2024 Volume 2, Issue 2
Big Data Analytics for Healthcare
ISSN: 2630-4767 (Online)

Bioinformatics Evaluation of the Clinicopathological and Prognostic Significance of PD-L1 Expression in Lung Adenocarcinoma

Nana Du[1], Lu Zhang[2], Jingting Min[1], Ran An[3], Zhenghong Li[2]*
[1]School of Basic Medical Sciences, Bengbu Medical College, Bengbu, Anhui 233030, China
[2]School of Life Sciences, Bengbu Medical College, Bengbu, Anhui 233030, China
[3]School of Public Foundation, Bengbu Medical College, Bengbu, Anhui 233030, China

***Corresponding author:** Zhenghong Li, lizhbbmc@163.com

Abstract:

Objective: To evaluate the clinicopathological and prognostic significance of PD-L1 expression in lung adenocarcinoma using bioinformatics and validate its expression and clinicopathological correlation through immunohistochemistry. *Methods:* The GEPIA database was utilized to analyze the quantitative expression of PD-L1 mRNA, which was further validated using immunohistochemistry. The impact of PD-L1 expression on the prognostic value of lung adenocarcinoma was assessed using the Kaplan-Meier Plotter. Immune cell infiltration in lung adenocarcinoma was analyzed with Timer 2.0. Additionally, the differential expression of various PD-L1 subtypes in lung adenocarcinoma was examined using the TCGA module of the UALCAN database. *Results:* Bioinformatics analysis revealed that PD-L1 expression was significantly lower in cancer tissues compared to normal tissues. Immunohistochemistry results demonstrated high expression of PD-L1 in lung adenocarcinoma tissues, whereas low or no expression was observed in adjacent normal tissues. No significant differences in PD-L1 expression were found among different ages, genders, clinical stages, or lymph node metastasis statuses. However, PD-L1 expression was significantly correlated with overall survival and first progression-free survival of patients. *Conclusion:* The expression of PD-L1 is associated with the survival prognosis of patients with lung adenocarcinoma.

Keywords:

PD-L1
Lung adenocarcinoma
Immunohistochemistry
Bioinformatics

Online publication: December 27, 2024

1. Introduction

Lung cancer is one of the cancers with the highest incidence and fatality rates worldwide, and it is also the most common cause of cancer-related deaths. Lung cancer mainly includes small cell lung cancer and non-small cell lung cancer (NSCLC), with approximately 80% of lung cancers being NSCLC. NSCLC further comprises lung adenocarcinoma and lung squamous cell carcinoma, with lung adenocarcinoma accounting for the majority [1]. This study primarily focuses on lung adenocarcinoma.

PD-L1, also known as B7-H1, is a type I transmembrane glycoprotein encoded by the calcium glycoprotein gene on human chromosome 9. It belongs to the B7 superfamily of costimulatory ligands and consists of 7 exons. Its expression is considered an effective immunosuppressant mediated by T-cells in antitumor responses [2]. Abnormal expression of PD-L1 can occur in cancer cells of some malignancies [3,4]. Tumor-associated PD-L1 expression is correlated with poor pathological features and prognosis [5]. The PD-1/PD-L1 pathway is recognized as an effective immunosuppressive checkpoint, and inhibiting the interaction between PD-L1 and its receptor PD-1 can enhance T-cell responses *in vitro* and mediate clinical antitumor activity. It has been applied in lung cancer, primary renal cell carcinoma, and ovarian cancer [7,8]. Studies have found that PD-L1 expression is associated with patient prognosis [9]. Overexpression of PD-L1 in NSCLC often indicates a poor prognosis. However, there is no consensus on the relationship between PD-L1 protein expression in NSCLC and its clinicopathological features [8]. Therefore, this study aims to evaluate the relationship between PD-L1 expression in lung adenocarcinoma and clinicopathological characteristics and prognosis through bioinformatics and validate it using immunohistochemical staining methods.

