Pathology & Oncology Research (2019) 25:1437–1443
https://doi.org/10.1007/s12253-018-0501-x

ORIGINAL ARTICLE

Expression of Programmed Death-Ligand 1 in Laryngeal Carcinoma and its Effects on Immune Cell Subgroup Infiltration

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Received: 24 October 2017 / Accepted: 15 October 2018 / Published online: 25 October 2018

Abstract

To study the expression of programmed death-ligand 1 (PD-L1), and its effects on $CD8^+$ tumor infiltrating lymphocytes (TILs) and tumor associated macrophages (TAMs) in human laryngeal squamous cell carcinoma. Sixty-nine patients with laryngeal carcinoma and 10 with vocal cord leukoplakia received tumor resection at Neck Surgery Department in the Second Affiliation Hospital of Jilin University (Changchun, Jilin) from Jan. 2010 to Dec. 2015. The expressions of PD-L1, CD8, CD16 and CD206 in laryngeal carcinoma, paracancerous and vocal cord leukoplakia tissues were detected with immunohistochemistry. The associations between PD-L1 expression and clinicopathologic features, expression of TAMs and $CD8^+$ T cell infiltration were analyzed. Expression of PD-L1 is significantly higher in laryngeal carcinoma than in paracancerous or leukoplakia tissue. The expression of PD-L1 is closely associated with stage of laryngeal cancer, histological differentiation and neck lymphatic metastasis. PD-L1 expression is negatively correlated with the number of $CD8^+$ TILs and $CD16^+$ cells (M1 type TAMs), while is positively associated with $CD206^+$ (M2 type TAMs). PD-L1 is highly expressed in the laryngeal cancer with the tumor microenvironment immunosuppression.

Keywords PDL-1 · TAMs · Laryngeal squamous cell carcinoma · Immunosuppression

Introduction

Immunosuppression plays an important role in the occurrence and development of laryngeal carcinoma, including immune surveillance defects, abnormal function of antigen presenting cells (APC) and T cells, and abnormal secretion of immune cells [1]. Recent investigations reveal that programmed death-ligand 1 (PD-L1) is highly expressed in the tumor microenvironment and related with infiltration of tumor-associated macrophages (TAMs), which plays important roles in immunosuppression [2, 3]. PD-L1 binds to programmed death 1 (PD-1), one of the members of CD28 family and inhibits the activation, proliferation and cytokine secretion of CD8+ cytotoxic T lymphocytes through different mechanisms [4, 5]. In this condition, effector T cells turn into non-reaction cells or program into apoptosis [6]. TAMs account for about 30–50% in the tumor stroma, and take part in the occurrence, invasion and metastasis of tumor cells and influence the prognosis of the cancer patients. In tumor tissues, TAMs are more like M2 macrophages in function and phenotypes [7]. Moreover, it was reported that infiltration of TAMs has a close relationship with the high expression of PD-L1 in in hepatocellular carcinomas [8]. In this study, we investigated the expression of PD-L1 in human laryngeal squamous cell carcinoma and disclosed the association of PD-L1 with the infiltration of CD8 + T lymphocytes and TAMs. Our study would reveal the biological and clinical significance of PD-L1 in laryngeal carcinoma.

Patients and Methods

Patients

Sixty-nine patients (52 male and 17 female aging from 54 to 68 years) of laryngeal carcinoma (as Laryngeal carcinoma group) and 10 patients of vocal cord leukoplakia (as leukoplakia group) received tumor resection at Neck Surgery Department in the Second Affiliation Hospital of Jilin

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Table 1 Basic information of and PD-L1 expression in patients with laryngeal carcinoma or with voice cord leukoplakia

Variables	Laryngeal cancer, $n = 69$	Leukoplakia patients, $n = 10$	P value
Age (years), Mean ± SD.	62.87 ± 8.84	54.9 ± 7.28	0.008
Male, n(%)	52 (75.36)	10 (100.0)	0.174
PD-L1 expression in diseased tissue, n(%)			<0.001
–	0 (0.00)	8 (80.00)	
+	15 (21.74)	2 (20.00)	
++	33 (47.83)	0 (0.00)	
++++	21 (30.43)	0 (0.00)	
PD-L1 expression in paracancerous tissue, n(%)			<0.001
–	20 (28.99)	10 (100.0)	
+	41 (59.42)	0 (0.00)	
++	8 (11.59)	0 (0.00)	

University (Changchun, Jilin) from Jan. 2010 to Dec. 2015. Laryngeal carcinoma was diagnosed according to the medical history, physical examination, image examination, intraoperative findings and postoperative pathological results. All 69 cases received surgery, without preoperative radiotherapy / chemotherapy.

