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REVIEW

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Regulation of PD-L1 expression in the tumor microenvironment

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Abstract

Programmed death-ligand 1 (PD-L1) on cancer cells engages with programmed cell death-1 (PD-1) on immune cells, contributing to cancer immune escape. For multiple cancer types, the PD-1/PD-L1 axis is the major speed-limiting step of the anti-cancer immune response. In this context, blocking PD-1/PD-L1 could restore T cells from exhausted status and eradicate cancer cells. However, only a subset of PD-L1 positive patients benefits from α-PD-1/PD-L1 therapies. Actually, PD-L1 expression is regulated by various factors, leading to the diverse significances of PD-L1 positivity. Understanding the mechanisms of PD-L1 regulation is helpful to select patients and enhance the treatment effect. In this review, we focused on PD-L1 regulators at the levels of transcription, post-transcription, post-translation. Besides, we discussed the potential applications of these laboratory findings in the clinic.

Keywords: Cancer immunology, PD-L1, PD-1, Transcriptional regulation, Post-transcriptional modification

Background

In physiological conditions, the activities of T cells are intricately regulated. T cell immunity selectively eliminates pathogens and abnormal cells but avoids attacking normal cells, termed immune homeostasis [1]. Programmed cell death-1 (PD-1, which is encoded by *PDCD1*) and programmed death-ligand 1 (PD-L1, which is encoded by *CD274*) are vital proteins in maintaining immune homeostasis [2]. The PD-1/PD-L1 pathway restrains the hyperactivation of immune cells and prevents autoimmune diseases [3]. However, in the tumor microenvironment (TME), the PD-1/PD-L1 axis is hijacked by cancer cells to escape immune surveillance [4]. The overexpressed PD-L1 on cancer cells binds to the PD-1 on tumor-infiltrating lymphocytes (TILs), which counteracts the TCR-signaling cascade by phosphorylating SHP-2 [5, 6]. As a result, T cell activation is impaired. Apart from cancer cells, some other types of cells in the TME, such as macrophages, dendritic cells (DCs), activated T cells, as well as cancer-associated fibroblasts, also express PD-L1 [7]. These components orchestrate an immunosuppressive microenvironment, supporting tumor growth.

Inhibiting the PD-1/PD-L1 signaling is a feasible strategy to normalize the dysregulated TME [8]. Up to now, α-PD-1/PD-L1 treatments have exhibited potent antitumor activities in various cancers, such as melanoma, non-small cell lung cancer (NSCLC), gastric cancer, liver cancer, urothelial cancer, lymphoma, and all MSI-high cancers [2, 9–19]. Commonly, the PD-L1 protein level is the primary standard to select patients who are more likely to respond to α-PD-1/PD-L1 treatments [20, 21]. However, the PD-L1 level is determined by several factors, which results in the different significances of PD-L1 positivity or negativity. The PD-L1 positivity might result from immune response-induced PD-L1 expression or oncogenic constructive PD-L1 upregulation [22]. For the latter, in the absence of pre-existing immune response, patients with PD-L1 positive tumors commonly are resistant to α-PD-1/PD-L1 therapies [20].

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On the contrary, patients with PD-L1 negative tumors might respond to α-PD-1/PD-L1 treatment when undergoing combination therapies that promote T cell infiltration [22]. Therefore, an in-depth understanding of PD-L1 regulation is valuable for efficacy prediction and patient selection. In this review, we summarized the latest advances of PD-L1 regulation, including genomic alterations, epigenetic modification, transcriptional regulation, post-transcriptional modification, and post-translational modification. Moreover, we discussed the potential applications of these findings in the clinic.

Genomic alterations of *CD274*

In some cancers such as classical Hodgkin lymphoma and small-cell lung cancer, the copy number of chromosome 9p24.1 (where *CD274* resides) was increased [23, 24]. The chromosome rearrangement caused *CD274* amplification without influences on the open reading frame (Fig. 1) (Table 1) [24]. Besides, in mediastinal large B-cell lymphoma, the increased transcriptional expression of *CD274* was related to an adjacent ectopic promoter or enhancer by translocation [25]. These findings indicated that genomic alterations contributed substantially to cancer immune escape, which might be a potential biomarker for patient selection.