2. Materials and methods

2.1. Bioinformatics analysis

The quantitative expression of mRNA in the GEPIA (Gene Expression Profiling Interactive Analysis) database was used to analyze the differences in PD-L1 expression between tumor tissues and normal tissues, as well as its impact on pathological staging. The online tool Kaplan-Meier Plotter was utilized to evaluate the effect of PD-L1 expression on the prognosis of lung adenocarcinoma, employing Kaplan-Meier survival curves to analyze the relationship between PD-L1 expression and overall survival (OS), first-progression survival (FP), and post-progression survival (PPS) in lung adenocarcinoma patients.

Timer 2.0 is an online resource database that uses deconvolution as a statistical method to analyze immune cell infiltration in various cancers. The correlation between PD-L1 expression and the infiltration levels of immune cells (B cells, CD8+ T lymphocytes, CD4+ T lymphocytes, macrophages, neutrophils, and dendritic cells) was examined through gene analysis in the immune association module. The UALCAN database is an interactive web portal primarily used for cancer genomics data analysis. The TCGA module was used to analyze PD-L1 expression in lung adenocarcinoma and its differences related to gender and age.

2.2. Immunohistochemical staining

Sixty-two cases of lung adenocarcinoma tissues were selected from patients who visited the First Affiliated Hospital of Bengbu Medical College in 2020 and underwent surgical treatment. Twenty cases of adjacent normal tissues were chosen as controls. Immunohistochemical staining was performed using PD-L1 antibodies. The sample included 24 males and 38 females, with 3 cases aged 50 or below and 59 cases aged above 50. The clinicopathological data of the patients were obtained through the case management system of the First Affiliated Hospital of Bengbu Medical College.

2.2.1. Main reagent

PD-L1 antibody was purchased from Wuhan Sanying (Proteintech Group) Biotechnology Co., Ltd.

2.2.2. Immunohistochemical detection of PD-L1 expression

Lung adenocarcinoma tissues and normal tissues were fixed with 4% neutral formalin, embedded in paraffin, and sectioned at 4 μm. After dewaxing and hydration, cell permeabilization and blocking, antigen retrieval, and serum blocking, the primary antibody was diluted at 1:2000 and applied to the slide for 16 hours at 4℃,

followed by incubation with a goat anti-rabbit secondary antibody for 15 minutes. After DAB staining, the sections were dehydrated with a gradient concentration of ethanol, cleared with xylene, mounted with neutral balsam, and then observed under a microscope.

2.2.3. Result analysis

The results were evaluated by two pathologists using a blinded method. Evaluation was based on the positive rate of cell staining and staining intensity. The positive rate was scored as 0 (0%), 1 (0–10%), 2 (10–50%), and 3 (> 50%). The staining intensity was scored as 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The final score was calculated by multiplying the positive rate score by the staining intensity score. Scores of 0–1 were considered negative (-), 2 was considered weakly positive (±), 3–5 were considered positive (+), 6–8 were considered strongly positive (++), and > 8 was considered very strongly positive (+++).

2.3. Statistical analysis

Data were analyzed using SPSS 26.0 software. The chi-square test was used to compare differences in PD-L1 expression between different groups. Kaplan-Meier method and log-rank test were used to describe and compare survival rates between different groups. The significance level was set at $\alpha = 0.05$.

3. Results

3.1. Expression of PD-L1 in lung adenocarcinoma tissues and normal tissues

3.1.1. Bioinformatics

Analysis of PD-L1 protein expression levels in lung adenocarcinoma tissues using the GEPIA and UALCAN databases showed that PD-L1 protein expression was lower in lung adenocarcinoma tissues compared to adjacent normal tissues ($P < 0.001$) (**Figure 1**).

3.1.2. Immunohistochemistry

Since PD-L1 is a transmembrane protein, its positivity is determined by the presence of brown-yellow granules in the cytoplasm and/or cell membrane. Immunohistochemical staining showed that the positive expression rate of PD-L1 was 87.1% (54/62) in 62 cases of lung adenocarcinoma tissues, while no positive expression of PD-L1 was observed in 20 cases of adjacent normal tissues. There was a statistically significant difference in the positive expression rate of PD-L1 between the two groups ($\chi^2 = 51.014$, $P < 0.001$) (see **Table 1** and **Figure 2**).

