Regulation of PD-L1 expression in cancer and clinical implications in immunotherapy

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Received December 16, 2019; Accepted December 26, 2019; Epub January 1, 2020; Published January 15, 2020

Abstract: PD-1/PD-L1 immune checkpoint blockade therapy has become an effective method for the treatment of cancers in the clinic. It has great clinical advantages and therapeutic effects in the treatment of various cancers. However, a considerable number of cancer patients currently have relatively low response rates and drug resistance to PD-1/PD-L1 immunotherapy. Therefore, an in-depth understanding of the regulatory mechanism of PD-L1 expression in tumor cells will provide new insights into PD-1/PD-L1 immunotherapy. This review will systematically review the regulatory mechanisms of PD-L1 including genomic amplification, epigenetic regulation, transcriptional regulation, translational regulation and posttranslational modification. We will also discuss PD-L1 expression regulation in clinical applications. Finally, we hope to provide new routes for PD-1/PD-L1 immunotherapy in the clinic.

Keywords: Immune checkpoint blockade therapy, PD-1/PD-L1, gene expression, regulatory mechanism

Introduction

In recent years, immunotherapy has become a new method of cancer treatment. Currently, immune checkpoint blockade therapy is one of the most widely used methods of tumor immunotherapy. The pathway involving programmed death protein 1 (PD-1) and its ligand (PD-L1) is a well-characterized immune checkpoint and has been applied in the clinical treatment of various cancers. Antibodies targeting the PD-1/PD-L1 pathway have been approved for various cancers, including melanoma, non-small cell lung cancer (NSCLC), Hodgkin’s lymphoma, bladder cancer, renal cell carcinoma (RCC), head and neck squamous cell carcinoma (HNSCC), breast cancer, Merkel cell carcinoma, hepatocellular carcinoma (HCC) and gastric cancer (GC) [3]. However, these antibodies are only efficacious in a small portion of patients with certain cancers.

At present, the understanding of the resistance mechanism of immune checkpoint blockade therapy and the regulation of PD-L1 expression is quite limited. To develop a more effective and lasting immune checkpoint blocking therapy strategy, it is necessary to gain insights into the multiple roles and complex regulatory mechanisms of PD-L1 in cancers. In this review, we will discuss the molecular mechanisms of PD-L1 expression in cancer cells at the levels of genomic amplification, epigenetic regulation, transcriptional regulation, posttranscriptional regulation, translational regulation, and post-translational modification. These findings may provide new insights into targeting tumor immune escape after immunotherapy in the clinic.

Classification of PD-L1 expression in tumor cells

The expression of PD-L1 can be divided into constitutive expression and inducible expression depending on the extrinsic or intrinsic stimuli (Figure 1). Constitutive expression of PD-L1 in tumor cells is induced by dysregulation of oncogenic or tumor suppressor gene signaling pathways, by activation of abnormal transcription factors, or by genomic aberrations or gene amplifications. Many oncogenic transcr-
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Promoter to regulate PD-L1 expression [4, 11] (Figure 1).

Inducible expression refers to the expression of PD-L1-controlled inflammatory signals from tumor cells or other immune cells, such as APCs and T cells, in the tumor microenvironment. A number of inflammatory cytokines have been found to induce the expression of PD-L1. These inflammatory factors include IFN-γ, TNF-α, IL-17, IL-27, IL-10, IL-4, IL-2 and IL-10 [12, 13] (Table 1).

Regulation of PD-L1 expression by genomic amplification

PD-L1 and PD-L1 are located on chromosome 9p24.1. The amplification of the 9p24.1 region is closely related to an increase in PD-L1 levels in a wide range of cancers [14].

It has been found that copy number alterations (CNAs) of PD-L1 occur in various types of tumors, which lead directly to up-regulation of PD-L1 expression [15].

The highest frequency of CNAs of PD-L1 has been found in primary mediastinal B-cell lymphoma (PMBCL), classical Hodgkin lymphoma (CHL), and triple-negative breast cancer (TNBC), at 63% [16], 40% [17] and 29% [18], respectively. However, in GC, small cell lung cancers, NSCLCs and diffuse large B-cell lymphoma (DLBCL), the CNAs were much lower, with frequencies of 15% [19], 1.9% [20], 5.3% [21] and 3% [22], respectively. In general, the increase in CNAs is positively correlated with PD-L1 protein levels [23] (Figure 2).