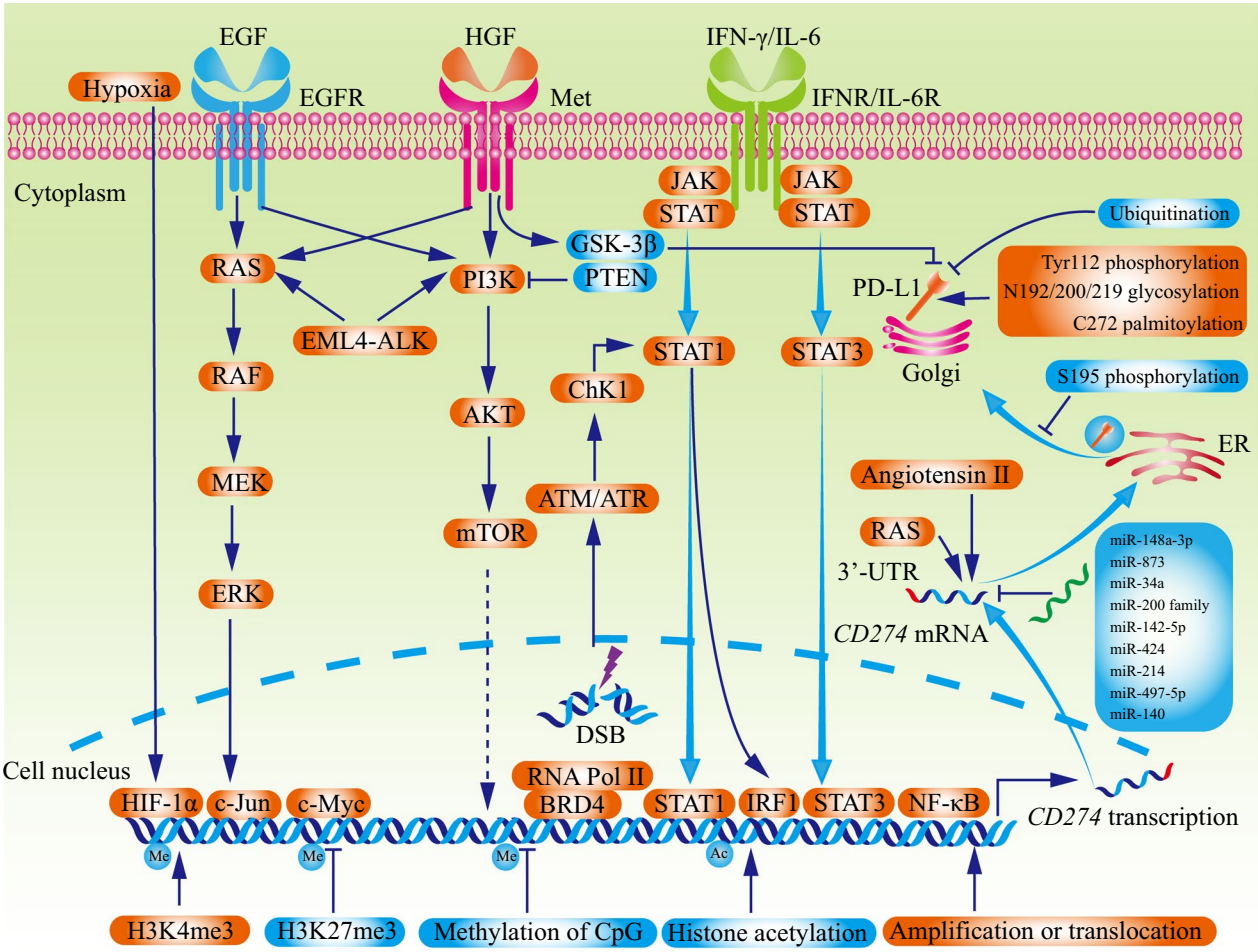

Fig. 1 The regulators of PD-L1 expression. PD-L1 abundance is regulated by genomic alterations (amplification or translocation), epigenetic modifications (methylation of histone or CpG island, and histone acetylation), transcriptional regulation (inflammatory stimuli and oncogenic signals), post-transcriptional regulation (miRNA, the status of 3′- UTR, RAS, and Angiotensin II), and post-translational modification (ubiquitination, phosphorylation, glycosylation, palmitoylation). H3K4me3: tri-methylation of histone H3 on lysine 4; H3K27me3: tri-methylation of histone H3 on lysine 27; EGFR: epidermal growth factor receptor; IRF: interferon-responsive factor; IFN: interferon; DSB: double-strand break; GSK3β: glycogen synthase kinase 3β; PI3K: phosphoinositide 3-kinase; NF-κB: Nuclear factor kappa-B; HIF-1α: hypoxia-inducible factor-1α; ALK: Anaplastic lymphoma kinase; ER: endoplasmic reticulum

Table 1 The mechanisms of PD-L1 regulation in the tumor microenvironment

Regulatory stage	Regulator	The change of PD-L1	Cancer type	References
Genomic alterations	Gene amplification or translocation	Up	Classical Hodgkin lymphoma; Small-cell lung cancer; Large B-cell lymphoma	[23–25]
Epigenetic regulation	H3K4me3	Up	Pancreatic cancer	[27]
	H3K27me3	Down	Hepatocellular carcinoma	[29]
	Methylation of some CpG loci in *CD274* promoter	Down	Melanoma; Head and neck squamous cell carcinomas; Colorectal cancer	[33–35]
	Histone acetylation	Up	Drug-resistant cancer cells;	[39]
Transcriptional regulation	IFN-α, IFN-β, IFN-γ	Up	Melanoma; Hepatocellular carcinoma; Gastric carcinoma	[44, 46, 48]
	IL-6	Up	Prostate cancer; Hepatocellular carcinoma; Glioblastoma; Non-small cell lung cancer	[50–53]
	TNF-α	Up	Prostate cancer; Colon cancer; Renal cell carcinoma	[54, 55]
	IL-10	Up	Oral squamous cell carcinoma	[56]
	IL-27	Up	Epithelial ovarian cancer; Prostate cancer; Non-small cell lung cancer	[57]
	TGF-β	Up	Lung cancer	[60, 61]
	EGFR	Up	Lung cancer	[62]
	MAPK	Up	Lung cancer; Melanoma; Pancreatic cancer; Triple-negative breast cancer	[67–70]
	PTEN	Down	Triple-negative breast cancer	[74]
	PI3K	Up	Gastric cancer; Her2-overexpressing cell lines; Colorectal cancer; Head and neck squamous cell carcinomas; Non-small cell lung cancer	[75–79]
	JAK-STAT	Up	Natural killer/T-cell lymphoma; Non-small cell lung cancer; Triple-negative breast cancer;	[80–83]
	NF-κB	Up	Natural killer/T-cell lymphoma; Gastric carcinoma; Non-small cell lung cancer; Triple-negative breast cancer	[84, 87–90]
	HIF-1	Up	Prostate cancer; Breast cancer; Nasopharyngeal carcinoma	[92, 93]
	Myc	Up	Leukemia and lymphomas; Melanoma; Non-small cell lung cancer; Hepatocellular carcinoma; Renal cell carcinoma; Colorectal carcinoma; Esophageal squamous cell carcinoma; Pancreatic cancer	[96–99]
	ALK	Up	Non-small cell lung cancer; Anaplastic large cell lymphoma	[103, 104]
	Met	Up	Non-small cell lung cancer	[107–110]
	BRD4	Up	Ovarian cancer	[112]

