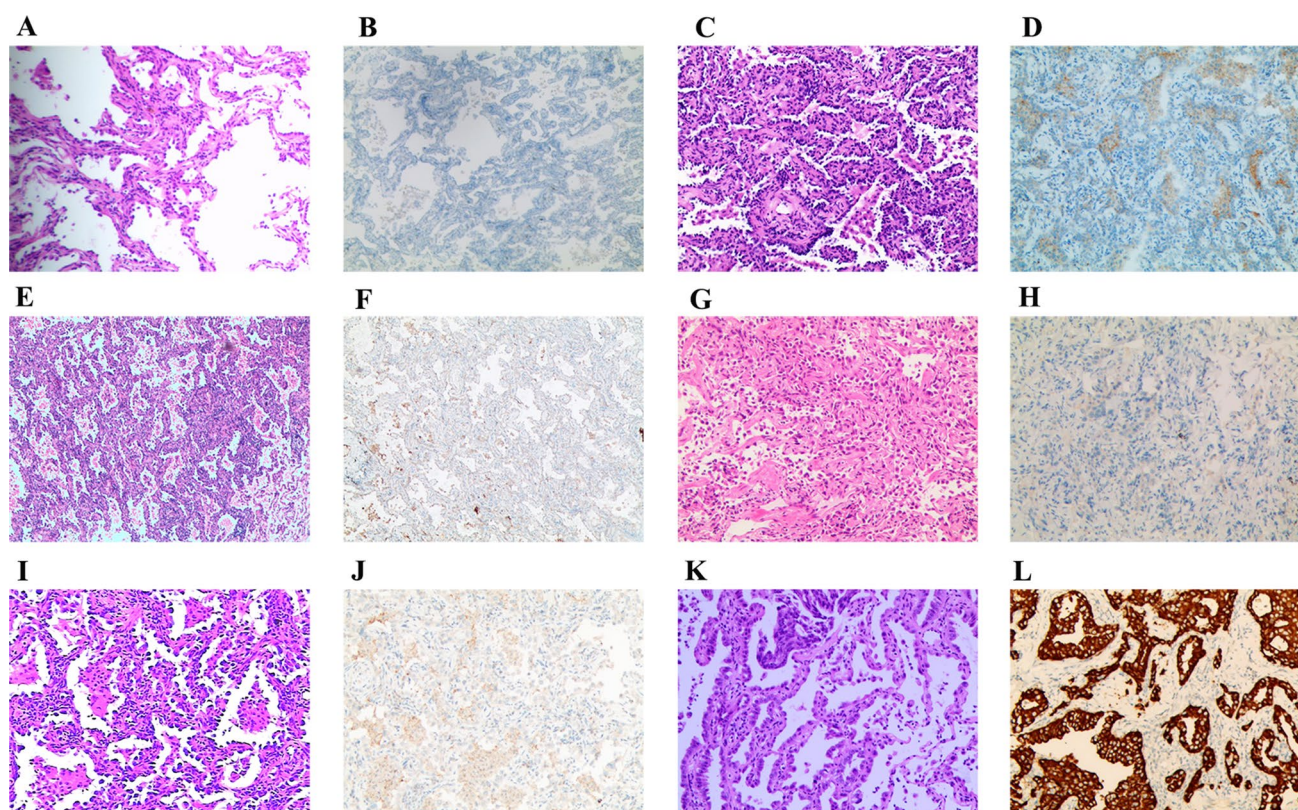


FIG. 2. HE stain and PD-L1 (22C3) immunohistochemical expression in patients with AIS, MIA and ADC. **A** AIS (HE stain) and **B** AIS (PD-L1 negative), same patient. **C** AIS (HE stain) and **D** AIS (PD-L1 positive), same patient. **E** MIA (HE stain) and **F** MIA (PD-L1 negative), same patient. **G** MIA (HE stain) and **H** MIA (PD-L1

positive), same patient. **I** ADC (HE stain) and **J** ADC (PD-L1 negative), same patient. **K** ADC (HE stain) and **L** ADC (PD-L1 positive), same patient. HE hematoxylin-eosin, AIS adenocarcinoma in situ, MIA minimally invasive adenocarcinoma, ADC invasive adenocarcinoma, PD-L1 programmed death ligand 1

