Who Will Benefit Most From PD-L1 Detection and Immunotherapy in Non-small Cell Lung Cancer?

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Abstract

Background: Lung cancer is one of the most lethal cancers worldwide, but studies have shown that the higher the expression of programmed cell death protein 1 ligand 1 (PD-L1) in non-small cell lung cancer (NSCLC), the more likely it will benefit from anti-PD-L1 immunotherapy. The purpose of our study was to collect and analyze abundant clinical samples in order to provide evidence for clinicians and patients who might consider anti-PD-L1 immunotherapy while jointly formulating treatment plans.

Methods: On the one hand, we obtained cases from The Cancer Genome Atlas (TCGA)database, including 498 squamous cell carcinoma of lung cancer (LUSC) patients and 515 adenocarcinoma of lung cancer (LUAD) patients. A heat map of gene expression was drawn. On the other hand, PD-L1 expression was detected in 1,008 NSCLC patients with immunohistochemistry staining (IHC), and we studied the correlation between PD-L1 expression and clinicopathological characteristics.

Results: PD-L1 expression was higher in LUSC than in LUAD at the mRNA level in the TCGA database. In univariate analysis, PD-L1 expression was higher in patients who were males, were LUSC, were smokers, had a tumor diameter >3 cm, had poor differentiation, or had stage III~IV disease. In multivariate analysis, PD-L1 expression was higher in patients who were LUSC or in poor differentiation.

Conclusion: PD-L1 expression was higher in patients who were LUSC or in poor differentiation. We recommend that PD-L1 detection can be mandated for patients who might be suitable candidates for immunotherapy.

1. Introduction

Lung cancer is one of the major causes of cancer deaths worldwide. It is estimated that over the disease has killed more than 1.76 million individuals annually in recent years[1]. There are no obvious symptoms in early-stage lung cancer, thus the disease is typically discovered in the advanced stage. Hence, diagnosis and treatment play significant roles in all research on lung cancer. With the exception of hematoxylin and eosin (HE) staining, immunohistochemical (IHC) staining and even genetic testing are gradually being used for better diagnosis of lung cancer. More precise tumor classification makes it more likely for patients to obtain better prognoses and accurate individual therapy.

Lung cancer has traditionally been divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with the former accounting for approximately 20% and the latter accounting for approximately 80% of all cases. The main subtypes of NSCLC include lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and large cell cancer (LCC)[2]. With great developments in molecular biology and precision medicine, the treatment of non-small cell lung cancer patients has changed from traditional surgery, radiotherapy, and chemotherapy to molecular-targeted therapy and immunotherapy. Due to the discovery of biomarkers and driver genes, a growing number of drugs for targeted therapy and immunotherapy have been approved by the FDA. Three of them have been listed in China and are aimed at PD-L1, EGFR, or ALK [3-5]. Compared with EGFR and ALK, PD-L1 is relatively novel; arguably, the advent

of anti-programmed death cell protein-1 (PD-1) and anti-programmed death ligand 1 (PD-L1) are the most important innovations in the past decade; these innovations have proven to evade the immune system and promote remarkable antitumor activity in NSCLC [4, 6]. Naturally, many researchers have focused on all aspects of PD-L1. Regarding the expression of PD-L1, some studies have shown that the higher the PD-L1 expression, the better the therapeutic effect of PD-L1 immune checkpoint drugs [7-9]. In fact, a phase 3 trial has suggested an association of increased tumoral PD-L1 expression with better clinical efficacy. In particular, KEYNOTE-024 showed that in advanced NSCLC patients with no EGFR mutations or no ALK fusion mutation who showed high tumoral PD-L1 expression (≥ 50%), pembrolizumab seemed to be superior to platinum-based chemotherapy [10]. Additionally, KEYNOTE-010 showed that for advanced NSCLC with tumoral PD-L1 expression ≥ 1%, pembrolizumab was superior to docetaxel [11].

Many papers have researched factors related to the expression of PD-L1, but their conclusions are controversial. Skov BG et al. report that the expression of PD-L1 is only related to the clinical stage of non-small cell lung cancer, which suggests that a lower stage is associated with a lower prevalence of PD-L1 positivity [12], while Zhang et al. detected an association between high PD-L1 expression and male sex and smoking history [13]. To a certain extent, the reason for the dispute is that, on the one hand, the detection reagents are different, some are genetic testing at the mRNA level, while some are immunohistochemistry testing at the protein level, and there are currently three kinds of antibodies in the detection of protein level [14-16]. On the other hand, there are subjective differences in the interpretation of pathological doctors [17]. Therefore, the purpose of our study is to collect and analyze a large number of clinical samples, interpret the mechanism on the basis of a great number of studies in the literature, and come to a conclusion on the correlation between the expression of PD-L1 and the sex, age, histological type smoking history, tumor maximum diameter, TNM stage, and clinical stage of patients. Our aim is to provide evidence for clinicians and patients when they are jointly formulating treatment plans as to whether anti-PD-L1 immunotherapy might be an option.

2. Materials & Methods

2.1 Patients in the study

We established a database of patients who were diagnosed with non-small cell lung cancer in the pathology department of Xiangya Hospital from January 2016 to June 2021. The histological analysis was based on the WHO classification standard of lung tumors in 2015 [18]. Among them, a total of 1,008 patients were selected for PD-L1 immunohistochemistry staining. Next, we analyzed the correlation between PD-L1 expression and patients' clinicopathological characteristics, including sex, age, lung cancer histological type, smoking history, maximum tumor diameter, level of differentiation, TNM stage, and clinical stage. The clinical stage was determined according to the TNM stage of lung cancer in the 8th edition of IASLC in 2017 [19]. In addition, we obtained 1,013 cases from the TCGA database, including 498 squamous cell carcinoma patients and 515 adenocarcinoma patients, and a heat map of gene expression was drawn. Approval to review, analyze, and publish the data in this study was given by the

Ethics Board of XiangYa Hospital of Central South University. Written informed consent for the collection of medical information was obtained from all patients at their first visit.