Epigenetic regulation of PD-L1 expression

Epigenetic modifications, such as microRNAs (miRNAs), promoter DNA methylation and histone modifications, can regulate the recognition and binding of transcription factors to DNA elements without affecting DNA sequences, thereby altering chromatin structure and regulating PD-L1 expression [24] (Figure 2).
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### Table 1. Classification of PD-L1 expression

<table>
<thead>
<tr>
<th>Class</th>
<th>Inducer</th>
<th>Type of cancers</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constitutive</td>
<td>MYC</td>
<td>NSCLC, lymphoma, HCC, melanoma</td>
<td>[3-5, 8]</td>
</tr>
<tr>
<td></td>
<td>KRAS</td>
<td>NSCLC, lung cancer</td>
<td>[9, 10, 35, 71]</td>
</tr>
<tr>
<td></td>
<td>STAT3</td>
<td>HNSC, lymphoma, melanoma</td>
<td>[4, 11, 72, 73]</td>
</tr>
<tr>
<td></td>
<td>JUN</td>
<td>Lymphoma, melanoma, medulloblastoma</td>
<td>[53, 72, 74]</td>
</tr>
<tr>
<td></td>
<td>PTEN</td>
<td>Glioma, colorectal cancer, melanoma, breast cancer</td>
<td>[72, 75-78]</td>
</tr>
<tr>
<td></td>
<td>EGFR</td>
<td>Head and neck cancer, breast cancer, NSCLC</td>
<td>[10, 61, 79]</td>
</tr>
<tr>
<td></td>
<td>MEK-ERK</td>
<td>Melanoma, lymphoma, multiple myeloma</td>
<td>[67, 80, 81]</td>
</tr>
<tr>
<td>Inducible</td>
<td>IFN-γ</td>
<td>Pancreatic cancer, colon cancer, HCC, melanoma, lung cancer, gastric cancers</td>
<td>[82-86]</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>HCC, lung cancer, prostate cancer</td>
<td>[87-89]</td>
</tr>
<tr>
<td></td>
<td>IL-27</td>
<td>Lung cancer, epithelial ovarian cancer</td>
<td>[88, 90]</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>Breast cancer, HCC, prostate and colon cancer cells</td>
<td>[52, 83, 91]</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>Gastric cancers</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>EGF</td>
<td>NSCLC, breast cancer</td>
<td>[10, 61, 71, 93]</td>
</tr>
<tr>
<td></td>
<td>IL-8</td>
<td>Gastric cancers, NSCLC, melanoma</td>
<td>[94, 95]</td>
</tr>
</tbody>
</table>

Figure 2. Regulation of PD-L1 expression in cancer cells at different levels. PD-L1 expression can be regulated by genomic amplification, transcriptional regulation, epigenetic regulation and transcriptional regulation.

miRNAs are a class of non-coding single-stranded RNAs that contain 22-24 nucleotides. miRNAs inhibit translation or degradation of target mRNA by binding to the 3’ untranslated region (3’UTR) of the target mRNA. A number of miRNAs have been found to regulate PD-L1.
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expression in different types of cancers [24]. They can regulate PD-L1 expression directly or indirectly.

Direct effectors regulate PD-L1 expression primarily by binding to PD-L1 mRNA. miRNAs that directly regulate PD-L1 expression include miR513 [25], miR-34 [26], miR-570 [27, 28], miR-152 [29], miR-200 [30], miR-138 [31], miR-142-5p [32], miR-424 [33], miR-193a [34] and miR-140/142/340/383 [35]. Indirect effects mainly occur through affecting the expression of other PD-L1 regulators. miRNAs that indirectly regulate PD-L1 expression include miR-20b, miR-21, miR-130b [36], and miR-197 [37].

Recently, it was found that the promoter methylation of PD-L1 was negatively correlated with PD-L1 expression in a number of cancers [38-42]. PD-L1 promoter methylation has been found in many cancers, including acute myeloid leukemia [38], HNSCC [43-45], glioblastoma [41], glioma [42, 43], colorectal cancer [40], and prostate cancer [46]. Analysis of PD-L1 promoter methylation has clinical significance for predicting the outcome of PD-1/PD-L1 immune checkpoint blockade therapies. In PD-1/PD-L1 targeted drug-treated patients, increased PD-L1 promoter methylation is associated with overall patient survival and recurrence-free survival [40].

In addition, histone modifications, including methylation, acetylation, phosphorylation, adenylation, ubiquitination, and ADP ribosylation, can also regulate PD-L1 gene expression [24]. The histone acetylation of the promoter region of the PD-L1 gene is essential for the expression of PD-L1 [24].