$\geq 50\%$ (highly positive). All tumors showing $\text{TPS} \geq 1\%$ were considered to indicate positive expression (Fig. 2). The clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expression ($\text{TPS} \geq 1\%$) in previously treated patients with NSCLC is based on the KEYNOTE-010 study.¹³ The clinical validity of PD-L1 IHC 22C3 pharmDx in identifying high PD-L1 expression ($\text{TPS} \geq 50\%$) in previously untreated patients with NSCLC is based on the KEYNOTE-024 study.²⁵ When PD-L1 expression ($\text{TPS} \geq 1\%$) was evaluated in NSCLC, PD-L1 IHC 28-8 pharmDx was used as the gold standard, and the sensitivity and specificity of PD-L1 IHC 22C3 pharmDx were 0.88 and 0.93, respectively.²⁶ When PD-L1 expression ($\text{TPS} \geq 1\%$) was evaluated in NSCLC, Ventana PD-L1 (SP263) was used as the gold standard, and the sensitivity and specificity of PD-L1 IHC 22C3 pharmDx were 0.84 and 0.91, respectively.²⁶ When PD-L1 expression ($\text{TPS} \geq 50\%$) was evaluated in NSCLC, Ventana PD-L1 (SP263) was used as the gold standard, and the sensitivity and specificity of PD-L1 IHC 22C3 pharmDx were 0.57 and 0.99, respectively.²⁶ Meanwhile, the Blueprint Phase (BP) 2 Project consolidates the analytical evidence for interchangeability of the 22C3, 28-8, and SP263 assays.²⁷ Strictly speaking, the current clinically used PD-L1 IHC assays have not been validated for all sample types encountered in their clinical application. Consequently, the diagnostic companies that commercialize the various PD-L1 assays have not recommended their use on cytological specimens, and the training offered to pathologists for these assays has not included examples of cytological specimens. Although many pathologists do not consider any difference in their approach to assessment of PD-L1 expression on TCs when using cytological cell block or tissue biopsy/resection sections, a few studies with small sample sizes have reported on the concordance of PD-L1 assessment on cytological versus matching surgical specimens.^{28–32}

EGFR mutations were assessed using amplification-refractory mutation systems (ARMS) or next-generation sequencing (NGS). ALK expression in tumor tissues was detected using Ventana ALK (D5F3) IHC, fluorescence in situ hybridization, or NGS.

Radiological Evaluation

According to the manifestation of the lung window, the ground-glass opacity (GGO) component was defined as an area with increased foggy attenuation in the lungs and retained bronchial and vascular margins, whereas the solid component was defined as a plaque completely covering the lung parenchyma. Subsolid nodules were distinguished

from pure solid nodules on the basis of their GGO components. In subsolid nodules, pure GGO (pGGO) nodules were defined as lesions with no solid components, and mixed GGO (mGGO) nodules were defined as lesions with solid components.

Predominant Histological Pattern and the International Association for the Study of Lung Cancer (IASLC) Grading System

On the basis of the relationship between the main histological patterns and prognosis of LUAD, the 2015 World Health Organization (WHO) guidelines divided LUAD into three prognostic groups: low (lepidic predominant), intermediate (acinar or papillary predominant), and high grade (solid or micropapillary predominant).³³ The IASLC Pathology Committee recently proposed a novel grading system for invasive pulmonary adenocarcinoma.³⁴ The model comprises grade 1, lepidic-predominant tumor; grade 2, acinar- or papillary-predominant tumor, both with $< 20\%$ high-grade patterns; and grade 3, any tumor with $\geq 20\%$ high-grade patterns (solid, micropapillary, or complex gland).³⁴ The relationship between the expression level of PD-L1 in subcentimeter ADC and both the WHO and IASLC grading systems was investigated in this study.

Statistical Analysis

The data are presented as mean $\pm$ standard deviation or mean $\pm$ standard error of the mean. Data from the three groups were evaluated using analysis of variance (ANOVA). Categorical data were analyzed using the chi-squared test or Fisher's exact test. Correlations between continuous variables were assessed using Spearman's correlation analysis. P value < 0.05 was considered statistically significant. All data were statistically analyzed using R software version 3.6.0 (Lucent Technologies, Murray Hill, NJ, USA). More statistical details are as follows: In Table 1, Fisher's exact test was used for statistics of variables such as sex, smoking status, GGO component, location, *EGFR*, and ALK. In Table 1, ANOVA test was used for statistics of variables such as age and size. In Table 2, Fisher's exact test was used for the statistics of variables such as WHO grading system and novel IASLC grading system. In Fig. 5, chi-squared test was used for the statistics of variables such as pathology, GGO component, and ALK. In Fig. 5, Fisher's exact test was used for the statistics of the variable such as novel IASLC grading system.