Immunohistochemistry

The expression of PD-L1, CD8, CD16 and CD206 were detected by immunohistochemistry in laryngeal carcinoma and paracancerous tissues. All the resected specimens were fixed in 4% formalin and embedded in paraffin tissue. Slices were continuously (4 m-thickness) sectioned. CD8, CD16 and CD206 primary antibodies were purchased from Boster Company (Wuhan, China). The samples were incubated with the antibodies at 4 °C in a dilution of 1:100. Immunohistochemical SP method was performed according to the instruction of the kit. 3, 3 - diaminobenzidine (DAB) staining was utilized to color the target protein and nuclei were stained by hematoxylin. The slides were observed under light microscope.

Five fields in each specimen were randomly selected. Cells in brown yellow or brown granules were determined as positive staining. The positive cell numbers and dyeing strength were calculated. PD-L1 expression was evaluated as follows: 0 point (positive rate: < 6%), 1 point (positive rate: 6% - 25%), 2 points (positive rate: 26% - 50%), 3 points (positive rate: 51% - 75%) and 4 points (positive rate > 75%). Semi-quantitative staining intensity was divided into 4 grades: grade 0 (negative), grade 1 (weak positive), 2 (moderately positive), and 3 (strongly positive). A final comprehensive rate was

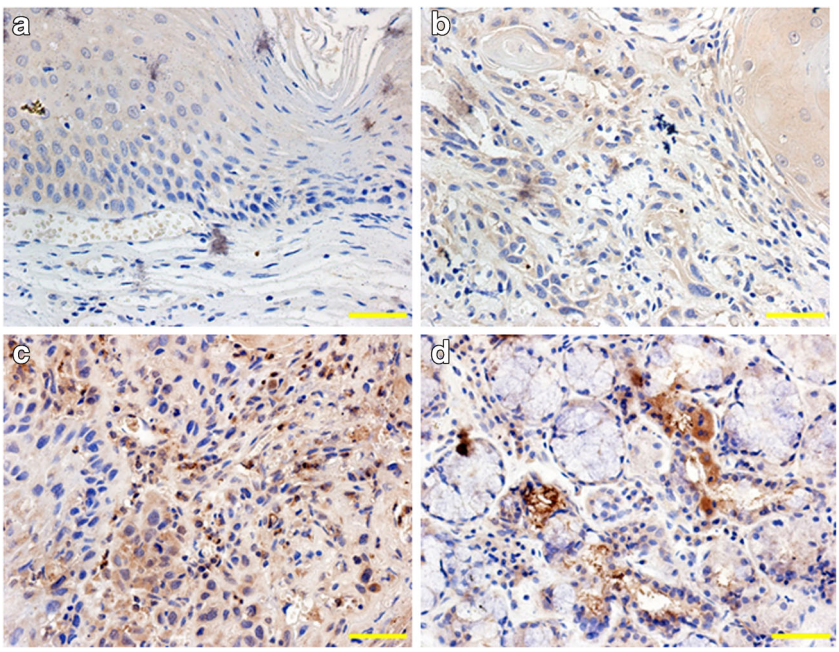

Fig. 1 Expression of PD-L1 (shown in brown) in laryngeal squamous cell carcinoma and laryngo-leukoplakia tissue. **a** PD-L1 was rarely expressed in laryngo-leukoplakia tissue. **b** PD-L1 was lowly expressed in the high differentiation laryngeal carcinoma. **c** PD-L1 was highly expressed in the tissue medium and low differentiation laryngeal carcinoma. **d** PD-L1 was also expressed in the paracancerous tissue (400×)

established: 0 (negative), + [1, 2], + + [3, 4] and + + + [5–7]. "0" or "+" was considered as no expression or low expression. "++" and "+++" were deemed as medium and high expression, respectively.

$CD8^+$T cell invasion was evaluated. According to the distribution of tumor epithelial cells and stromal tumor, the $CD8^+$T cell invasion was divided into strong infiltration (> 10/100 of epithelial cells; >20/100 stromal cell infiltration) and weak infiltration (< 10/100 epithelial cells; < 20/100 stromal cell infiltration). CD16 and CD206 staining were detected in tumor stroma using a similar method.