Transcriptional activation of PD-L1 expression

A number of transcription factors have been found to regulate PD-L1 transcriptional activation. These transcription factors include MYC, STAT3, NF-kB, AP1, and HIF-1 (Figure 2).

The oncogene MYC is a transcription factor that is overexpressed and activated in a variety of tumors and involved in tumorigenesis [47]. However, there is controversy about the regulation of PD-L1 expression by MYC. Casey et al. found that inhibition of MYC in tumor cells resulted in a decrease in PD-L1 mRNA and protein expression. MYC can bind directly to the promoters of PD-L1 and enhance the anti-tumor immune response [3]. In contrast, Hogg and Durand-Panteix et al. reported that MYC transcriptional levels inhibited PD-L1 mRNA expression [48, 49]. Future research is also needed to clarify these discrepancies.

STAT3 is another reported transcription factor that is involved in the regulation of PD-L1 expression. In chimeric nucleophosmin (NPM)/ALK-carrying T cell lymphoma, STAT3 upregulates PD-L1 expression by binding to the PD-L1 promoter. This effect can be suppressed by silencing STAT3 with siRNA [49]. It was also reported that latent membrane protein-1 (LMP1) of the Epstein–Barr virus can induce PD-L1 expression through inducing the phosphorylation of STAT3 [50].

NF-kB is a nuclear transcription factor that also regulates PD-L1 expression. However, the mechanism of regulation is still unclear. In natural killer/T-cell lymphoma (NKTCL), inhibition of the NF-kB signaling pathway reduces PD-L1 expression [51]. Recently, Lim et al. found that the inflammatory factor TNF-α activates the NF-kB signaling pathway and activates COP9 signalosome 5 (CSN5) to inhibit ubiquitination and degradation of PD-L1 protein [52].

The transcription factor AP-1 is a dimeric complex composed of c-Jun, FOS, MAF, or ATF. Expression of PD-L1 in Hodgkin's lymphoma is induced by AP1 via binding to the enhancer region of the first intron of the PD-L1 gene [53].

Hypoxia-inducible factor 1α (HIF-1α) is another important carcinogenic factor and has clinical significance in regulating the expression of PD-L1 in tumor cells [54]. Binding of HIF-1α to the PD-L1 proximal promoter stimulates transcription of PD-L1. Overexpression of HIF-1α induces an increase in PD-L1 levels [54, 55].

Translation-level regulation of PD-L1

It has been found that ubiquitination, deubiquitination, glycosylation and phosphorylation can affect the stability of PD-L1 protein in cancer cells, thereby regulating the expression of PD-L1 protein (Figure 2).

Several proteins were reported to regulate the stability of the PD-L1 protein through ubiquitination. CSN5 is the fifth component of the CSN complex, which contains a conserved JAMM
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motif. CSN5 has deubiquitination activity through the JAMM motif and plays an important role during tumorigenesis. Lim et al. found that macrophages secrete TNF-α to activate NF-kB and then induce transactivation of CSN5. Activation of CSN5 results in deubiquitination of PD-L1 in breast cancer cells and enhances the stability of PD-L1 [52]. Cyclin-dependent kinase 4/6 is a key regulator of the cell cycle. Cyclin D-CDK4 induces ubiquitination degradation of PD-L1 via cullin 3-SPOP to control therapeutic efficacy in human cancers [56]. CMTM6 was a recently identified type 3 transmembrane protein involved in regulating PD-L1 expression [57, 58]. A genome-wide CRISPR-Cas9 screening technology revealed that CMTM6 inhibits ubiquitination and inhibits lysosomal-mediated degradation of PD-L1 by interacting with PD-L1 on the surface of tumor cells [57]. In addition to CMTM6, its closest family member, CMTM4, has similar functions [58]. Epidermal growth factor (EGF) treatment also induces ubiquitination of PD-L1 and regulates PD-L1 protein expression [59].

Glycosylation is an important posttranslational modification of proteins. N-linked glycosylation is a key protein modification that determines the structure and function of proteins and plays an important role in regulating membrane proteins. N-linked glycosylation of PD-L1 was shown to stabilize the PD-L1 protein and prevent degradation by the 26S proteasome [60, 61]. In triple-negative breast cancer, β-1,3-N-acetylglucosaminyl transferase (B3GNT3) was required for the interaction between PD-L1 and PD-1 [60].