TABLE 1 Patient characteristics by PD-L1 expression groups

	PD-L1 ^a < 1%	PD-L1 1–49%	PD-L1 ≥ 50%	<i>P</i>
<i>N</i> = 2022 ^b	1826 (90.30%)	187 (9.25%)	9 (0.45%)	
Race (<i>n</i>)				
East Asian (2022)	1826 (90.30%)	187 (9.25%)	9 (0.45%)	
Sex (<i>n</i>)				
Male (531)	470 (88.52%)	58 (10.92%)	3 (0.56%)	0.247
Female (1491)	1356 (90.95%)	129 (8.65%)	6 (0.40%)	
Age (years)				
Median (range)	49.62 (14–86)	51.46 (23–82)	52.56 (43–71)	0.237
Smoking status (<i>n</i>)				
Never (1880)	1701 (90.47%)	170 (9.05%)	9 (0.48%)	0.438
Current/former (142)	125 (88.03%)	17 (11.97%)	0 (0.00%)	
Location (<i>n</i>)				
Right upper lobe (597)	544 (91.12%)	49 (8.21%)	4 (0.67%)	0.648
Right middle lobe (186)	167 (89.78%)	18 (9.68%)	1 (0.54%)	
Right lower lobe (424)	378 (89.15%)	43 (10.14%)	3 (0.71%)	
Left upper lobe (518)	468 (90.35%)	50 (9.65%)	0 (0.00%)	
Left lower lobe (297)	269 (90.57%)	27 (9.09%)	1 (0.33%)	
Size (mm)				
Median (range)	7.27 (3–10)	7.47 (4–10)	7.78 (6–9)	0.178
Pathology (<i>n</i>)				
AAH/AIS (378)	367 (97.09%)	11 (2.91%)	0 (0.00%)	<0.001
MIA (1016)	940 (92.52%)	73 (7.19%)	3 (0.30%)	
ADC (628)	519 (82.64%)	103 (16.40%)	6 (0.96%)	
GGO component (<i>n</i>)				
pGGO (1377)	1258 (91.35%)	113 (8.21%)	6 (0.44%)	0.043
mGGO (591)	523 (88.49%)	66 (11.17%)	2 (0.34%)	
Solid (54)	45 (83.33%)	8 (14.81%)	1 (0.19%)	
<i>EGFR</i> (<i>n</i>) ^c				
Mutation (411)	372	39	0	0.454
Wild-type (538)	484	51	3	
Unknown (1073)	970	97	6	
<i>ALK</i> (<i>n</i>) ^c				
Positive (39)	27	7	5	<0.001
Negative (1878)	1699	176	3	
Unknown (105)	100	4	1	

Bold values indicate a statistical difference in *P* values

PD-L1 programmed death ligand-1, *AAH* atypical adenomatous hyperplasia, *AIS* adenocarcinoma in situ, *MIA* minimally invasive adenocarcinoma, *ADC* invasive adenocarcinoma, *GGO* ground-glass opacity, *EGFR* epidermal growth factor receptor, *ALK* anaplastic lymphoma kinase

^aImmunohistochemistry was performed using the PD-L1 kit (PD-L1 IHC 22C3 pharmDX; Dako, Carpinteria, CA, USA) according to the manufacturer's instructions

^bStatistical methods and dimensions was the number of nodules

^cData with unknown gene status were not included in the statistical analysis

Ethics Approval and Consent to Participate

All procedures involving collection of tissue were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable

ethical standards. This study was approved by the ethics committee of Guangdong Provincial People's Hospital, the Third Affiliated Hospital of Sun Yat-sen University, and General Hospital of Southern Theater Command. Written informed consent was obtained from individual participants or their guardians.

TABLE 2 Patient characteristics by PD-L1 expression group among patients with ADC

	PD-L1 ^a < 1%	PD-L1 1–49%	PD-L1 ≥ 50%	<i>P</i>
<i>N</i> = 628	519 (82.64%)	103 (16.40%)	6 (0.96%)	
WHO grading system (<i>n</i>)				0.433
Low grade (17)	16 (94.12%)	1 (5.88%)	0 (0.00%)	
Intermediate grade (611)	503 (82.32%)	102 (16.69%)	6 (0.99%)	
High grade (0)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Novel IASLC grading system (<i>n</i>) ^b				< 0.001
Grade 1 (17)	16 (94.12%)	1 (5.88%)	0 (0.00%)	
Grade 2 (606)	503 (83.00%)	99 (16.34%)	4 (0.66%)	
Grade 3 (5)	0 (0.00%)	3 (60.00%)	2 (40.00%)	

Bold value indicates a statistical difference in *P* values

PD-L1 programmed death ligand-1, ADC invasive adenocarcinoma, IASLC International Association for the Study of Lung Cancer

^aImmunohistochemistry was performed using the PD-L1 kit (PD-L1 IHC 22C3 pharmDX; Dako, Carpinteria, CA, USA) according to the manufacturer's instructions.