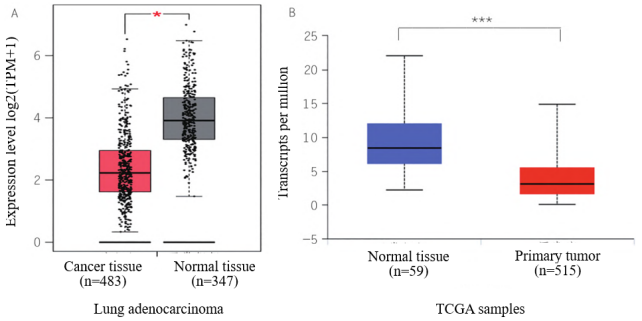

Figure 1. Analysis of PD-L1 expression in lung adenocarcinoma using GEPIA and UALCAN databases. (A) The expression level of PD-L1 in normal tissues and cancer tissues of lung adenocarcinoma in the GEPIA database; (B) The expression level of PD-L1 in normal tissues and primary tumors of lung adenocarcinoma in the UALCAN database. *$P < 0.001$.

Table 1. Expression of PD-L1 in lung adenocarcinoma tissue and normal tissue

Grouping	PD-L1 pathological score					
	-	±	+	++	+++	Total
Lung adenocarcinoma tissue	2	6	18	36	0	62
Normal tissue	20	0	0	0	0	20

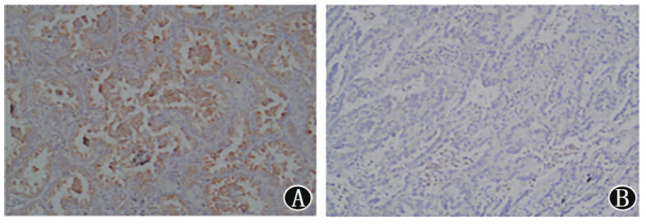

Figure 2. Immunohistochemical detection of PD-L1 expression in lung adenocarcinoma tissue and normal tissue. (A) Immunohistochemical staining image of a clinical specimen of lung adenocarcinoma tumor (SP × 400); (B) Immunohistochemical staining image of a clinical specimen of normal tissue (SP × 400).

3.2. Impact of PD-L1 expression on the prognosis of lung adenocarcinoma patients

The Kaplan-Meier Plotter was used to evaluate the prognostic value of PD-L1 expression in lung adenocarcinoma. Survival curve analysis was performed

to assess the overall survival (OS), first progression (FP), and post-progression survival (PPS) of lung adenocarcinoma patients with different PD-L1 expression levels. The results suggested that high PD-L1 expression levels were significantly associated with OS and FP ($P < 0.05$). Moreover, higher PD-L1 expression levels in tumor tissues were associated with lower OS and FP in lung adenocarcinoma patients (**Figure 3A** and **Figure 3B**). However, PD-L1 expression levels were not correlated with PPS (**Figure 3**).

3.3. Relationship between PD-L1 expression and clinicopathological features of lung adenocarcinoma patients

3.3.1. Bioinformatics

The association between PD-L1 expression in lung adenocarcinoma and gender and age was analyzed using the TCGA module in the UALCAN database. The relationship between PD-L1 expression in lung adenocarcinoma tissue and pathological grading was analyzed using the GEPIA database. The results showed statistically significant differences in CD274 expression of the PD-L1 gene between genders, ages, and lymph node metastasis compared to normal tissue ($P < 0.05$), as shown in **Figure 4**. However, the GEPIA database indicated that there was no statistically significant difference between PD-L1 expression and pathological staging ($P > 0.05$), as illustrated in **Figure 5**.