Statistical Analysis

Quantitative data were expressed as mean and standard deviation (S.D.) and analyzed with the t-test between two groups. When the comparisons were among three groups, one-way ANOVA followed by Student-Newman-Keuls Test was applied. The enumeration data were analyzed using the Chi-square test. The association between PD-L1 expression and tumor differentiation, TNM classification, CD8 expression or CD16+ and CD206+ cell infiltration was analyzed using Cochran-Mantel-Haenszel non-zero correlation. When the correlation was statistically significant, Spearman's correlation coefficient was calculated. All statistics were carried out using SAS 9.3. $P < 0.05$ was considered as significant difference.

Results

Sixty-nine cases of laryngeal cancer and 10 leukoplakia patients were included in the study. The basic information of the patients was listed in Table 1. The age in two groups were 68 ± 8.84 (laryngeal cancer) and 54.9 ± 7.28 (leukoplakia), respectively. There was a significant difference when comparing the age ($P = 0.008$). The gender ratio in two groups was comparable. PD-L1 expression was significantly higher in

Table 2 Clinical characteristics in tissues with different level of PD-L1 expression

Variables	Tumor PD-L1 expression level			*P* value
	+, *n* = 15	++, *n* = 33	+++, *n* = 21	
Male, n(%)	10 (66.67)	23 (69.70)	19 (90.48)	0.152
Age (years), mean ± SD	60.8 ± 8.55	63.91 ± 8.42	62.71 ± 9.79	0.533
Tumor diameters (cm), mean ± SD	1.99 ± 0.94	2.58 ± 1.33	3.21 ± 1.49[a]	0.029
Growth location[b], n(%)				0.113
Other	1 (6.67)	3 (9.38)	4 (19.05)	
Supraglottic	3 (20.00)	13 (40.63)	11 (52.38)	
Glottic	11 (73.33)	16 (50.00)	6 (28.57)	
Lymph node metastasis, n(%)	1 (6.67)	7 (21.21)	9 (42.86)	0.037
Differentiation, n(%)				0.001[c]
High	12 (80.00)	17 (51.52)	7 (33.33)	
Medium	3 (20.00)	16 (48.48)	10 (47.62)	
Low	0 (0.00)	0 (0.00)	4 (19.05)	
T typing[d], n(%)				0.087
1	4 (26.67)	7 (21.88)	3 (17.65)	
2	8 (53.33)	16 (50.00)	5 (29.41)	
3	2 (13.33)	4 (12.50)	5 (29.41)	
4	1 (6.67)	5 (15.63)	4 (23.53)	
N typing[d], n(%)				0.066
0	14 (93.33)	27 (84.38)	11 (64.71)	
1	1 (6.67)	4 (12.50)	6 (35.29)	
2	0 (0.00)	1 (3.13)	0 (0.00)	
M typing[d], n(%)				0.051
0	15 (100.0)	32 (100.0)	15 (88.24)	
1	0 (0.00)	0 (0.00)	2 (11.76)	
Death in 3 years [b], n(%)	0 (0.00)	2 (6.25)	3 (14.29)	0.355

[a] compared with PD-L1 (+), P < 0.05

[b] loss of one case

[c] Spearman's correlation coefficient was 0.372 ± 0.107

[d] loss of five cases

Table 3 Consistence of the PD-L1 in tumor and paracancerous tissue

PDL1 in paracancerous tissue	PD-L1 in tumor tissue				Total	*P* value
	–	+	++	+++		
–	0 (0.00)	8 (53.33)	12 (36.36)	0 (0.00)	20	0.006
+	0 (0.00)	7 (46.67)	20 (60.61)	14 (66.67)	41	
++	0 (0.00)	0 (0.00)	1 (3.03)	7 (33.33)	8	
+++	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0	
Total	0	15	33	21	69	

Kappa0.2020 (95CI,0.012–0.391)

laryngeal cancer than in leukoplakia ($P < 0.001$) (Fig.1, Table 1). Moreover, PD-L1 was highly expressed in paracancerous tissue from laryngeal cancer group compared with that in leukoplakia group ($P < 0.001$). These data implicated that PD-L1 expression was related to laryngeal cancer.