2.2 Immunohistochemical staining

Immunohistochemistry staining of PD-L1 (22c3) was performed on Dako Link 48. Specimens were cut into 4 mm slices, mounted on a slide (Trajan ® series 3), dewaxed and rehydrated with xylene, washed with graded alcohol, and dried in an oven at 60 °C for 60 minutes. Antigen recovery and inhibition of endogenous peroxidase activity were performed in accordance with the manufacturer's instructions. According to FDA approval, Dako.22c3 was used to detect PD-L1, and when the cell membrane was stained, the tumor cells were considered positive. When the cytoplasmic immune response occurred, the tumor cells were considered negative[14, 20]. The expression of PD-L1 was determined by the tumor proportion score (TPS), which refers to the percentage of living tumor cells showing partial or complete membrane staining (Figure 1). The specimens were divided into 3 types according to TPS: negative (<1%); low expression (1%-49%); and high expression (50%-100%) [21-24]. Each patient's slide reading was completed by two qualified and blinded pathologists. Disagreements were resolved with the help of a third and senior pathologist. Pathologists interpreted the results of PD-L1 IHC by combining hematoxylin and eosin staining slides.

2.3 Statistical methods

Statistical analysis was performed using SPSS 26.0 statistical software. Rank transformation nonparametric tests or Spearman rank correlation tests were conducted to analyze whether PD-L1 expression was different among different groups, such as age and sex. When a difference existed, the $\chi 2$ test was used for linear trend analysis. P values < 0.05 were considered statistically significant.

3. Results

3.1 PD-L1 expression in NSCLC patients

We obtained the gene expression information of NSCLC patients from the TCGA database and drew a heat map (Figure 2A). There is an apparent difference in the mutation status of EGFR and ROS-1 between LUAD and LUSC, while on KRAS, RET, and ALK, obvious discrepancies do not exist. Regarding PD-L1, although the expression level is not very high in general, there are still divergences between the two subtypes: compared to LUSC, PD-L1 is more likely to be close to 0 in LUAD. Furthermore, we made a scatter plot of PD-L1 expression in LUADs and LUSCs to compare the differences (Figure 2B). PD-L1 expression was higher in LUSC than in LUAD (P=0.0155). Of course, this result refers to the expression of PD-L1 at the mRNA level.

3.2 Correlation Between PD-L1 Expression and Clinicopathologic Characteristics of NSCLC Patients

Overall, 1,008 clinical samples were included in our study, and the patients' relevant clinicopathological characteristics are shown in Table 1. From univariate analysis, the expression of PD-L1 was significantly

different from the patients' sex, subtype, smoking history, maximum tumor diameter, differentiation degree, tumor (T), regional lymph node (N), metastasis (M), and clinical stage. The expression of PD-L1 was higher in patients who were male, had lung squamous cell carcinoma, a smoking history, a tumor diameter >3 cm, poor differentiation, and stage III~IV disease (P<0.001). From multivariate analysis, the expression of PD-L1 was significantly different between lung cancer types and differentiation degrees. The expression of PD-L1 was higher in patients who had LUSC or poor differentiation (P 0.001).

3.3 PD-L1 expression differs by subtype and degree of differentiation

LUSCs and LUADs are well known to be the main subtypes of non-small cell lung cancer. In 337 LUSC patients, we found that PD-L1 expression was correlated with T stage, N stage, and clinical stage. In 671 LUAD patients, we found that PD-L1 expression was correlated with sex, smoking history, differentiation degree, maximum tumor diameter, tumor (T), regional lymph node (N) metastasis (M), and clinical stage (Table 2).

In 282 cases of patients with well~ medium differentiation (LUSCs or LUADs), we found that PD-L1 expression was correlated with age, sex, histological type, tumor maximum diameter, tumor (T), regional lymph node (N), metastasis (M), and clinical stage. In 611 patients with poor differentiation (LUSCs or LUADs), we found that PD-L1 expression was correlated with age, tumor (T) and regional lymph node (N) status (Table 3).

The prevalence of PD-L1 expression is demonstrated in Figure 3, where we can see the distribution. Among the 1,008 patients, 14.7% had a TPS ≥50%, 31.1% had a TPS 1% to 49%, and 54.3% had a TPS 1%. In particular, we conducted stratified analysis on LUSCs and LUADs or populations with poor differentiation and well to medium differentiation, and calculated the corresponding negative expression and high expression rates, as well as the 95% confidence interval (Table 4). In a well to medium differentiated adenocarcinoma patient in the early clinical stage (~) or with no regional lymph node metastasis, the possibility of PD-L1 negative expression was 78.4%. In an LUSC patient with lymph node metastasis (N1~N3), the probability of high PD-L1 expression was higher (R2=0.375, 95% CI 21.8%-53.2%). For a patient with a smoking history in the N3 stage, the probability of high PD-L1 expression was as high as 62.5% (95% CI 35.9%-89.1%).

3.4 Pathological characteristics of patients with high expression of PD-L1

We want to screen out the population with high expression of PD-L1, which is the purpose of our study. Therefore, we summarized all the characteristics of the high expression population. Compared with the total 1,008 cases, the high expression group shows something different (Figure 4). NSCLC patients with PD-L1 high expression are more likely to be male, over 65 years old, LUSC, smoker, tumor maximum diameter >3 cm, poor differentiation, T1-T3 stage, regional lymph node metastasis, tumor metastasis or clinical stage III-IV.