^b Novel IASLC grading system consists of: grade 1: lepidic predominant tumor; grade 2: acinar or papillary predominant tumor, both with no or less than 20% of high-grade patterns; grade 3: any tumor with 20% or more of high-grade patterns (solid, micropapillary and or complex gland).

RESULTS

Patient Characteristics

From January 2018 to August 2021, 2058 patients were screened at the Guangdong Provincial People's Hospital, Guangdong Lung Cancer Institute, General Hospital of the Southern Theater Command, and Third Affiliated Hospital of Sun Yat-sen University (Fig. 3). A total of 166 and 71 nodules were excluded as they were pathologically confirmed to be benign pulmonary nodules and pulmonary metastatic nodules, respectively. A total of 184 nodules were excluded because they did not show PD-L1 expression or did not show detection of PD-L1 expression using the 22C3 anti-PD-L1 antibody. Finally, 2022 nodules from 1844 patients were included in the analysis.

Most of the dataset included nonsmoking female patients (Table 1). The mean age of patients was 49.8 (range 14–86) years. Most patients were younger than 65 years of age (89.81%). Upper lobe nodules accounted for 55.14% of nodules (1115/2022). PD-L1 expression was detected in only 196 (9.69%) subcentimeter nodules in patients with early lung ADC and precancerous lesions (Fig. 4).

PD-L1 Expression Pertaining to Pathology

Among the 2022 nodules, 378 (18.69%), 1016 (50.25%), and 628 (31.06%) were diagnosed as atypical adenomatous hyperplasia (AAH)/AIS, MIA, and ADC by postoperative pathology, respectively (Table 1). The expression levels

of PD-L1 were significantly different between AAH/AIS, MIA, and ADC ($P < 0.001$; Fig. 5). In ADC nodules, there was no statistically significant difference between the predominant histological patterns and PD-L1 expression levels (Table 2, $P = 0.433$). There was a statistical difference between the expression of PD-L1 and novel International Association for the Study of Lung Cancer (IASLC) grading system ($P < 0.001$; Fig. 5).

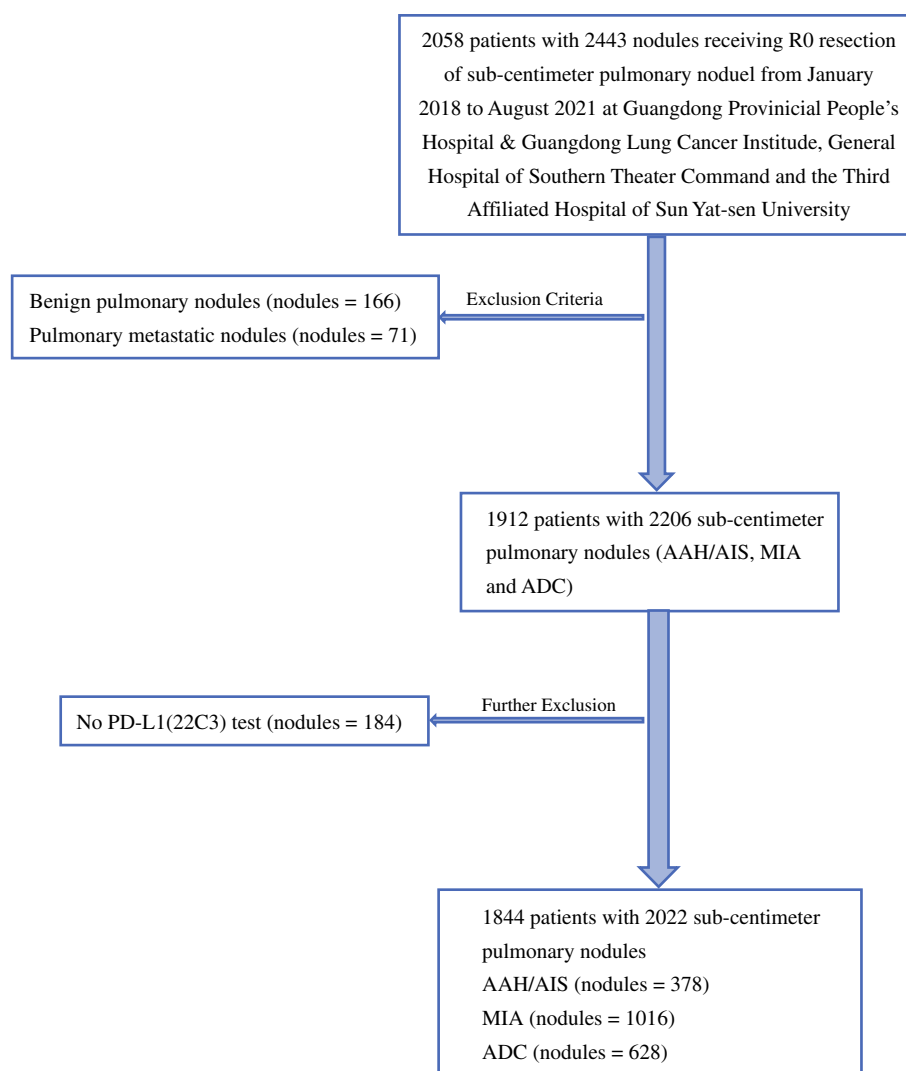
PD-L1 Expression Pertaining to the GGO Component