The association between PD-L1 expression and tumor differentiation was also investigated. As shown in Table 2, tumor diameter was significantly different in the groups divided based upon the expression PD-L1 (Table 2). The patients with a high expression of PD-L1 also had a large tumor. PD-L1 expression did not determine the location of tumor growth. However, PD-L1 expression influenced lymph node metastasis and tumor differentiation. By contrast, PD-L1 expression did not influence tumor grading (T, N, M) and 3-year mortality rate.

The consistence of the PD-L1 in tumor tissue and paracancerous tissue was also analyzed. As shown in Table 3, the consistence of PD-L1 expression in tumor tissue and paracancerous tissue was significant (Kappa: 0.2020, (95CI, 0.012–0.391)) ($P = 0.006$) (Table 3).

PD-L1 expression in tumor tissue was negatively and weakly correlated with CD8 expression in epithelial cells ($P < 0.001$, Spearman's correlation coefficient: -0.550 ± 0.083) (Fig. 2, Table 4), very low correlated with CD16+ infiltration ($P = 0.016$, Spearman's correlation coefficient: -0.290 ± 0.102) and with CD206+ infiltration ($P < 0.001$, Spearman's correlation coefficient: 0.426 ± 0.095) (Fig. 3, Table 4).

Discussion

In this study, we demonstrated that PD-L1 expression in laryngeal cancer tissue was significantly higher compared with the expression in leukoplakia patients. Moreover, PD-L1 was up-regulated with the deterioration of the cancer. Additionally, PD-L1 expression was associated with the pathological degree, cervical lymph node metastasis and clinical classification.

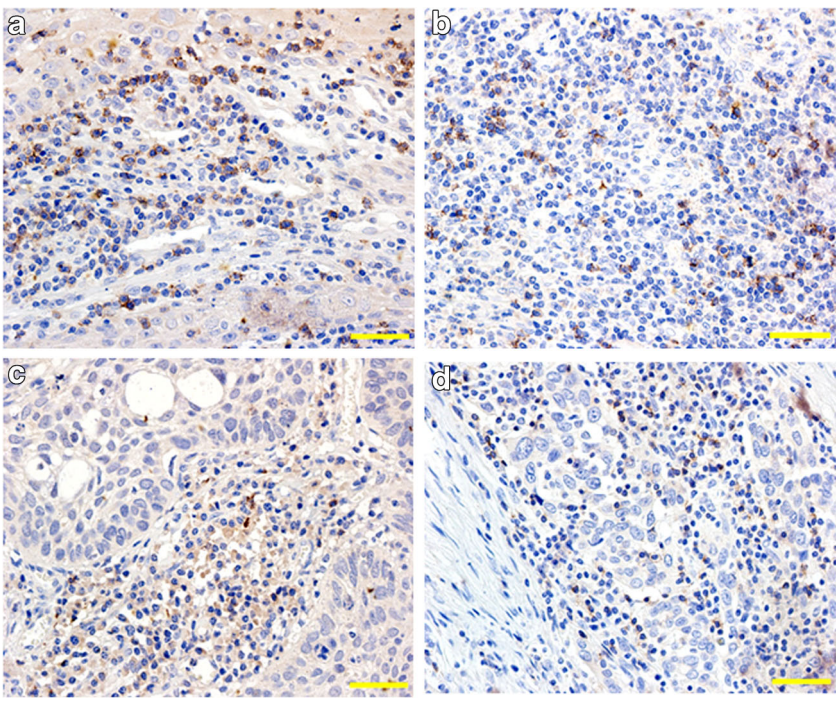

Fig. 2 Infiltration of CD8 + T (shown in brown) in laryngeal carcinoma with high and low expression of PD-L1. **a** Infiltration in stroma of CD8 + T in low expression of PD-L1 group. **b** Intraepithelial infiltration of CD8 + T in low expression of PD-L1 group. **c** Infiltration in stroma of CD8 + T in high expression of PD-L1 group. **d** Intraepithelial infiltration of CD8 + T in high expression of PD-L1 group (400×)