Table 1 (continued)

Regulatory stage	Regulator	The change of PD-L1	Cancer type	References
	DSB	Up	Osteosarcoma; Non-small cell lung cancer; Prostate cancer	[114]
Post-transcriptional regulation	miR-148a-3p	Down	Colorectal cancer	[116]
	miR-873	Down	Breast cancer	[117]
	miR-34a	Down	B-cell lymphoma; Acute myeloid leukemia	[118, 126]
	miR-200 family	Down	Non-small cell lung cancer; Hepatocellular carcinoma	[119, 120]
	miR-142-5p	Down	Pancreatic cancer	[121]
	miR-424	Down	Ovarian cancer	[122]
	miR-214	Down	Diffuse large B-cell lymphoma	[123]
	miR-497-5p	Down	Clear cell renal cell carcinoma	[124]
	miR-140	Down	Non-small cell lung cancer	[125]
	miR-23a-3p/PTEN axis	Up	Liver Cancer	[127]
	miR-200a/PTEN axis	Up	Osteosarcoma	[128]
	miR-27a-3p/ MAGI2/PTEN axis	Up	Breast cancer	[129]
	miR-145/c-Myc axis	Down	Ovarian cancer	[130]
	miR-18a/ PTEN, WNK2, SOX6 axis	Up	Cervical cancer	[131]
	miR-BART5/PIAS3/pSTAT3	Up	Gastric cancer	[133]
	RAS-tristetraprolin	Up	RAS mutant cancer	[135]
	Angiotensin II/human antigen R	Up	Non-small cell lung cancer	[136]
Post-translational modification	Ubiquitination	Down	Multiple cancers	[138, 141, 142]
	Y112 phosphorylation	Up	Hepatocellular carcinoma	[143]
	S195 phosphorylation	Down	Breast cancer	[144]
	T180 and S184 phosphorylation	Down	Breast cancer	[145]
	Glycosylation	Up	Breast cancer; Glioma	[145, 149, 150]
	Palmitoylation	Up	Breast cancer; Colon cancer	[151, 152]

H3K4me3 tri-methylation of histone H3 on lysine 4, *H3K27me3* tri-methylation of histone H3 on lysine 27, *EGFR* epidermal growth factor receptor, *MAPK* mitogen-activated protein kinase, *PTEN* phosphatase and tensin homolog, *PI3K* phosphoinositide 3-kinase, *NF-κB* nuclear factor kappa-B, *HIF-1α* hypoxia-inducible factor-1α, *ALK* anaplastic lymphoma kinase, *DSB* double-strand break

Epigenetic regulations

Epigenetic regulations such as methylation and histone acetylation determine the PD-L1 expression as well (Fig. 1). Tri-methylation of histone H3 on lysine 4 (H3K4me3) is generally believed as a histone modification promoting gene transcriptions [26]. In pancreatic cancer, MLL1 protein could bind to the *CD274* promoter to catalyze H3K4me3, leading to the increased expression of PD-L1 [27]. In agreement, the MLL1 inhibitor had a synergistic effect with α-PD-1/PD-L1 therapy [27]. On the contrary, tri-methylation of histone H3 on lysine 27 (H3K27me3) relates to transcription suppression [28]. In hepatocellular carcinoma, enhancer of zeste homolog 2 negatively regulated PD-L1 expression by promoting H3K27me3 [29].

Besides the methylation of histone, the methylation of DNA at CpG islands regulated PD-L1 expression [30]. Inhibiting methylation of DNA by DNA methyltransferase inhibitors (DNMTis) increased PD-L1 level in cancer cells [31, 32]. The authors assumed that DNMTis elevated the expression of hypermethylated endogenous retroviruses in cancer cells, which might activate the innate immune response and lead to IFN-γ-stimulated PD-L1 expression [30]. Moreover, the methylation of some specific CpG loci in the *CD274* promoter determined the level of *CD274* mRNA [33–35]. In NSCLC, TGF-β1 impaired the activity of DNMTs, demethylated the *CD274* promoter, and increased PD-L1 expression [36]. Notably, in patients with recurrent gastric cardia adenocarcinoma, PD-L1 expression was reduced after

α-PD-1/PD-L1 treatment [37]. Further investigations indicated that the *CD274* promoter was more hypermethylated in the relapsed tumors than in the primary tumors without α-PD-1/PD-L1 treatment [37]. In murine tumor models, the combination therapy of hypomethylating agent azacytidine and α-PD-1 showed a more significant antitumor effect than α-PD-1 monotherapy [37].