Among the 2022 nodules, 1377 (68.10%), 591 (25.67%), and 54 (2.67%) were diagnosed as pGGO, mGGO, and solid nodules, respectively, on CT imaging (Table 1). There was a statistically significant difference between the expression of PD-L1 and GGO components ($P < 0.001$; Fig. 5).

PD-L1 Expression Pertaining to Common Driver Genes

EGFR mutation status was detected in 949 nodules, and *EGFR* mutations were detected in 411 nodules (Table 1). There was no statistically significant difference between *EGFR* mutation status and PD-L1 expression ($P = 0.454$). A total of 1917 nodules were tested for *ALK* status, and 39 nodules were *ALK* positive. There was a statistically difference between PD-L1 expression and *ALK* mutation status ($P < 0.001$; Fig. 3).

FIG. 3. Diagram of patient selection and study design. *AAH* atypical adenomatous hyperplasia, *AIS* adenocarcinoma in situ, *MIA* minimally invasive adenocarcinoma, *ADC* invasive adenocarcinoma, *PD-L1* programmed death ligand 1



PD-L1 Expression Pertaining to the WHO and IASLC Grading Systems

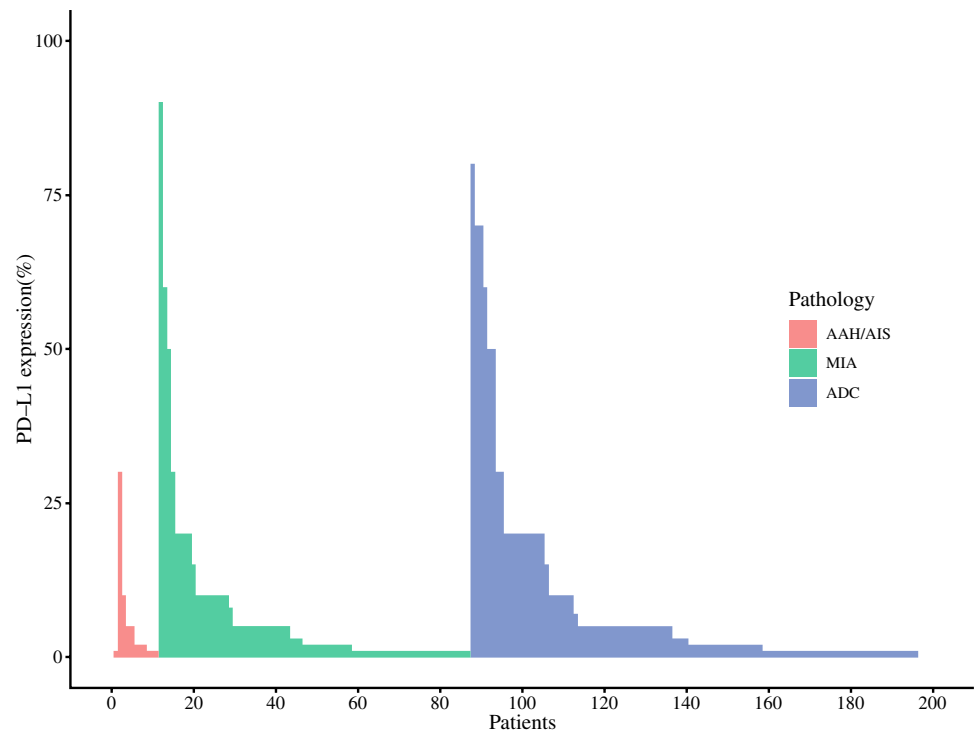
In the 628 subcentimeter ADC nodules, PD-L1 expression levels were not statistically different from those of the WHO grading system ($P=0.433$). However, there were significant differences compared with those in the IASLC grading system ($P<0.001$).