Table 4 Correlation of PD-L1expressions with CD8, $CD16^+$, $CD206^+$

Variables	PD-L1 in tumor tissue			*P* value
	+, n = 15	++, n = 33	+++, n = 21	
Epithelial CD8, n(%)				<0.001[a]
+	1 (6.67)	10 (30.30)	15 (71.43)	
++	4 (26.67)	12 (36.36)	5 (23.81)	
+++	10 (66.67)	11 (33.33)	1 (4.76)	
CD8 in stroma, n(%)				0.142
–	0 (0.00)	1 (3.03)	0 (0.00)	
+	6 (40.00)	7 (21.21)	14 (66.67)	
++	7 (46.67)	19 (57.58)	5 (23.81)	
+++	2 (13.33)	6 (18.18)	2 (9.52)	
$CD16^+$ expression				0.016[b]
+	1 (6.67)	12 (36.36)	9 (42.86)	
++	9 (60.00)	14 (42.42)	10 (47.62)	
+++	5 (33.33)	7 (21.21)	2 (9.52)	
$CD206^+$expression				<0.001[c]
+	9 (60.00)	15 (45.45)	3 (14.29)	
++	6(40.00)	14 (42.42)	10 (47.62)	
+++	0(0.00)	4 (12.12)	8 (38.10)	

[a] Spearman correlation coefficient: −0.550 ± 0.083

[b] Spearman correlation coefficient was −0.290 ± 0.102

[c] Spearman correlation coefficient was 0.426 ± 0.095

PD-L1, one kind of type I transmembrane glycoprotein is a member of the B7 superfamily, which is extensively expressed in a variety of immune cells, epithelial cells and tumor cells. As reported, PD-L1 plays an important role in tumor immunity [9]. High expression of PD-L1 in tumor cells, bind to PD-1 receptor on cytotoxic T lymphocytes (CTLs) to transfer the negative regulatory signals to regulate expression and secretion of cytokine, leading to apoptosis of CTLs. Subsequently, the tumor cells escape from the immune monitor and survive [10]. The experimental data also demonstrated that PD-L1 expression was related to tumor stage and tumor differentiation. Moreover, the high expression of PD-L1 is one of the adverse prognostic factors and PD-L1 has become a new biological marker for cancer detection and prognosis prediction [11, 12]. Our data revealed that PD-L1 was still expressed in paracancerous tissue, but lower than that in tumor tissue.

The effector $CD8^+$ TILs are important executors of antitumor immunity in vivo. Studies have shown that a high number of infiltrations of $CD8^+$ TILs in tumor epithelial cells or stroma are often associated with a positive prognosis [13]. The binding of PD-L1 to PD1 induces apoptosis of effector CD8 + T cells in tumor tissue and leads to a decrease in the number of TILs in tumor tissue. In other words, depletion of TILs in tumor microenvironment is related to the binding of PD-1 with PD-L1 [14]. In order to investigate the correlation between PD-L1 and CD8 + T cells in laryngeal carcinoma, this study utilized continuous section and detected PD-L1 expression by immunohistochemical staining. The numbers of infiltrating tumor epithelial and interstitial cells of CD8 + T computing in low expression and high expression of PD-L1 group were compared. The results showed that the expression of PD-L1 was correlated with infiltration of tumor CD8 + T cells in laryngeal carcinoma. More infiltrations