Histone acetylation is an epigenetic modification enhancing gene transcription [38]. In some drug-resistant cancer cells, hyperactivated JNK/c-Jun signaling suppressed the histone deacetylase 3 (HADC3) expression, thereby elevating the histone H3 acetylation of the *CD274* promoter [39]. The HADC inhibitor had a synergistic effect with α-PD-1 in the B16F10 tumor model [40]. Furthermore, HADC inhibitor-mediated PD-L1 upregulation was observed in other types of cancers [41, 42]. These findings provide a rationale to combine α-PD-1/PD-L1 treatments with HDAC inhibitors.

Transcriptional regulation

Inflammatory Signaling

Interferon (IFN) and IL-6

As a negative feedback for inflammation, PD-L1 could be upregulated by multiple inflammatory signaling pathways to restrain T cells' hyperactivity (Fig. 1). Generally believed, IFN-γ is the prominent stimulator contributing to the inducible PD-L1 expression [43].

During cancer progression, the IFN-γ-derived PD-L1 promotes cancer immune escape [3]. In the TME, activated T cells and NK cells generate most IFN-γ. Then, IFN-γ binds to type II interferon receptor, activating the JAK-STAT signaling (mainly through STAT1) [44, 45]. Subsequently, the expression of several transcriptional factors is upregulated, especially interferon-responsive factors (IRFs). IRF-1 is the vital downstream component of STAT1 upon IFN-γ treatment [46, 47]. In hepatocellular carcinoma, it was identified that two elements (IRE1/2) in the 5′-flanking region of the *CD274* promoter were the binding sites of IRF-1, which participated in regulating PD-L1 transcription [48]. Notably, the intactness of JAK-STAT-IRF1 pathway is also related to the response to α-PD-1/PD-L1 therapy. The effect of α-PD-1/PD-L1 treatment is limited in tumors with mutations in *JAK1* and *JAK2* [49]. It was speculated that these tumors might not rely on the PD-1/PD-L1 pathway to escape immune surveillance [49].

Besides IFN-γ, other inflammatory stimuli such as IFN-α, IFN-β, and IL-6 could induce PD-L1 expression as well. However, IFN-α and IFN-β had a more significant effect on PD-L2 regulation than PD-L1 regulation [44]. In prostate cancer, the IL-6-JAK-STAT3 pathway promoted PD-L1 expression and led to the resistance to immune killing [50]. Moreover, in hepatocellular carcinoma, increased IL-6 activated the STAT3/c-MYC/miR-25-3p pathway, which resulted in the decreased protein tyrosine phosphatase receptor type O (PTPRO) [51]. The downregulated PTPRO-enhanced PD-L1 expression by deregulating the activation of JAK2-STAT1/3 [51]. Furthermore, the glioblastoma-derived IL-6 could induce the local and systemic myeloid PD-L1 expression by STAT3 phosphorylation [52]. Besides, in lung cancer, it was detected that IL-6-derived PD-L1 expression was related to multiple pathways, especially the MEK-ERK signaling [53].

Other inflammatory signals

Tumor necrosis factor-α (TNF-α) increased *CD274* mRNA by activating nuclear factor kappa-B (NF-κB) pathway [54]. In renal cell carcinoma, TNF-α cooperated with IL-4 to enhance *CD274* transcription by activating NF-κB, IκB, and STAT6 [55]. Moreover, in oral squamous cell carcinoma, the IL-10 level in the TME was positively correlated to the abundance of PD-L1 on tumor-associated macrophages [56]. Blocking IL-10 suppressed PD-L1 expression [56]. Furthermore, in several human cancer cells, IL-27 increased *CD274* transcription by promoting the tyrosine phosphorylation of STAT1 and STAT3 [57].

The effect of TGF-β on PD-L1 regulation is still unclear. Although some previous studies indicated that TGF-β downregulated PD-L1 expression in renal tubular epithelial cells and monocytes [58, 59], TGF-β mainly had a positive impact on the PD-L1 expression in the TME. In NSCLC cells, exogenous TGF-β increased the *CD274* transcription probably by Smad-binding elements [60]. The expression of phosphorylated-Smad2 was significantly increased in PD-L1 positive NSCLC patients [60]. Apart from cancer cells, TGF-β could increase PD-L1 expression on DCs in the TME [61].

Oncogenic Signaling

Besides inflammatory stimuli, growing evidence suggests that hyperactive oncogenic pathways play a vital role in PD-L1 expression (Fig. 1). Therefore, α-PD-1/PD-L1 therapies might have a synergistic effect with oncogenic signal-targeting treatments.

Epidermal Growth Factor Receptor (EGFR)

In lung epithelial cells, the mutated EGFR pathway (EGFR T790M) increased PD-L1 expression [62]. For lung cancer cells, PD-L1 expression was impaired after EGFR tyrosine kinase inhibitor (TKI) treatment [62]. In murine EGFR-driving lung cancer models, α-PD-1 effectively reversed T cell exhaustion and retarded tumor growth [62]. The results indicated that the mutant EGFR pathway facilitated tumor to escape from immune surveillance [62]. However, a clinical study showed that

EGFR-mutant NSCLC patients tended to resist α-PD-1 therapy [63]. The authors found that although some EGFR-mutant NSCLCs were PD-L1 positive, the concurrent PD-L1 upregulation and abundant TILs were rare [63]. The lack of a pre-existing inflammatory TME might limit the effect of α-PD-1/PD-L1 treatment [63]. The low response rate in EGFR-mutant patients was reported by other investigators [64, 65].