4. Discussion

4.1 PD-L1 expression in NSCLC

The most comprehensive study regarding PD-L1 expression was the EXPRESS study, which included 2,435 patients and evaluated PD-L1 expression in samples obtained from local laboratories located in 45 centers in 18 countries. The percentages of patients with PD-L1 TPS ≥50% and TPS ≥1% were 22% vs 51% in Europe, 22% vs 53% in the Asia-Pacific region, 22% vs 47% in the Americas, and 24% vs 54% in other countries. In our study, the ratio is 14.7% vs 54.3%. The prevalence of PD-L1 was similar across geographic regions and broadly consistent with central testing results from a clinical trial screening population[25]; naturally, our results are no exception.

In 2016, Aggarwal et al. found a larger proportion of patients with TPS < 1% in the subgroup with non-squamous cell carcinoma compared with squamous cell carcinoma (26% vs 19%); that is, in LUADs, patients' PD-L1 is more likely to be negative compared with LUSCs [26], which is similar to our findings. In our study, 59.46% of LUADs expressed PD-L1 negatively, while 43.91% of LUSCs expressed PD-L1 negatively. Because PD-L1 is increasingly familiar and recognized by the medical community and testing is affordable, a growing number of patients can access PD-L1 detection. Therefore, with the increase in detection rates, the overall negative rate has also increased, which is understandable. Similarly, in 2017, an association was observed between adenocarcinoma and low PD-L1 expression in NSCLC samples, including Chinese, Japanese, Korean, American, Canadian, British, and French individuals. Additionally, the results indicated that PD-L1 expression was also associated with sex, smoking status, histology, differentiation, tumor size, lymph node metastasis, TNM stage, and EGFR mutation [12]. Moreover, this is one of the few studies that is consistent with our conclusion. However, Chen et al. concluded that a significant difference in PD-L1 expression between LUSCs and LUADs was observed, but in contrast, age, sex, and smoking history were not statistically significant[27]. Gelatti et al. were the first to comprehensively describe PD-L1 expression in the Brazilian population [28]. In China, a study by Song et al. showed that PD-L1 expression was associated with advanced stage, lymph node (LN) metastasis, solid predominant subtype and wild-type epidermal growth factor receptor (EGFR) gene expression [29].

Taken together, there are some divergences in certain aspects between these studies of patients in various regions. However, this may be due to differences in the regional population, sample size, PD-L1 detection reagent, and PD-L1 expression interpretation threshold [17]. Therefore, we should try to find other experiments with small differences for analogy analysis. Despite the differences, a relatively unified opinion is that the expression of PD-L1 is related to the histological subtype of lung cancer. Generally, the expression of PD-L1 in LUADs is more likely to be lower than that in LUSCs, which is consistent with our research results.

4.2 Statistical differences mainly exist in subtypes and differentiation degree

Until now, no general pathway to control PD-L1 expression has been discovered. Depending on stimulus and cell type, the expression of PD-L1 was found to connect with various signaling molecules: p44/42 and/or p38 MAPKs 26, 27, or STAT-1, STAT-3, and IRF-1 28-30 [30]. Also discovered was that STAT3 in LUSC tissues showed increased expression level rather than in LUAD in the Talbot database [31].

Intercellular communication between tumor cells, immune cells, and the stroma characterizes the tumor microenvironment [32]. On the one hand, accumulating data have established that STAT3 directly regulates the expression of PD-L1 [33, 34]; on the other hand, recent findings have shown that STAT3 represses CD8+ T cell chemotaxis and activation [35]. Additionally, PD-L1 expression has bearing on CD8 T cell infiltration within the squamous cell carcinoma microenvironment [36, 37]. Based on these studies, many experiments on the expression of PD-L1, T cells, and STAT3 in squamous cell carcinoma have been carried out. Dun et al. obtained tumor-associated MDSCs derived from patients with head and neck squamous cell carcinoma, and it was found that pSTAT3 was at higher levels of expression to and suppressed T cell proliferation through the actions of arginase 1[38]. Another trial conducted in oral squamous carcinoma cells by Xia revealed that pSTAT3 levels are elevated in CAFs cocultured with oral squamous carcinoma cells [39]. According to these studies, activation of the STAT3 pathway directly upregulates the expression of PD-L1. However, in different lung cancer subtypes, the expression of STAT family members is different. Hence the influence of the STAT family on the expression of PD-L1 in lung cancer and adenocarcinoma is worthy of further discussion.

Consistent with most studies, we concluded that the expression of PD-L1 is related to the type of NSCLC. However, we also believe that a significant difference exists in the differentiation states of lung cancer tissues. We know that cellular differentiation, or simply cell differentiation, is the process through which a cell undergoes changes in gene expression to become a more specific type of cell. These changes are largely due to highly controlled modifications in gene expression and are the study of epigenetics [40-43]. In other words, during the process of cell differentiation, cell morphology tends to exhibit certain histology features, which is the fundamental basis for the microscopic diagnosis of lung squamous cell carcinoma or lung adenocarcinoma. In our study, many statistical results showed that tumor differentiation is related to PD-L1 expression; in particular, a patient with well to medium differentiation, has a 76.0% to 78.4% probability of having a negative PD-L1 expression. Although findings in other studies differ, there are subjective distinctions in the judgment of differentiation in various studies. It is worth noting that our research sample is as large as possible to reduce this error, so we are more confident in our conclusions. Because there is no research on the molecular mechanism as theoretical support for this controversy, more work needs to be conducted.