DISCUSSION

To the best of the authors' knowledge, this is the largest study to demonstrate changes in PD-L1 expression from precancerous lesions to invasive adenocarcinoma in subcentimeter pulmonary nodules. Using the PD-L1 IHC 22C3 pharmDx assay antibody, we found that the expression level of PD-L1 increased with increasing invasiveness of pulmonary nodules. The EXPRESS study was a global, multicenter, retrospective observational study involving tumor

tissue samples from patients over 18 years of age diagnosed with stage IIIB/IV primary NSCLC prior to systemic therapy.¹⁷ PD-L1 expression was assessed at each center using the PD-L1 IHC 22C3 pharmDx kit. A total of 2617 patients who met the inclusion criteria were included in this study, of whom 2368 (90%) had PD-L1 data.¹⁷ The most common reason for not having PD-L1 data ($n=249$) was insufficient tumor cells on the slides ($n=170$ [6%]).¹⁷ Therefore, a total of 530 patients (22%) with PD-L1 TPS $\geq 50\%$, 1232 patients (52%) with PD-L1 TPS $\geq 1\%$, and 1136 patients (48%) with PD-L1 TPS $< 1\%$ were included in this study.¹⁷ The proportions of PD-L1 patients with TPS $\geq 50\%$ and TPS $\geq 1\%$ were 22%/52%, respectively, 22%/53% in Asia Pacific, 21%/47% in the Americas, and 24%/55% in other countries.¹⁷ The EXPRESS study used counts and percentages to summarize the prevalence of PD-L1 with TPS $\geq 50\%$, TPS $\geq 1\%$, and TPS $< 1\%$. Since there were no prior hypotheses, no P value is determined.¹⁷ Li et al.²⁰ retrospectively analyzed Chinese patients with NSCLC from a prospectively maintained

FIG. 4. Distribution of PD-L1⁺ in different pathological types. *AAH* atypical adenomatous hyperplasia, *AIS* adenocarcinoma in situ, *MIA* minimally invasive adenocarcinoma, *ADC* invasive adenocarcinoma, *PD-L1* programmed death ligand 1



database. PD-L1 expression was assessed in this study using VENTANA (SP142) anti-PD-L1 antibody.²⁰ A total of 1370 patients who met the inclusion criteria were included in this study, of whom 122 (8.9%) had PD-L1 TPS $\geq 50\%$ and 405 (29.6%) had PD-L1 TPS $\geq 1\%$.²⁰ There was a statistical difference between PD-L1 TPS $\geq 1\%$ and driver gene mutation in NSCLC patients ($P=0.002$).²⁰ There was a statistical difference between PD-L1 TPS $\geq 50\%$ and driver gene mutation in NSCLC patients ($P=0.041$).²⁰ Zheng et al.³⁵ reported a large, multicenter, real-world Chinese cohort study of 6295 non-small cell lung cancer specimens from six centers across the country using the PD-L1 (22C3) assay. This study showed that positive expression of PD-L1 is more common in LUSC and other histological types of NSCLC than in LUAD. In LUAD, the expression of PD-L1 is associated with sex, histological type, metastatic status, and pathological features of lymphatic vascular invasion and visceral pleural invasion ($P<0.001$, $P<0.001$, $P<0.001$, $P<0.001$, and $P<0.001$, respectively).³⁵ Compared with other pathological subtypes, solid and micropapillary LUAD were more likely to have PD-L1 positive expression ($P<0.01$ and $P<0.01$, respectively).³⁵ PD-L1 expression significantly differed between the biopsy and resected samples, especially in patients at stage I ($P=0.018$) and stage IV ($P=0.034$).³⁵

In our study, PD-L1 TPS $\geq 1\%$ accounted for 20.07% of patients with ADC subcentimeter nodules, which was far lower than the findings of previous studies (52.03% and 29.56%),^{17,20} possibly because some patients with lung squamous cell carcinoma (LUSC) were included in

these studies,^{17,35,36} and the expression of PD-L1 in LUSC was higher than that in LUAD (59.44% versus 36.72%, $P<0.001$).³⁵ Moreover, the expression of PD-L1 in the early stages (stage I, 23.06%) of NSCLC is lower than that in advanced stages (stage IV, 59.39%, $P<0.001$).³⁵ Zheng et al.³⁵ also reported a correlation between PD-L1 expression and tumor size (T1 versus T4, 27.70% versus 49.25%, $P<0.001$).

Previous large-scale studies on the expression status of PD-L1 have mainly focused on NSCLC^{17,35,36} and did not independently analyze the expression of PD-L1 in patients with LUAD, especially in those in the very early stages. A few studies have included a small number of patients with AAH/AIS or MIA, all of whom tested negative for PD-L1 expression.^{21,37} However, 378 AAH/AIS nodules and 1016 MIA nodules were included in our study, with PD-L1 expression rates of 2.21% and 7.73%, respectively. This may also indicate immune tolerance in a subset of very early LUAD.³⁸