Mitogen-activated protein kinase (MAPK)

MAPK is a well-studied oncogenic pathway, which counts for nearly 40% of human cancer cases [66]. According to TCGA database, the *CD274* mRNA level was significantly positively related to RAS- or MEK-activation scores in NSCLC patients [67]. In lung adenocarcinoma cells, activating EGF-MAPK signaling increased the mRNA and protein levels of PD-L1 [67]. Inhibiting MAPK signaling by MEK inhibitor (Selumetinib) counteracted the EGF- and IFN-γ-stimulated upregulation of *CD274* mRNA and PD-L1 protein [67]. In melanoma cells, the activated NRAS-RAF-MEK1/2-ERK-c-Jun axis enhanced the transcription of *CD274* [68]. Moreover, in pancreatic cancer, myeloid cells induced PD-L1 expression on tumor cells by activating EGFR-MAPK pathway [69]. After MEK inhibitor treatment, the levels of p-ERK and PD-L1 were decreased, and this reduced PD-L1 led to a higher sensitivity to α-PD-1 treatment in murine pancreatic tumors [69].

On the contrary, in murine breast cancer cells, suppressing MAPK signaling by Trametinib (a MEK inhibitor) potentiated the IFN-γ-stimulated upregulation of PD-L1 and major histocompatibility complex (MHC) [70]. Furthermore, in cancer cell lines, including KYSE30, TE-1, MKN7, PC-9, SNU-475, OE19, and BT-549, there was no significant alteration when cancer cells were treated with MAPK inhibitor [71]. Besides, the MAPK inhibitor had no significant impact on IFN-γ-stimulated PD-L1 expression [71]. The role of MAPK pathway in PD-L1 regulation might depend on cell types [72].

PTEN/PI3K-AKT pathway

As a well-studied tumor suppressor, PTEN is a vital regulator of the oncogenic signaling pathway PI3K-AKT [73]. PTEN loss and PI3K activation have been identified in multiple types of cancers, including hepatocellular carcinoma, prostate cancer, and breast cancer [73]. Deficient PTEN was detected in nearly half of PD-L1 positive triple-negative breast cancer samples [74]. Knocking down PTEN resulted in a rise of PD-L1 expression [74]. Moreover, activating the PI3K-AKT pathway in gastric cancer cells increased PD-L1 abundance, while PI3K inhibitor (LY294002) reduced PD-L1 level [75]. Besides, in head and neck cancer cells, melanoma cells, colorectal cancer cells, and Her2-amplified cancer cells (SNU216, NCI-N87, and SKBR3), PD-L1 expression was suppressed by PI3K inhibition [68, 76–78]. Moreover, inhibiting mTOR (the downstream of PI3K-AKT) by rapamycin reduced PD-L1 level in NSCLC cells [79].

JAK-STAT pathway

Mutations in *JAK1*, *JAK3*, and *STAT3* were prevalent in mature T-cell lymphomas [80]. Some *STAT3* mutations, such as p.E616K, increased the STAT3 phosphorylation and STAT3-mediated transcription [80]. In the meanwhile, silencing STAT3 or STAT3 inhibitor reduced PD-L1 expression [80]. Further chromatin immunoprecipitation qPCR assay indicated the p.E616K mutation might increase the transcription activity of *CD274* promoter by a stronger STAT3 binding [80]. Besides, in breast and lung cancer cells, the PD-L1 expression was hampered by JAK and STAT3 inhibitors [81–83].

NF-κB pathway

Activated NF-κB signaling was related to the high level of PD-L1 in several cancers [36, 84–88]. Multiple oncogenic signals could impair immune surveillance by activating the NF-κB-PD-L1 axis. In lung cancer cells, overexpressed MUC1-C increased the occupancy of NF-κB p65 in *CD274* promoter, which enhanced *CD274* transcription [89]. Besides, in breast cancer, reactive oxygen species (ROS) inducers (paclitaxel, glutathione synthesis inhibitor, and buthionine sulphoximine) led to the accumulation of ROS, subsequently activating the downstream NF-κB pathway [90]. In a murine breast cancer model, paclitaxel treatment induced PD-L1 upregulation in tumor-associated macrophages by the NF-κB p65-PD-L1 pathway [90].

Hypoxia-inducible factor-1 (HIF-1)

Hypoxia facilitates the drug resistance and distant metastasis of tumor cells [91]. Besides, a hypoxic TME undermines host immunity activities and contributes to immune escape [92]. Hypoxia upregulated PD-L1 expression by HIF-1α [92]. The hypoxia-induced upregulation of *CD274* mRNA was hampered when HIF-1α was silenced [92]. Further investigations suggested the cellular colocalization of PD-L1 and HIF-1α [92]. In the meanwhile, inhibiting HIF-1 signaling could reduce PD-L1 expression in multiple types of cancers [93, 94].