In our univariate analysis, we also found that PD-L1 expression was significantly different in smoking, sex, and lymph node metastasis. It was found that the efficacy of pembrolizumab, an antibody targeting programmed cell death-1 (PD-1), is correlated with the molecular smoking signature [44-46]. Smoking status may be a predictive marker for better survival as PD-1/PD-L1 inhibitors [47] and total smoking duration may be a predictor of a PD-L1 TPS $\geq$ 50% (P= 0.001)[48], which is consistent with our study. In our study, nearly half of the patients were smokers, and there was a significant difference in their PD-L1 expression (P=0.031). We all know that in China, the proportion of male smokers is much larger than that of female smokers, and our data also show the same (the correlation analysis between smoking history and sex: P = 0.015). So, to some extent, it is comprehensible when smoking history and sex are factors related to the expression of PD-L1. However, gender differences themselves have an impact on the diseases [49-51]. Preclinical studies suggest that the expression of PD-L1 is modulated in an estrogen-

dependent and sex-dependent manner [49, 50]. Sex-related differences in the anticancer immune response have been described in tumor expression levels of PD-L1 across a large spectrum of tumors, including NSCLC [52-54]. This difference in lung cancer treatment based on gender differences also verifies our experimental results and affirms the significance of our study. In our research, there were 99 female patients who were medium or well differentiated with no lymph node metastasis; their negative expression rate was as high as 78.8% (95% CI: 70.6%-87.0%).

Lymph node metastasis is the most common method of metastasis of lung cancer [55-57]. Recent studies have demonstrated that the expression of PD-L1 may be mediated by the PI3K/AKT/PTEN pathway [58-60]. The activated PI3K-AKT pathway plays a central role in the translation of interferon-γ, which is a key regulator of PD-L1 transcription [60, 61]. Up to 50% of penile cancers positively express PD-L1, and PD-L1 is positively related to LNM [62, 63]. In addition, Hu et al. first showed a positive linear correlation between NLR and PD-L1 in penile cancer. Such a linear correlation was also reported in cholangiocarcinoma [64]. These conclusions combined with our experimental results show that there is a relationship between PD-L1 expression and lymph node metastasis. Although the specific mechanism is not completely clear, it also provides guidance for future research. In our study, the expression of PD-L1 in patients without lymph node metastasis was usually negative or low. In contrast, while a smoker patient was in the N3 stage with no distant metastasis, he showed high expression of PD-L1 (R2=62.5%, 95% CI: 35.9%-89.1%).

4.3 Anti-PD-1/PD-L1 immunotherapy

The biological function of PD-1 is to limit T cell activity in peripheral tissues in the inflammatory response of infection and to limit autoimmunity [65, 66]. However, this function translates into the main immune resistance mechanism in the tumor microenvironment. The PD-1/PD-L1 signaling pathway plays an important role in tumor cells escaping immunosurveillance[67, 68]. To evade host immune surveillance, tumors express PD-L1, which interacts with PD-1 on T cells to decrease immune responses. In the past decade, programmed death-1 receptor (PD-1) and programmed death ligand-1 (PD-L1) monoclonal antibodies have shown great potential in the treatment of lung cancer; they have many advantages, including significant antineoplastic activity, induction of long-lasting responses, and good safety. However, the side effects and factors influencing therapy have also been noticed, and only a subset of lung cancer patients will respond to anti-PD-1/PD-L1 therapy [69]. Although four anti-PD1/PDL1 agents-nivolumab, pembrolizumab, atezolizumab, and durvalumab-have been approved by the FDA as significant therapeutic effects for lung cancer [70-73], they have their challenges. For example, the effective cure ratio is not met. Due to the high expense and long treatment period, there is no doubt that the low effective ratio fails to satisfy doctors and patients. Hence, how to identify patients most likely to benefit from the treatment and how to improve its therapeutic effects are questions that remain to be answered.

In clinical practice, inhibitors targeting programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) are gradually being used to treat non-small cell lung cancer, while radiotherapy combined with

chemotherapy is the main treatment for small cell lung cancer in China. Early concurrent chemoradiation is the standard of care for limited-stage SCLC (LS-SCLC). A combination of etoposide and cisplatin or carboplatin remains the mainstay of first-line treatment for ES-SCLC, with the addition of atezolizumab, now becoming standard [74]. Although chemotherapy combined with PD-L1 antibody was approved by the FDA in 2019, it has not been widely used, and there is no routine PD-L1 detection in patients with SCLC. Therefore, this study only recruited patients with squamous cell carcinoma and adenocarcinoma in SCLC.

5. Conclusion

By analyzing the correlation between the expression level of PD-L1 and clinicopathological parameters in 1,008 LUSCs or LUADs, we conclude that the expression of PD-L1 seems to be higher in males, smokers, squamous cell carcinoma tumors with a maximum diameter >3 cm, patients with poor differentiation and/or high TNM stage. Among patients with well- to moderate-differentiated lung adenocarcinoma, clinical stages ～ , with no smoking history, lymph node metastasis or distant metastasis, we would not routinely recommend PD-L1 testing. However, we strongly recommend testing for patients with LUSCs who have lymph node metastasis or poorly differentiated NSCLC in N3, especially smokers in the N3 stage. If high PD-L1 expression is revealed, it will be effective in guiding targeted therapy. Naturally, other categories that are not mentioned should be considered according to the needs of the patient.

Compared with similar studies, our study has a larger sample size. At the same time, we have more detailed groups of patients with the goal of trying to determine which patient populations have a high expression PD-L1 high expression, the focus of our study. However, there are limitations worth noting. We cannot analyze the relationship between PD-L1 expression and prognosis. We also selected cases from 2016 to the present, and the time span causes deviation in clinical information. In the future, our team will continue to study lung cancer-related molecules and hope to compensate for the deficiencies in this study.