Toyokawa et al. reported that, among 47 LUAD harboring PD-L1 expression, the frequencies of PD-L1 expression for consolidation/tumor ratios of 0, 0.1, 0.25, 0.26–0.5, and ≥ 0.51 were 6.4%, 2.1%, 4.3%, and 87.2%, respectively ($P=0.007$).³⁷ In our study, PD-L1 was expressed in 9.51%, 9.41%, and 33.33% of pGGO, mGGO, and solid nodules, respectively ($P=0.043$). Consistent with the findings of Toyokawa et al., the expression rate of PD-L1 in solid nodules was much higher than that in pGGO or mGGO nodules. Previous studies have also reported that positive PD-L1

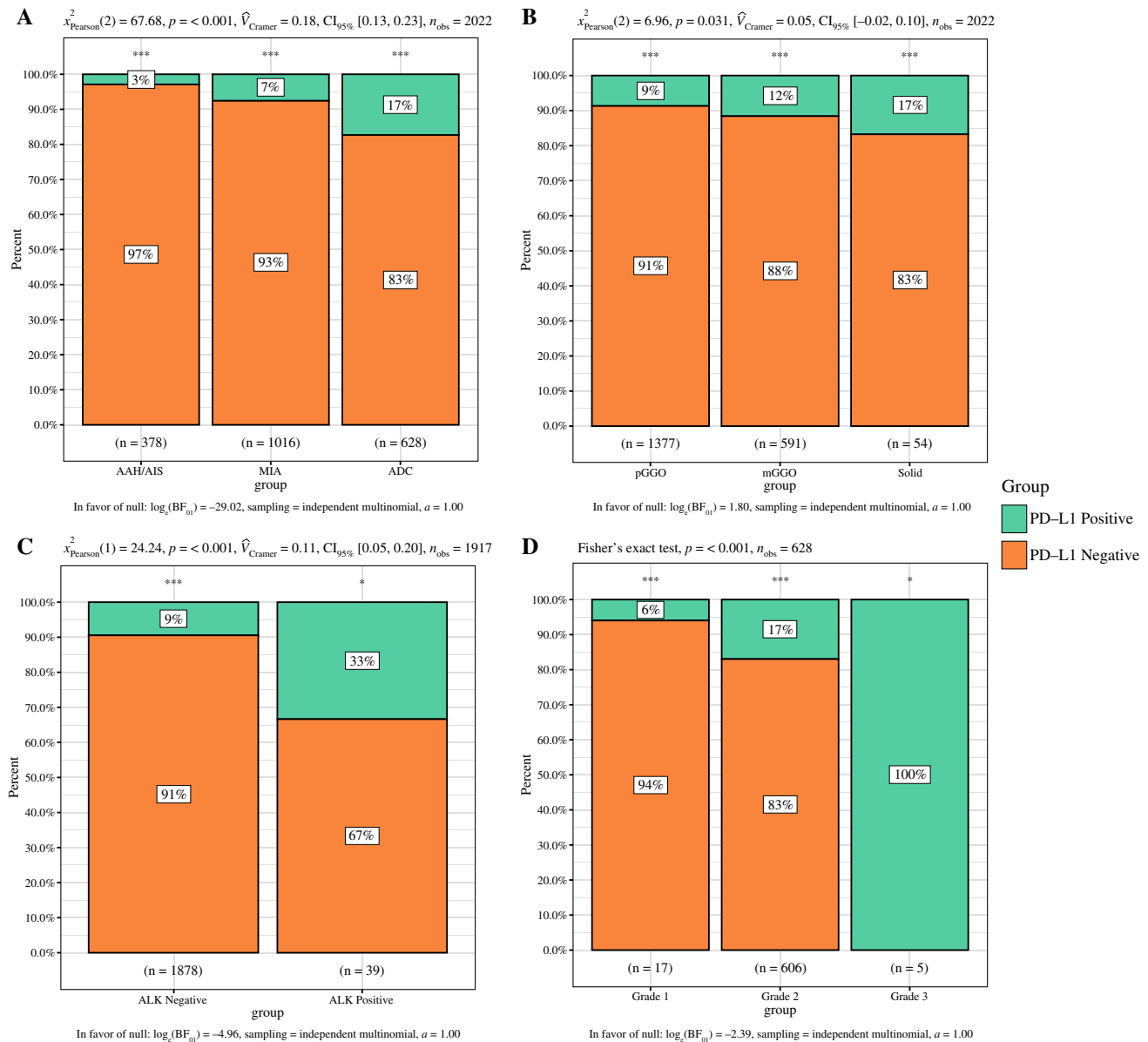


FIG. 5. PD-L1 expression with respect to pathology (A), GGO component (B), ALK rearrangement (C), and the novel IASLC grading system (D). AAH atypical adenomatous hyperplasia, AIS adenocarcinoma in situ, MIA minimally invasive adenocarcinoma, ADC invasive

adenocarcinoma, PD-L1 programmed death ligand 1, GGO ground-glass opacity, pGGO pure GGO, mGGO mixed GGO, ALK anaplastic lymphoma kinase, IASLC International Association for the Study of Lung Cancer. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns $P > 0.05$

expression is an indicator of poor prognosis in early NSCLC; therefore, the expression rate of PD-L1 can represent tumor invasiveness to a certain extent.