3.3.2. Immunohistochemistry

The results showed no statistically significant difference in PD-L1 expression based on gender, age, lymph node metastasis, pathological stage, and degree of differentiation in patients with lung adenocarcinoma ($P > 0.05$).

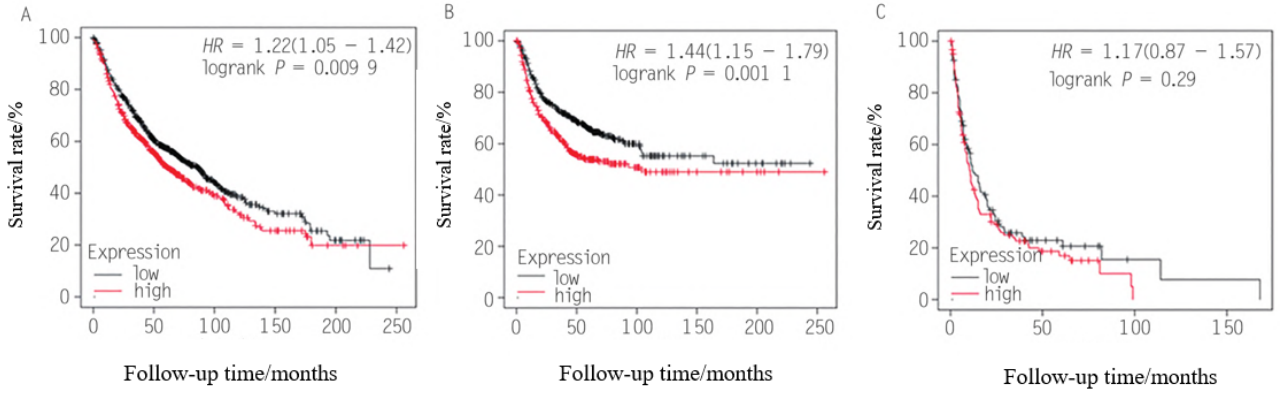

Figure 3. Bioinformatics analysis of the relationship between PD-L1 expression and prognosis in patients with lung adenocarcinoma. (A) The relationship between PD-L1 expression and overall survival rate; (B) The relationship between PD-L1 expression and first progression survival; (C) The relationship between PD-L1 expression and progression-free survival.

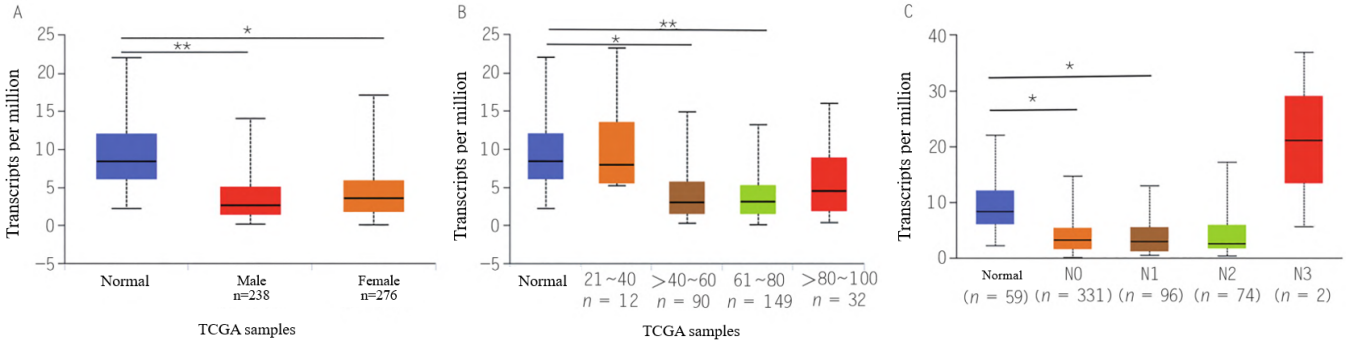

Figure 4. Relationship between PD-L1 expression and clinicopathological features in lung adenocarcinoma. (A) CD274 expression levels based on gender differences in lung adenocarcinoma patients; (B) CD274 expression levels based on age differences in lung adenocarcinoma patients; (C) CD274 expression levels based on lymph node metastasis in lung adenocarcinoma patients. *$P < 0.01$, $P < 0.05$.