Abbreviation

PD-L1, programmed cell death protein 1 ligand 1; TCGA, The Cancer Genome Atlas; NSCLC, non-small cell lung cancer; HE, hematoxylin and eosin; IHC, immunohistochemical; SCLC, small cell lung cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; LCC, large cell cancer; FDA, Food and Drug Administration; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; PD-1, programmed cell death protein 1; TNM stage, Tumor, Region lymph node, Metastasis stage; WHO, World Health Organization; IASLC, International Association for the Study of Lung Cancer; TPS, the tumor proportion score; LN, lymph node

Declarations

Consent for publication

All authors consented for the publication.

Competing interests

There are no competing interests.

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Authors' contributions

Conceived and designed the experiments: LL, X-DS, T-YG. Performed the experiments: LL, XB, ZW, H-QY, H-YL, LS. Analyzed the data: LL, XB, ZW, H-QY. Contributed reagents/materials/analysis tools: H-YL, LS, Z-HJ, X-DS, T-YG. Wrote the paper: LL, XB, X-DS, T-YG. All authors contributed to the article and approved the submitted version.

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Tables

Table 1. Correlation Between PD-L1 Expression and NSCLC Patients Clinicopathologic Characteristic

Variable	n (%)	PD-L1 TPS, n (%)			Univariate analysis		Multivariate analysis
		<1%	1%~49%	≥50%	P value	R value	P value
Sex(n=1008)					0.000	-0.139**	0.497
Male	686(68.1)	345	217	124			
Female	322(31.9)	202	96	24			
Age, y(n=1008)					0.644	0.015	0.873
≤ 65	713(70.7)	388	227	98			
>65	295(29.3)	159	86	50			
Histological type(n=1008)					0.000	-0.168**	0.044
LUSCs	337(33.4)	148	114	75			
LUADs	671(66.6)	399	199	73			
Smoking history(n=435)					0.031	0.103*	0.868
No	218(50.1)	136	64	18			
Yes	217(49.9)	120	56	41			
Tumor maximum diameter(n=411)					0.000	0.213**	0.708
≤3	224(54.5)	157	53	14			
>3	187(45.5)	97	52	38			
Level of differentiation (n=893)					0.000	0.191**	0.003
Well~ Medium	282(31.6)	119	98	65			
Poor	611(68.4)	370	176	65			
Tumor(T) (n=408)					0.000	0.240**	0.172
T1	208(51.0)	114	52	12			
T2	94(23.0)	59	21	14			
T3	61(15.0)	38	13	10			
T4	45(11.0)	11	19	15			
Region lymph node (N) (n=408)					0.000	0.329**	0.089
N0	269(65.9)	192	63	14			
N1	29(7.1)	14	9	6			

N2	63(15.4)	32	17	14			
N3	47(11.5)	14	16	17			
Metastasis(M) (n=408)					0.000	0.255**	0.351
M0	363(89.0)	240	84	39			
M1	45(11.0)	12	21	12			
Clinical Stage(n=408)					0.000	0.328**	0.148
~	282(69.1)	201	65	16			
~	126(30.9)	51	40	35			

Table 2. Difference of PD-L1 distribution in LUADs and LUSCs

Histological type	Variable	P value	R value
LUSC			
	Tumor (T)	0.049	0.187*
	Region lymph node (N)	0.001	0.323**
	Clinical Stage	0.003	0.278**
LUAD			
	Sex	0.003	-0.116**
	Smoking history	0.035	0.118*
	Differentiation	0.000	-0.200**
	Tumor Maximum diameter	0.001	0.195*
	Tumor (T)	0.002	0.184**
	Region lymph node (N)	0.000	0.337**
	Metastasis (M)	0.000	0.324**
	Clinical Stage	0.000	0.361**

Table 3. Difference of PD-L1 distribution in different differentiation groups

Differentiation	Variable	P value	R value
Well~ Medium			
	Age	0.045	-0.081*
	Sex	0.000	-0.158**
	Histological type	0.000	-0.226**
	Tumor Maximum diameter	0.010	0.146*
	Tumor (T)	0.027	0.127*
	Region lymph node (N)	0.000	0.280**
	Metastasis(M)	0.000	0.246**
	Clinical stage	0.000	0.306**
Poor			
	Age	0.017	0.142*
	Tumor (T)	0.048	0.200*
	Region lymph node (N)	0.029	0.219*

Table 4. Summary of Groups with High Negative-Rate(R0) or High-Expression Rate(R2)

Classification	PD-L1 TPS, n (%)			Sum	R0(95%CI)	R2(95%CI)
	<1%	1%~49%	≥50%			
Well~ Medium differentiation &Clinical stage ~ &LUADs	145	36	4	185	0.784(0.724-0.884)	0.022(0.000-0.430)
Well~ Medium differentiation &LUADs &N0	138	36	5	179	0.771(0.709-0.833)	0.028(0.004-0.052)
No Smoking &LUADs &M0	113	33	4	150	0.753(0.684-0.823)	0.027(0.001-0.053)
Well~ Medium differentiation &N0 &M0	173	44	7	224	0.772(0.717-0.828)	0.031(0.008-0.054)
Well~ Medium differentiation &Clinical stage ~	184	48	10	242	0.760(0.706-0.814)	0.041(0.016-0.067)
N1~N3 & LUSC	15	10	15	40	0.375(0.218-0.532)	0.375(0.218-0.532)
Poor differentiation &N3	7	9	13	29	0.241(0.076-0.407)	0.448(0.256-0.641)
Smoking &N3 &M0	3	3	10	16	0.188(0.040-0.460)	0.625(0.359-0.891)

R0 means the rate of being negative in PD-L1 detection, R2 means the rate of being high expression in PD-L1 detection.