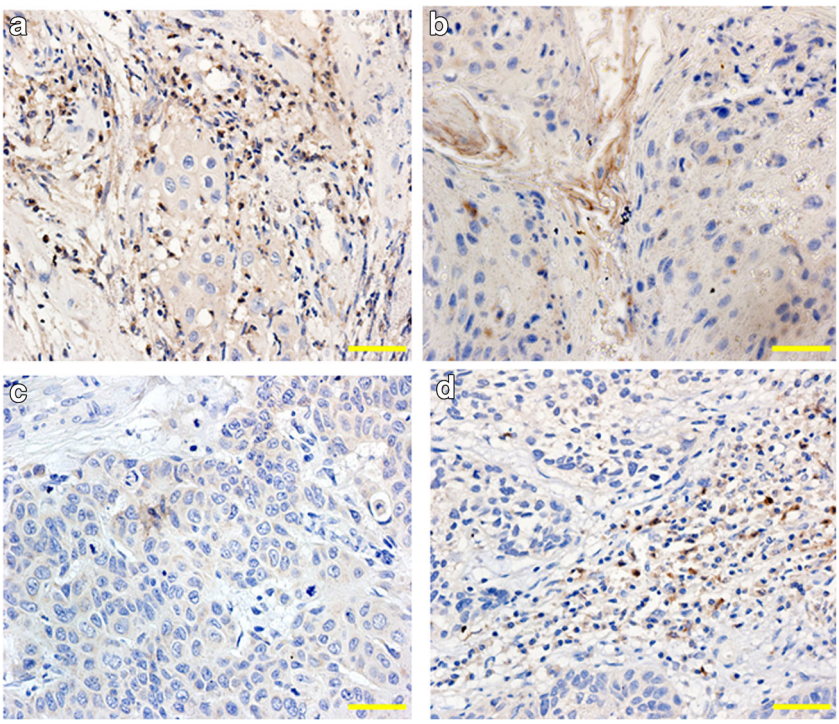

Fig. 3 Infiltration of $CD16^+$ (brown) and $CD206^+$ (brown) cells in laryngeal carcinoma with high and low expression of PD-L1. **a** CD16 was highly expressed in PD-L1 low expression group. **b** CD206 was lowly expressed in PD-L1 low expression group. **c** CD16 was lowly expressed in PD-L1 high expression group. **d** CD206 was highly expressed in PD-L1 high expression group. (400×)

of $CD8^+$T lymphocyte were found in the cancer tissue with high expression of PD-L1. Subsequently, tumor cells in low differentiated squamous cell carcinoma cannot be effectively killed due to immune escape.

Tumor associated macrophages are the main cells in the stroma of tumors, and are important components of inhibitory tumor microenvironment. Under the tumor microenvironment "domestication" function, TAMs tend to differentiate into M2 type macrophage [15]. The latter is the alternative activated macrophages, mainly involved in the Th2 immune response. The antigen presenting ability is low, through its complicated autocrine and paracrine IL-10, TGF-β and other cytokines to inhibit the immune defense function [16]. The relationship between PD-L1 expression and TAMs has attracted more and more attention. Many cytokines in TAMs and tumor microenvironment can regulate the phenotype of tumor cells. For example, Chen et al. [17] found that lung cancer cells can upregulate the expression of B7-H3 and B7-H4 under the action of TAMs, and that IFN-γ can upregulate the expression of PD-L1 in tumor cells. Kuang et al. [18] found that TNF-β and IL-10 induced PD-L1 expression in an autocrine manner. Kang et al. showed that the expression level of PD-L1 protein in hepatocellular carcinoma (HCC) tissues was significantly related to TAMs infiltration. In vitro culture of macrophages could upregulate the expression of PD-L1 in hepatoma cell line [19].

In order to investigate the correlation between TAMs clarification and PD-L1 expression, we selected CD16 as the antigen marker of M1 TAMs and CD206 as antigen marker of M2 TAMs. The expression of TAMs in cancers with high and low PD-L1 expression was compared. PD-L1 low expression group displayed higher degree of M1 TAMs, while PD-L1 high expression group displayed higher degree of M2 TAMs in the tumor stroma infiltration. These results reflect the inherent link between the expression of PD-L1 in laryngeal squamous cell carcinoma and the expression of TAMs classification. These data likely implicate that high expression of PDL1 associated tumor microenvironment TAMs is one of the important factors of immune suppression [20].

The high expression of PDL1 was associated with the increase of $CD8^+$ T infiltration and decrease of M1 type TAMs and increase of M2 TAMs. The relationship between PDL1 and immune cell subsets further confirmed its participation in the development and progression of laryngeal carcinoma and invasion. However, the exact role of PD-L1 in laryngeal carcinoma and its specific mechanism required further research. How PDL1 regulates TAMs still deserves further investigation. Moreover, the expression site of PDL1 (cytoplasm or cell membrane, epithelial or mesenchymal cells) might have different effects on CD8+ T.

Conclusion

The high expression of PD-L1 in laryngeal carcinoma is one of the important characteristics of tumor microenvironment with immunosuppression.

Acknowledgements Thanks to Dr. Xueshibojie Liu and Yuanzhang Zu for their contribution to the work!

Funding This work is supported by the National Natural Science Youth Foundation of China (81502348), the National Natural Science Foundation of China (81572653), International Cooperation Project from Science and Technology Department of Jilin Province (20180414054GH) and the Natural Science Foundation of Jilin Province (20160101039JC).

Compliance with Ethical Standards

Conflict of Interest Statements All the authors declare that they have no conflict of interest.