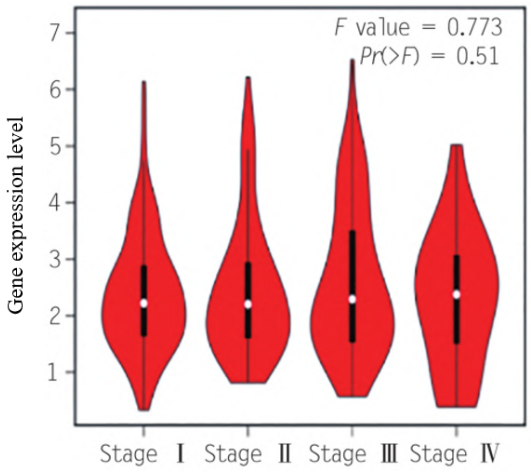

Figure 5. Relationship between PD-L1 expression and pathological staging.

3.4. Correlation between PD-L1 and the degree of immune infiltration

Based on the TIMER database, the correlation between PD-L1 and the degree of immune infiltration was analyzed and the infiltration levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells were measured. The results indicated that CD8+ T cells, B cells, NK cells, and dendritic cells (DCs) are associated with a good prognosis in lung adenocarcinoma. To evaluate the correlation between PD-L1 expression and immune cells in the tumor microenvironment of lung adenocarcinoma, the TIMER database was used to determine the infiltration levels of CD8+ T cells, B cells, NK cells, and DCs. The results showed that CD8+ T cells (R = 0.457, $P < 0.01$), B cells (R = -0.086, $P > 0.05$), NK cells (R = -0.261, $P < 0.001$), and DCs (R = 0.451, $P < 0.01$) had significant correlations with PD-L1 expression. Specifically, there was a positive correlation between the infiltration levels of CD8+ T cells and DCs and PD-L1 expression in lung adenocarcinoma, while there was a negative correlation between the infiltration levels of B cells and NK cells and PD-L1 expression (**Figure 6**).

4. Discussion

Lung cancer currently has the highest mortality rate among all cancers globally, with a 5-year survival rate of less than 10%. It is also very common in clinical practice [9]. Every year, there are 22 million new cases and 17.9 million deaths [10]. This study found inconsistencies between the bioinformatics of PD-L1 and immunohistochemistry results. This may be due to differences in the clone numbers of PD-L1 detection antibodies currently used, and there is no unified standard for positive interpretation. Therefore, there are large discrepancies in results among different studies, and a unified positive interpretation standard should be established as soon as possible [11]. Meanwhile, the TCGA database contains expression profile data obtained from mRNA expression analysis of tumor samples. Immunohistochemistry, on the other hand, is based on immunological and histochemical principles, allowing for *in situ* qualitative, localization, or quantitative studies of certain chemical components in tissue sections or cell samples. It utilizes the high specificity of the binding between antibodies and antigens, which may also contribute to the inconsistencies in the results. In summary, although the results of the two methods are