Figures

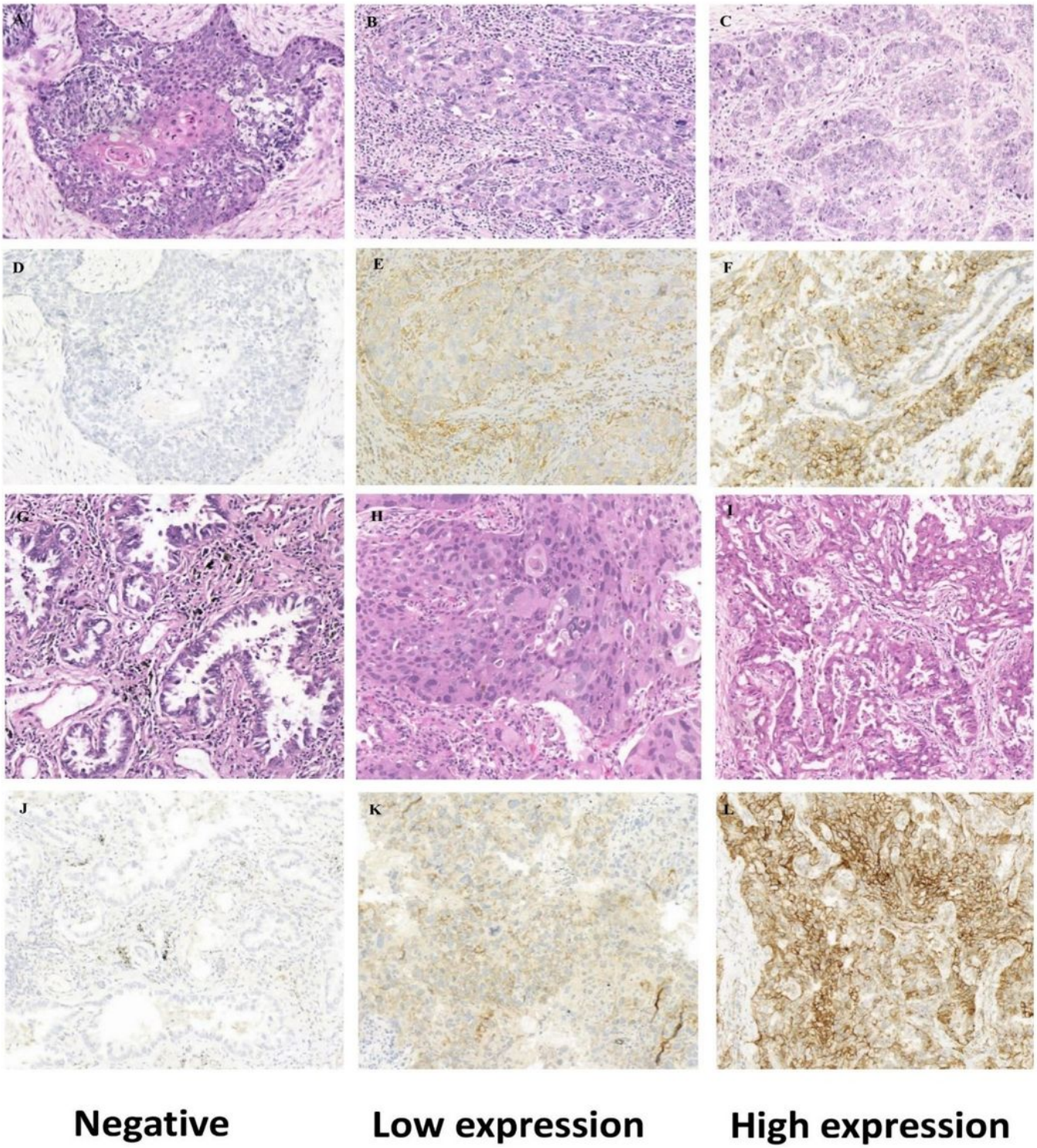

Figure 1

Typical patterns of HE staining and corresponding PD-L1 IHC staining (200X). A~F are about LUSCs. A, B and C are HE staining; D, E, F are the corresponding PD-L1 IHC staining: D is negative (<1%), E is low expression (1%~49%), and F is high expression (≥50%). G~L are about LUADs. G, H, and I are HE staining; J, K, L are the corresponding PD-L1 staining: J is negative, K is low expression, and L is high expression.