Myc

As a transcription factor regulating cell differentiation, proliferation, and apoptosis, Myc is overexpressed in various cancers [95]. Knocking down or inhibiting Myc in cancer cells reduced *CD274* mRNA and PD-L1 protein [96–99]. The results of the ChIP-seq assay showed that

Myc could bind to the *CD274* promoter [96]. However, in some particular types of cancer, Myc negatively regulated PD-L1 expression. In hepatocellular carcinoma cells, inhibiting Myc increased the IFN-γ-stimulated PD-L1 expression [100]. Besides, in the murine MycCaP tumor model, Myc inhibitor treatment promoted T cell infiltration, enhanced antitumor immune response, but simultaneously upregulated PD-L1 expression [101]. This PD-L1 upregulation was induced by immune response [101].

Anaplastic lymphoma kinase (ALK)

Chromosomal rearrangements in the *ALK* gene are an oncogenic driver for NSCLC [102]. In various NSCLC cell lines, the *CD274* mRNA and PD-L1 protein levels were higher in cells with echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* fusion [103]. Ectopic expressing EML4-ALK protein or blocking ALK phosphorylation positively or negatively regulated PD-L1 expression [103]. Besides, inhibiting PI3K-AKT or MEK-ERK pathways reversed the EML4-ALK-induced PD-L1 expression [103]. Apart from NSCLC, the PD-L1 level was higher in ALK-positive systemic anaplastic large cell lymphoma [104].

Met

Alterations in the *Met* gene were reported in multiple types of cancers [105, 106]. In primary lung cancer tissues, the level of PD-L1 was positively correlated to the *Met*-amplification [107, 108]. In a microarray assay, inhibiting or knocking down Met substantially reshaped the expression of several immune-related genes, including *CD274* [109]. On the contrary, activating Met by hepatocyte growth factor increased PD-L1 expression [109, 110].

BRD4

As a member of the bromodomain and extraterminal (BET) family, BRD4 acts as a super-enhancer of oncogenes [111]. In ovarian cancer cells, BET inhibitor reduced PD-L1 expression in a time- and dose-dependent manner [112]. Further, the ChIP assay showed a significant association of *CD274* promoter and BRD4 [112]. After BET inhibitor treatment, the associations of *CD274* promoter-BRD4 and *CD274* promoter-RNA Pol II were decreased, which contributed to the downregulated *CD274* transcription [112]. Besides, it was validated that BET inhibitor suppressed *CD274* transcription by reducing the BRD4 occupancy at *CD274* promoter, independent of c-Myc [113].

DNA double-strand break (DSB) repair pathway

After inducing DSB by ionizing radiation, PD-L1 expression was increased in multiple cancer cell lines [114]. In contrast, paclitaxel (a non-DNA damaging agent) treatment had no significant impact on PD-L1 expression [114]. DSB-activated ATM-ATR-ChK1-STAT1/3-IRF1 pathway led to the downstream PD-L1 upregulation [114].

Post-transcriptional regulation

microRNA (miRNA)

Cancer-derived miRNA is a vital post-transcriptional regulator for PD-L1 expression in the TME (Fig. 1) [115]. In colorectal cancer cells with mismatch repair deficiency or microsatellite instability-high, miR-148a-3p was decreased while PD-L1 was increased [116]. The results of the co-transfection of miR-148a-3p mimic and wild-type or mutant *CD274* 3′-untranslated region (UTR) luciferase reporter indicated that *CD274* mRNA was the direct target of miR-148a-3p [116]. Furthermore, in breast cancer cells, miR-873 suppressed PD-L1 expression by targeting *CD274* mRNA [117]. Up to now, it was identified that *CD274* mRNA was the direct target of multiple oncogenic miRNAs such as miR-34a, miR-200 family, miR-142-5p, miR-424, miR-214, miR-497-5p, miR-140 [118–126].

Besides, some cancer-derived miRNAs indirectly regulated PD-L1 expression [127–129]. In ovarian carcinoma cells, miR-145 downregulated PD-L1 by targeting *c-Myc* [130]. In cervical cancer cells, increased PD-L1 was related to the upregulation of miR-18a [131]. miR-18a promoted PD-L1 expression by targeting *PTEN* (inhibitor of PI3K-AKT), *WNK2* (inhibitor of MAPK), and *SOX6* (inhibitor of Wnt/β-catenin) [131]. Similarly, hepatocellular carcinoma cell-derived miR-23a-3p enhanced PD-L1 expression in macrophages via targeting *PTEN* [127]. In NSCLC cells, miR-3127-5p promoted PD-L1 expression by activating STAT3 [132]. Moreover, in gastric cancer, miR-BART5-5p increased PD-L1 by targeting *PIAS3* (inhibitor of STAT3) [133].

The stability of *CD274* mRNA

The variations in the 3′- UTR affected the stability of *CD274* mRNA [134]. Disturbing the 3′-UTR of *CD274* mRNA by Crisper-Cas9 could stabilize *CD274* mRNA [134]. Besides, oncogenic RAS activation inhibited tristetraprolin (AU-rich element-binding protein) by kinase MK2, stabilizing *CD274* mRNA [135]. As a result, RAS activation increased PD-L1 expressed on cancer cells [135]. Moreover, in NSCLC, Angiotensin II increased the stability of *CD274* mRNA and induced PD-L1 expression by human antigen R (also known as HuR, an AU-rich element-binding protein) [136].

Post-translational modification

Post-translational modifications, including ubiquitination, phosphorylation, glycosylation, palmitoylation, and SUMOylation, play a vital role in regulating protein stability, activation, localization, as well as interaction [137]. Aberrant post-translational modification patterns participated in PD-L1 upregulation in the TME (Fig. 1) [138].