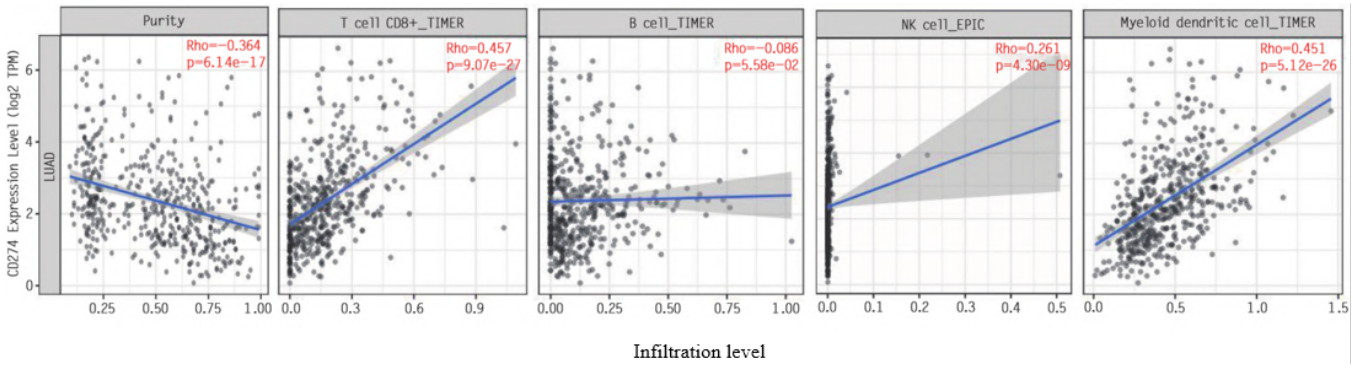

Figure 6. Correlation between PD-L1 and the degree of immune cell infiltration in lung adenocarcinoma.

inconsistent, they provide some indication for subsequent research. Through bioinformatics analysis, this study found that high PD-L1 expression levels are significantly correlated with patients' OS and FP. Higher PD-L1 expression levels in tumor tissues result in lower OS and FP for lung adenocarcinoma patients. PD-L1 expression in healthy individuals is higher than in lung adenocarcinoma patients, which may be due to the significant difference in the number of healthy individuals and tumor patients in the TCGA database. Nevertheless, previous studies have found that improvement in overall survival is not related to PD-L1 expression [12], while other studies have shown that PD-L1 expression is an unfavorable prognostic factor for non-small cell lung cancer [13].

A meta-analysis evaluating the correlation between PD-L1 expression and OS found that overexpression of PD-L1 is associated with shorter OS in lung cancer [14]. Although some results are inconsistent, studies have shown that PD-L1-positive patients have a longer survival period [15], indicating that it is reasonable to correlate PD-L1 expression with clinical outcomes. In this study, bioinformatics analysis revealed that PD-L1 expression is related to patients' age, gender, and lymph node metastasis, but not to pathological staging. Immunohistochemistry also showed that PD-L1 expression is not associated with the patient's age, gender, lymph node metastasis, pathological staging, or degree of differentiation. Immunohistochemistry detects whether there is a statistically significant difference in PD-L1 expression among lung adenocarcinoma tumor tissues. Compared to normal tissues, bioinformatics analysis yielded statistically significant results, but the findings between groups were almost consistent with those obtained through immunohistochemistry. Some literature suggests that PD-L1 expression is not related to patients' age, gender, or pathological staging but is associated with lymph node metastasis [12], while other studies indicate no correlation between PD-L1 expression and lymph node metastasis [11]. These contradictions may be due to differences in antibodies, detection techniques, and sample sizes. Bioinformatics analysis also demonstrated a positive correlation between PD-L1 expression and CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells, suggesting that the infiltration of immune cells has a certain impact on the occurrence and development of lung adenocarcinoma. Traditional treatment methods for lung adenocarcinoma include chemotherapy, radiotherapy, and surgery, but the prognosis for these patients remains poor. Therefore, a new therapeutic approach is needed to improve the current situation [16]. PD-L1 and PD-1 are important immunosuppressive molecules. The PD-L1/PD-1 pathway can inhibit T-cell activity, helping tumors achieve immune evasion. PD-L1/PD-1 activating inhibitors have shown significant efficacy in certain types of tumors and are widely recognized in clinical oncology treatment [17]. Therefore, this study provides valuable insights into patient prognosis and clinical treatment.

Funding

National Natural Science Foundation of China (Project No.: 81472656); Key Research and Development Program of Anhui Province (Project No.: 201904a07020019); Major Natural Science Research Projects of Colleges and Universities in Anhui Province (Project No.: 2022AH040224); Natural Science Project of Bengbu Medical College (Project No.: Byycx23084)

Disclosure statement

The authors declare no conflict of interest.