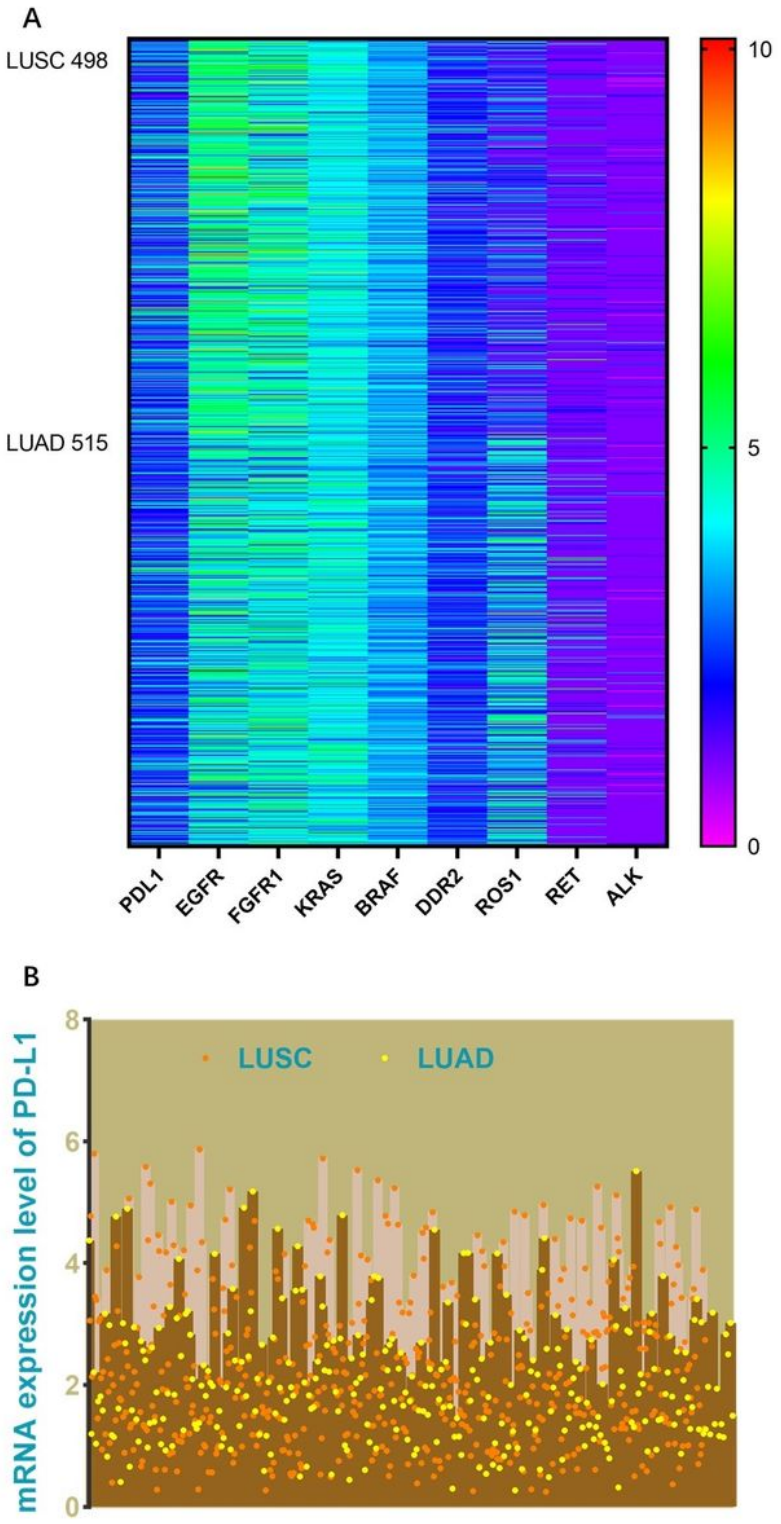

Figure 2

A heat map and a scatter plot for gene expression. (A) shows a heat map of the gene expression information of NSCLC patients from TCGA database. PD-L1 and other driving genes were detected in patients with LUAD or LUSC. Regarding PD-L1, although the expression level is not very high in general, there are still divergences between the two subtypes: compared to LUAD, PD-L1 expression is higher in

LUSC. (B) shows scatter plot of PD-L1 expression in LUADs and LUSCs. Compared to LUAD, PD-L1 expression is higher in LUSC at the mRNA level (P=0.0155).