Since more than 50% of the subcentimeter nodules in our study did not undergo *EGFR* mutation testing, it is impossible to judge the relationship between PD-L1 expression and *EGFR* mutations in very early LUAD or precancerous diseases. However, previous large-scale studies on Asian populations reported a negative correlation between PD-L1 expression and *EGFR* mutations.^{20,35} Li et al.²⁰ reported that *EGFR* mutations were present in 23.4% of cases of NSCLC

in patients with positive PD-L1 expression. In our study, the proportion of PD-L1 expression-positive patients with *EGFR* mutations was 9.49% (39/411 patients). Even in very early stage LUAD, 30.77% of patients with ALK rearrangements were positive for PD-L1 expression. Previous studies have also reported that ALK-mutated tumors have a higher proportion of PD-L1 expression than have wild-type cases.^{20,35,39}

According to the WHO grading system, solid and micropapillary subtypes are associated with poor prognosis in patients with early LUAD.³³ Several studies have reported

that high PD-L1 expression is mostly found in solid and micropapillary subtypes, suggesting that the expression of PD-L1 tends to increase in more aggressive tumors.^{35,40–42} In the real world, stage IA1 ADC rarely presents as a solid or micropapillary subtype. In our study, there were no WHO high-grade nodules among 628 IA1 stage ADC nodules. However, using the novel IASLC grading system, we found that, the higher the pathological grade, the higher the positive rate of PD-L1 expression. In our study, all patients with IASLC grade 3 tumors tested positive for PD-L1 expression. Previous studies have shown that the IASLC grading system has more advantages than the WHO grading system in differentiating the prognosis of LUAD.³⁴

Our study has several limitations. First, we used PD-L1 22C3 antibodies in all samples, which may have resulted in different results from those obtained with other PD-L1 antibodies, although both BP1 and BP2 studies confirmed similar staining for 22C3 and two other PD-L1 tests (28-8 and SP263).^{27,43} However, we did not take multiple samples from the same patient at the same time, so the consistency between different sampling techniques has not been verified. There is no doubt that we need more data on the expression characteristics of PD-L1 in different situations. Secondly, in the included data, *EGFR* mutation tests were only performed on some nodules, which may not properly represent clinical settings. Although this was a large study, the low incidence of *ALK* rearrangements among patients with early NSCLC may limit the reliability of PD-L1 prevalence estimates in this specific subgroup, although Zheng et al.³⁵ reported higher PD-L1 positive expression in *EGFR* wild-type patients (high: 15.0%, low: 28.9%) than in mutant patients (high: 6.0%, low: 15.3%) ($P < 0.001$). In addition, the frequency of PD-L1 expression was higher in *ALK* fusion cases (high: 18.5%, low: 38.3%) than in wild-type cases (high: 9.7%, low: 20.6%) ($P < 0.001$).³⁵ However, the type of data in our study differed greatly from the above study, and their results are for reference only.³⁵ In the future, we need more data to clarify the relationship between PD-L1 and driver gene expression in very early stage LUAD. Third, all patients included in this study were of East Asian ethnicity; therefore, there is limited reference for PD-L1 expression in patients with very early LUAD and precancerous lesions of other races. The EXPRESS study demonstrated that the prevalence of PD-L1 TPS $\geq 1\%$ and PD-L1 TPS $\geq 50\%$ were similar across regions: 52% for PD-L1 TPS $\geq 1\%$ in Europe, 53% in Asia Pacific, 47% in the Americas, and 55% in other countries; 22% for PD-L1 TPS $\geq 50\%$ in Europe, 22% in Asia Pacific, 21% in the Americas, and 24% in other countries.¹⁷ Therefore, in future studies, patients from multiple races with very early lung cancer should also be included to further elucidate the ethnic differences in PD-L1 expression.

CONCLUSIONS

This study provides a real-world association of PD-L1 expression changes from precancerous lesions to invasive adenocarcinoma in subcentimeter pulmonary nodules based on a large multicenter Chinese cohort. PD-L1 expression is significantly associated with radiologically and pathologically invasive tumors. PD-L1 expression suggests that immune escape occurs in precancerous lesions and very early LUADs. The complexity of the tumor immune micro-environment and significance of PD-L1 expression in the evolution of early-stage LUAD require further investigation.

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