Ubiquitination

Ubiquitination is related to proteasome-mediated protein degradation [139]. In a broad range of cancer cells, CMTM6 maintained PD-L1 expression by reducing PD-L1 ubiquitination and increasing PD-L1 half-life [138, 140]. Moreover, cyclin D/cyclin-dependent kinase 4 (CDK4) promoted PD-L1 ubiquitination by SPOP/Cullin 3-SPOP E3 ligase [141]. CDK4/6 inhibitor treatment increased PD-L1 abundance, which provided a potential for the combining therapy of CDK4/6 inhibitors and α-PD-1/PD-L1 agents [141]. Besides, the TNF-α-NF-κB pathway inhibited PD-L1 ubiquitination via upregulating COP9 signalosome 5 (CSN5) [142]. Inhibiting CSN5 impaired PD-L1 expression and sensitized tumor cells to the following immunotherapy [142].

Phosphorylation

IL-6-activated JAK1 promoted the phosphorylation of PD-L1 protein (Tyr112) [143]. Subsequently, Tyr112-phosphorylated PD-L1 recruited STT3A (N-glycosyltransferase) to catalyze the PD-L1 glycosylation [143]. Activating the IL-6-JAK1 signaling elevated PD-L1 stability by this phosphorylation modification [143]. Blocking the IL-6-JAK1 axis had a synergistic effect with α-Tim-3 treatment in murine tumor models [143]. Besides, metformin-activated AMP-activated protein kinase promoted the phosphorylation of PD-L1 (S195) [144]. The S195 phosphorylation led to the aberrant PD-L1 glycosylation, which undermined the PD-L1 translocation from endoplasmic reticulum to Golgi [144]. Apart from hampering the translocation of PD-L1 to cell membrane, the S195 phosphorylation enhanced endoplasmic reticulum-associated PD-L1 degradation [144]. The combination therapy of metformin and α-cytotoxic T Lymphocyte antigen 4 (CTLA-4) exhibited a robust antitumor activity [144]. Moreover, glycogen synthase kinase 3β (GSK3β) decreased the level of PD-L1 by promoting phosphorylation-dependent proteasome degradation [145, 146].

Glycosylation

Glycosylation modification is related to protein stability [147, 148]. The N192/200/219 glycosylation stabilized PD-L1 and suppressed the formation of GSK3β-β-TrCP-PD-L1 complex [145]. EGF increased PD-L1 expression by promoting glycosylation-induced GSK3β inactivation [145]. Additionally, in epithelial-mesenchymal transition, β-catenin transcriptionally induced the expression of N-glycosyltransferase STT3. The STT3 promoted PD-L1 N-glycosylation, stabilizing and upregulating PD-L1 [149]. Moreover, in glioma, FKBP51s (a co-chaperone) regulated PD-L1 expression by promoting glycosylation modification [150]. Overexpressing or silencing FKBP51s increased or decreased the level of glycosylated-PD-L1 [150].

Palmitoylation

Palmitoylation is a well-studied post-translational lipid modification. Palmitoylation at C272 increased PD-L1 stability by counteracting its ubiquitination [151, 152]. DHHC3 catalyzed C272 palmitoylation of PD-L1 [152]. Silencing DHHC3 enhanced antitumor immune response in vitro and in vivo [152].

Perspectives and conclusion

A growing body of evidence suggests that it is inaccurate to select patients merely by PD-L1 abundance. Understanding the difference between inflammation-induced PD-L1 and oncogenic signal-mediated constitutive PD-L1 is helpful to patient selection. For instance, for *EGFR* mutant NSCLC patients, α-PD-1 therapy's efficacy was poor despite the high level of PD-L1 [153]. The *EGFR* mutation-driving NSCLCs commonly harbor lower mutation burdens, and the lower immunogenicity leads to the resistance to α-PD-1 treatments [43]. This oncogenic EGFR-mediated PD-L1 expression could not reflect the real status of the TME. Alternatively, a comprehensive framework containing multiple surrogate markers such as tumor mutation burden would be valuable for selecting patients and predicting outcomes.

Besides, agents regulating PD-L1 expression might have a synergistic effect with the current immune checkpoint inhibitors (Fig. 2). Targeting therapies such as CDK4/6 inhibitor upregulated PD-L1 expression and promoted immune escape [141, 146]. This treatment-induced immune evasion could be overcome by combination therapies containing α-PD-1/PD-L1. Besides, adjuvant treatment regulating PD-L1 expression might elevate the sensitivity to α-PD-1/PD-L1 or other immune checkpoint inhibitors [144, 145]. For example, metformin downregulated PD-L1 by promoting endoplasmic-reticulum-associated degradation, and the combination therapy of metformin and α-CTLA-4 exhibited a synergistic antitumor activity [144].

Generally, in the TME, the expression of PD-L1 is regulated by numerous factors, including inflammatory stimuli and oncogenic pathways at the levels of transcription, post-transcription, and post-translation.