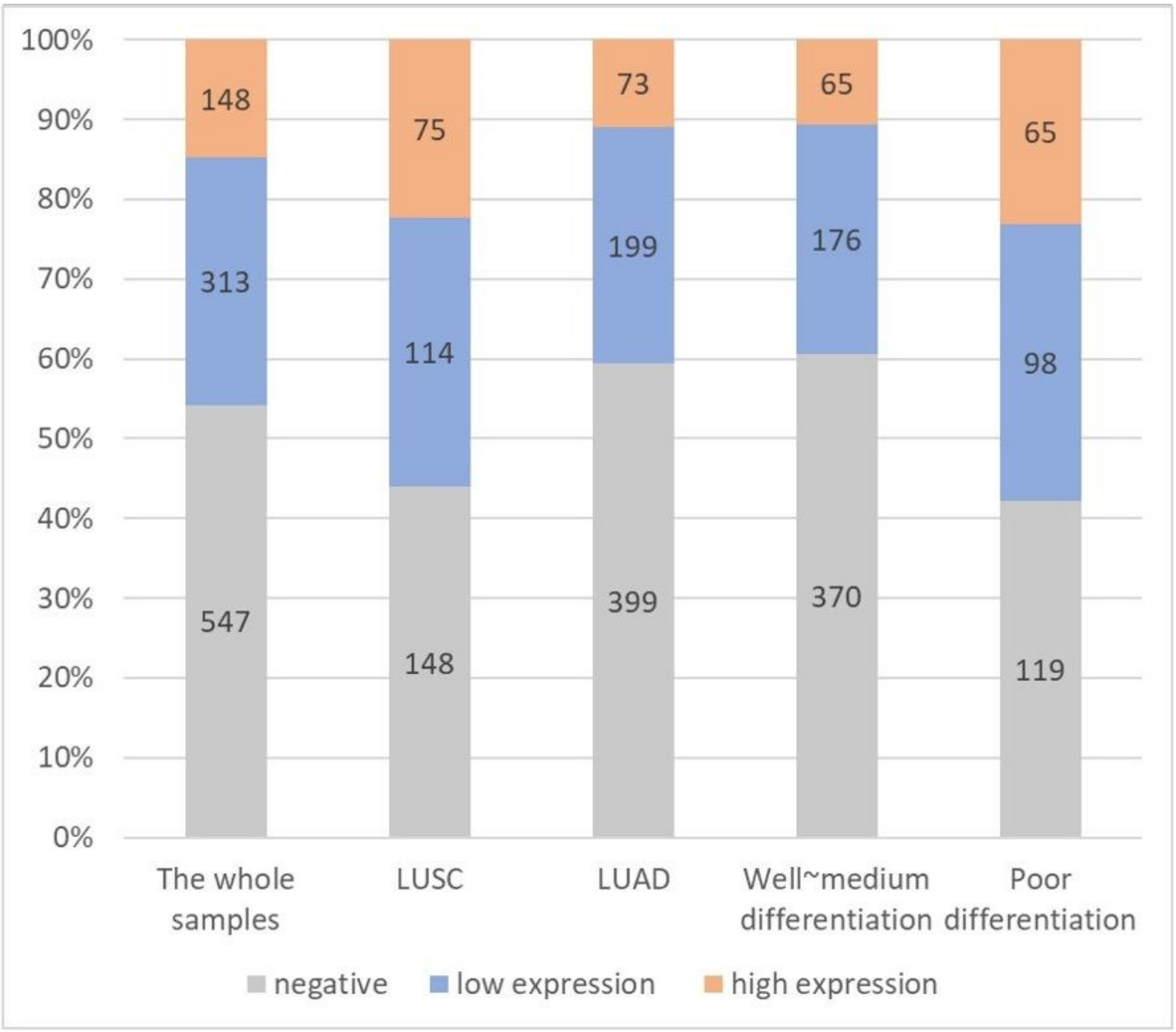

Figure 3

Distribution of PD-L1 expression in 1008 lung cancer samples. In the whole samples, almost half (547 in 1008) of PD-L1 expression was negative, while the proportion of high expression was 14.7%. By contrast, in patients with LUSC, the rate of PD-L1 ≥ 50% is higher than in the whole samples, while the rate of being negative is descending, as is the patients in poor differentiation. In contrast, in patients with LUAD or in well~ medium differentiation, the change in the rate of being negative and being high are completely opposite.

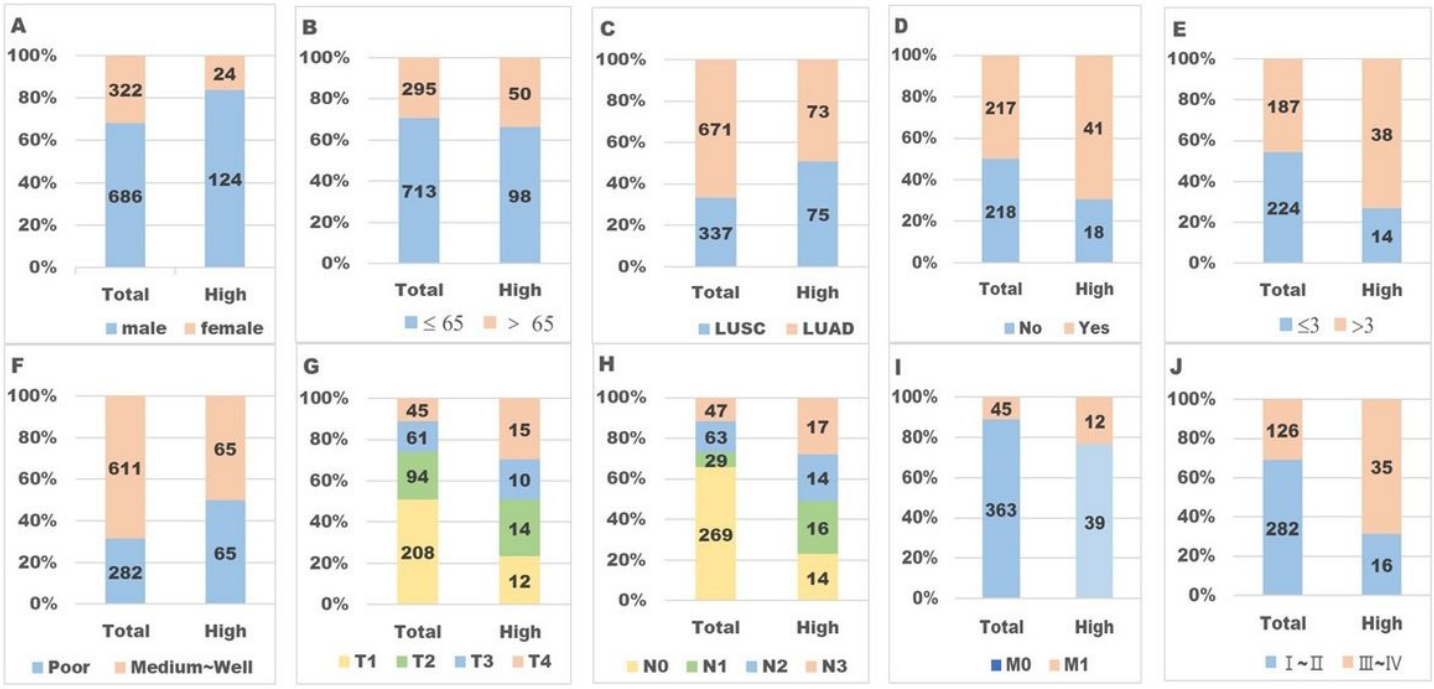

Figure 4

Pathological characteristics of patients with high expression of PD-L1. “Total” refers to the whole sample, and “high” refers to the high expression group. (A) male or female. (B) age <65 or age ≥65. (C)LUSC or LUAD. (D) NO smoking or smoking. (E) tumor maximum diameter ≤3 cm or >3 cm. (F) differentiation state: well~ medium or poor. (G) T stage. (H) N stage. (I) M stage. (J) clinical stage. By comparing patients’ different pathological characteristics between the whole samples and the high expression group, we found some significant differences ($p < 0.01$). The one expressing PD-L1 highly is more likely to be a patient who is male (A), over 65 years old(B), LUSC (C), a smoker (D), with tumor diameter >3 cm (E), in poor differentiation (F), with a higher TNM stage (G~ I), and in clinical stage ~ (J).