References

1. Ferris RL, Whiteside TL, Ferrone S (2006) Immune escape associated with functional defects in antigen-processing machinery in head and neck cancer. Clin Cancer Res 12(13):3890–3895
2. Zandberg DP, Strome SE (2014) The role of the PD-L1:PD-1 pathway in squamous cell carcinoma of the head and neck. Oral Oncol 50(7):627–632
3. Jinushi M, Komohara Y (2015) Tumor-associated macrophages as an emerging target against tumors: creating a new path from bench to bedside. Biochim Biophys Acta 1855(2):123–130
4. Lee YH, Martin-Orozco N, Zheng P, Li J, Zhang P, Tan H, Park HJ, Jeong M, Chang SH, Kim BS, Xiong W, Zang W, Guo L, Liu Y, Dong ZJ, Overwijk WW, Hwu P, Yi Q, Kwak L, Yang Z, Mak TW, Li W, Radvanyi LG, Ni L, Liu D, Dong C (2017) Inhibition of the B7-H3 immune checkpoint limits tumor growth by enhancing cytotoxic lymphocyte function. Cell Res 27:1034–1045
5. Liu Z, Zhao Y, Fang J, Cui R, Xiao Y et al (2017) SHP2 negatively regulates HLA-ABC and PD-L1 expression via STAT1 phosphorylation in prostate cancer cells. Oncotarget
6. Fife BT, Pauken KE, Eagar TN, Obu T, Wu J, Tang Q, Azuma M, Krummel MF, Bluestone JA (2009) Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. Nat Immunol 10(11):1185–1192
7. Zhang L, Li Z, Ding G, La X, Yang P et al (2017) GRP78 plays an integral role in tumor cell inflammation-related migration induced by M2 macrophages. Cell Signal 37:136–148
8. Chen J, Li G, Meng H, Fan Y, Song Y, Wang S, Zhu F, Guo C, Zhang L, Shi Y (2012) Upregulation of B7-H1 expression is associated with macrophage infiltration in hepatocellular carcinomas. Cancer Immunol Immunother 61(1):101–108
9. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, Gupta R, Tsai JM, Sinha R, Corey D, Ring AM, Connolly AJ, Weissman IL (2017) PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature 545(7655):495–499
10. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH (2009) PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med 206(13):3015–3029

11. Yvorel V, Patoir A, Casteillo F, Tissot C, Fournel P, Stachowicz ML, Karpathiou G, Tiffet O, Péoc'h M, Forest F (2017) PD-L1 expression in pleomorphic, spindle cell and giant cell carcinoma of the lung is related to TTF-1, p40 expression and might indicate a worse prognosis. PLoS One 12(7):e0180346
12. Roper E, Lum T, Palme CE, Ashford B, Ch'ng S, Ranson M, Boyer M, Clark J, Gupta R (2017) PD-L1 expression predicts longer disease free survival in high risk head and neck cutaneous squamous cell carcinoma. Pathology 49:499–505
13. Milstein O, Hagin D, Lask A, Reich-Zeliger S, Shezen E, Ophir E, Eidelstein Y, Afik R, Antebi YE, Dustin ML, Reisner Y (2011) CTLs respond with activation and granule secretion when serving as targets for T-cell recognition. Blood 117(3):1042–1052
14. Mischinger J, Comperat E, Schwentner C, Stenzl A, Gakis G (2015) Inflammation and Cancer: what can we therapeutically expect from checkpoint inhibitors? Curr Urol Rep 16(9):59
15. Ager CR, Reilley MJ, Nicholas C, Bartkowiak T, Jaiswal AR, Curran MA (2017) Intratumoral STING activation with T cell checkpoint modulation generates systemic antitumor immunity. Cancer Immunol Res 5:676–684
16. Fujii N, Shomori K, Shiomi T, Nakabayashi M, Takeda C, Ryoke K, Ito H (2012) Cancer-associated fibroblasts and CD163-positive macrophages in oral squamous cell carcinoma: their clinicopathological and prognostic significance. J Oral Pathol Med 41(6):444–451
17. Chen C, Qu QX, Shen Y, Mu CY, Zhu YB, Zhang XG, Huang JA (2012) Induced expression of B7-H4 on the surface of lung cancer cell by the tumor-associated macrophages: a potential mechanism of immune escape. Cancer Lett 317(1):99–105
18. Kuang DM, Zhao Q, Peng C, Xu J, Zhang JP, Wu C, Zheng L (2009) Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. J Exp Med 206(6):1327–1337
19. Kang FB, Wang L, Li D, Zhang YG, Sun DX (2015) Hepatocellular carcinomas promote tumor-associated macrophage M2-polarization via increased B7-H3 expression. Oncol Rep 33(1):274–282
20. Chen W, Jiang J, Xia W, Huang J (2017) Tumor-related exosomes contribute to tumor-promoting microenvironment: an immunological perspective. J Immunol Res 2017:1073947