Clinical application of PD-L1 expression regulation

Due to tumor heterogeneity and genetic differences between individuals, there are significant defects in the therapeutic effects of targeting the PD-1/PD-L1 pathway alone. Recent studies have found that combining PD-L1/PD1 immunotherapy with targeted therapy significantly improves therapeutic effects by regulating PD-L1 at a very low level [62]. This strategy inhibits PD-L1 expression by regulating key proteins in the signaling pathway, and it combines with the immunotherapy of PD-L1 or PD-1 antibody to achieve a greater therapeutic effect.

In NSCLC, EGFR mutations can induce PD-L1 expression. The combination of osimertinib and durvalumab in the treatment of NSCLC patients with EGFR mutations showed significant efficacy and an overall response rate (ORR) of up to 70% [63-65]. Patients with advanced NSCLC treated with nivolumab in combination with erlotinib for EGFR mutations showed a durable clinical benefit [66]. The use of the KRAS/MEK inhibitor trametinib in combination with anti-PD-1 antibodies also significantly reduced PD-L1 expression and showed better therapeutic effects than individual treatments in NSCLC [67, 68].

On the other hand, the expression of PD-L1 is also regulated by MAPK and PI3K/Akt signaling pathways, and inhibition of these pathways also reduces PD-L1 expression [69]. Inhibition of these signaling pathways can inhibit cell proliferation and regulate PD-L1 expression. Clinical studies have found that receptor tyrosine kinase inhibitors have a better therapeutic effect in lung cancers with high PD-L1 expression [70].

Conclusions and future challenges

Immunotherapies are a new direction in cancer therapy and have many advantages over traditional treatments. Currently, immunotherapy that targets the PD-1/PD-L1 axis has been clinically approved in many countries for the treatment of various human cancers. It has shown unprecedented efficacy in the treatment of a wide range of human cancers. However, only a small proportion of patients show an effect with PD-1/PD-L1 immune checkpoint blockade therapy. The expression of PD-L1 varies greatly in tumor tissues. At present, methods to detect PD-L1 expression in tumor tissues include immunostaining, Western blotting, qPCR and microarray. However, these methods for detecting the expression of PD-L1 vary greatly. An in-depth understanding of the regulatory mechanism of PD-L1 expression has been very helpful for PD-1/PD-L1 immunotherapy in the clinic. Although the regulatory mechanism of PD-L1 expression has been investigated to some extent, there are still many questions that need to be solved. For example, new mechanisms that regulate PD-L1 expression need to be investigated in future studies.

The expression of PD-L1 can be regulated at different levels; however, it is necessary to study which regulatory mechanism plays a critical role in certain types of cancer. A number of
transcription factors that regulate the expression of PD-L1 regulate it by binding to the PD-L1 promoter, but the transcription factors that play key roles in certain types of cancer also need to be identified. In addition, the expression of PD-L1 varies greatly in different stages of tumor development, such as in primary cancer and metastatic cancer. In addition to antibody drugs, it is also necessary to develop a small molecule inhibitor of PD-L1 for treatment of cancer patients. Finally, these studies will provide new ideas for immunological checkpoint blocking therapy.

The understanding of the regulatory mechanism of PD-L1 expression will continue to deepen and will finally provide more choices and more effective methods for tumor immunotherapy of the PD-1/PD-L1 pathway.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (8150-2621 and 81502088), the China Postdoctoral Science Special Foundation (2017M5654), medical clinical science and technology development fund of Jiangsu University (JLY20180033).

Disclosure of conflict of interest

None.

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References

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[52] Lim SQ, Li CW, Xia W, Cha JH, Chan LC, Wu Y, Chang SS, Lin WC, Hsu JM, Hsu YH, Kim T,
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[66] Neoadjuvant PD-1 Blockade in Resectable Lung Cancer; Nivolumab and Ipilimumab in Advanced Melanoma; Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma; Prolonged Survival in Stage III Melanoma with Ipilimumab Adjuvant Therapy; Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma; Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma; Rapid Eradication of a Bulky Mela-noma Mass with One Dose of Immunotherapy; Genetic Basis for Clinical Response to CTLA-4 Blockade; Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma; Nivolumab plus Ipilimumab in Advanced Melanoma; Safety and Tumor Responses with Lambrolizumab (Anti-PD-1) in Melanoma; Hepatotoxicity with Combination of Vemurafenib and Ipilimumab. N Engl J Med 2018; 379: 2185.
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