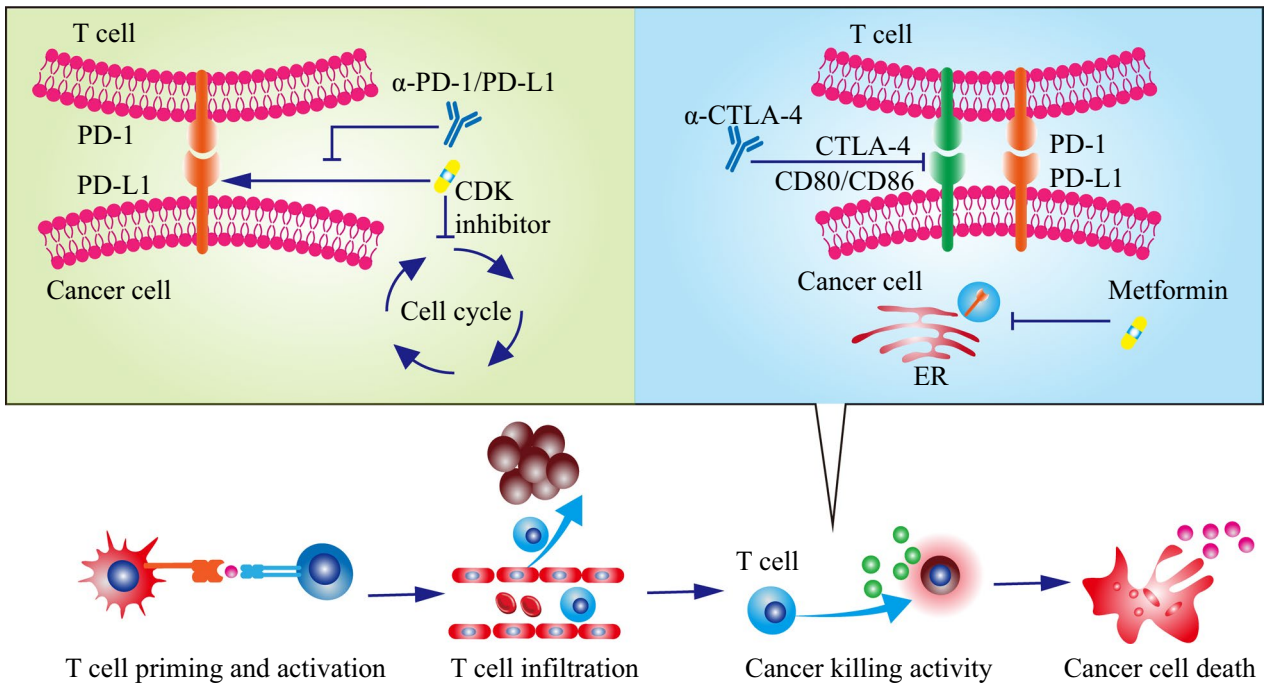

Fig. 2 The current immune checkpoint inhibitor therapy could be further enhanced by regulating PD-L1 expression. Agents regulating PD-L1 expression might have a synergistic effect with the current immune checkpoint inhibitors. For example, targeting therapies such as CDK4/6 inhibitor upregulated PD-L1 expression and promoted immune escape. This treatment-induced immune evasion could be overcome by combination therapies containing α-PD-1/PD-L1 (the left panel). Besides, adjuvant treatment regulating PD-L1 expression might elevate the sensitivity to α-PD-1/PD-L1 or other immune checkpoint inhibitors. For instance, metformin downregulated PD-L1 by promoting endoplasmic-reticulum-associated degradation, and the combination therapy of metformin and α-CTLA-4 had a synergistic antitumor activity (the right panel). CDK: cyclin-dependent kinase; CTLA-4: cytotoxic T Lymphocyte antigen 4; ER: endoplasmic reticulum

Exploring potential PD-L1 regulators helps select patients and overcome resistance to α-PD-1/PD-L1 treatments. Besides, the agents regulating PD-L1 expression might be possible adjuvant therapies for the current immune checkpoint inhibitors.

Abbreviations

PD-1: Programmed cell death-1; PD-L1: Programmed death-ligand 1; TME: Tumor microenvironment; NSCLC: Non-small cell lung cancer; H3K4me3: Tri-methylation of histone H3 on lysine 4; H3K27me3: Tri-methylation of histone H3 on lysine 27; EZH2: Enhancer of zeste homolog 2; DNMTi: DNA methyl-transferase inhibitors; HADC3: Histone deacetylase 3; IRF: Interferon-responsive factor; IFN: Interferon; PTPRO: Protein tyrosine phosphatase receptor type O; TNF-α: Tumor necrosis factor-α; EGFR: Epidermal growth factor receptor; TKI: Tyrosine kinase inhibitor; MAPK: Mitogen-activated protein kinase; MHC: Major histocompatibility complex; PTEN: Phosphatase and tensin homolog; PI3K: Phosphoinositide 3-kinase; NF-κB: Nuclear factor kappa-B; ROS: Reactive oxygen species; HIF-1α: Hypoxia-inducible factor-1α; ALK: Anaplastic lymphoma kinase; EML4: Echinoderm microtubule-associated protein-like 4; BET: Bromodomain and extraterminal; DSB: Double-strand break; miRNA: MicroRNA; UTR: Untranslated region; CDK: Cyclin-dependent kinase; CTLA-4: Cytotoxic T Lymphocyte antigen 4; GSK3β: Glycogen synthase kinase 3β.

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Authors' contributions

MY drafted the manuscript and prepared the figures. MN and LX collected the related references and participated in discussion. SL and KW designed this review and revised the manuscript. All authors contributed to this manuscript. All authors read and approved the final manuscript.

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Competing interests

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