References

[1] Ni W, Wang X, Wang QS, et al., 2020, Expression and Prognostic Significance of c-Met and GPC1 in Non-Small Cell

Lung Cancer. Journal of Practical Oncology, 35(6): 506.

[2] Thompson RH, Gillett MD, Cheville JC, et al., 2005, Costimulatory Molecule B7-H1 in Primary and Metastatic Clear Cell Renal Cell Carcinoma. Cancer, 104(10): 2084.

[3] Konishi J, Yamazaki K, Azuma M, et al., 2004, B7-H1 Expression on Non-Small Cell Lung Cancer Cells and Its Relationship with Tumor-Infiltrating Lymphocytes and Their PD-1 Expression. Clinical Cancer Research, 10(15): 5094.

[4] Xiao JX, Bai PS, Lai BC, et al., 2005, B7 Molecule mRNA Expression in Colorectal Carcinoma. World Journal of Gastroenterology, 11(36): 5655.

[5] Loos M, Langer R, Schuster T, et al., 2011, Clinical Significance of the Costimulatory Molecule B7-H1 in Barrett Carcinoma. The Annals of Thoracic Surgery, 91(4): 1025.

[6] Dumitru A, Dobrica EC, Croitoru A, et al., 2022, Focus on PD-1/PD-L1 as a Therapeutic Target in Ovarian Cancer. International Journal of Molecular Science, 23(20): 12067.

[7] Yu H, Boyle TA, Zhou CC, et al., 2016, PD-L1 Expression in Lung Cancer. Journal of Thoracic Oncology, 11(7): 964.

[8] Xie WY, He Y, Fu X, et al., 2022, Relationship Between Histological Features Related to Lung Adenocarcinoma and PD-L1 Expression and EGFR Mutation Types. Chinese Journal of Clinical Oncology, 49(15): 769.

[9] Zhou RG, Wang Y, Chen JS, et al., 2022, Relationship Between c-MET, BRAF, ROS1 Gene Mutations and Clinical Features in Non-Small Cell Lung Cancer. Journal of Molecular Diagnostics and Therapy, 14(7): 1129.

[10] Thai AA, Solomon BJ, Sequist LV, et al., 2021, Lung Cancer. Lancet, 398(10299): 535.

[11] Qin WH, Zhang HW, Yang BJ, et al., 2022, Study on the Correlation Between PD-L1 Expression and Clinicopathological Data as Well as Gene Mutations in Patients With NSCLC. Journal of Clinical Psychosomatic Diseases, 28(3): 11.

[12] Yang X, Qi C, Ji M, 2019, PD-L1 Status and Survival in Patients With Lung Cancer. JAMA, 322(8): 783.

[13] Zhu JM, 2022, Correlation Study of LAG-3, FGL1, PD-L1, and CD8-+T Cell Expression in Lung Adenocarcinoma, thesis, Xinjiang Medical University.

[14] Li H, Xu Y, Wan B, et al., 2019, The Clinicopathological and Prognostic Significance of PD-L1 Expression Assessed by Immunohistochemistry in Lung Cancer: A Meta-Analysis of 50 Studies With 11,383 Patients. Translational Lung Cancer Research, 8(4): 429.

[15] Shen X, Zhao B, 2018, Efficacy of PD-1 or PD-L1 Inhibitors and PD-L1 Expression Status in Cancer: Meta-Analysis. BMJ, 362: k3529.

[16] Gafeer MM, Hosny Mohammed K, Ormenisan-Gherasim C, et al., 2018, Diagnostic Utility of PD-L1 Expression in Lung Adenocarcinoma: Immunohistochemistry and RNA in Situ Hybridization. Applied Immunohistochemistry & Molecular Morphology, 26(8): e86.

[17] Zhou SM, Zhu JF, Xu JW, et al., 2022, Anti-Tumor Potential of PD-L1/PD-1 Post-Translational Modifications. Immunology, 167(4): 471.