Research progress on the intrinsic non-immune function of PD-L1 in tumors (Review)

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Abstract. Programmed death ligand 1 (PD-L1) is widely expressed in human tumors. It is widely known for its immunosuppressive function as it can help tumor cells evade T cell immune killing through the PD-1/PD-L1 signal. A number of clinical trials have proved that the destruction of the combination of PD-1 and PD-L1 by antibodies could significantly affect patients with advanced cancer. However, a number of patients with cancer still cannot benefit from PD-1/PD-L1 blocking therapy. The main reason is that PD-L1 also has some intrinsic regulatory functions to promote the progression of tumors. PD-L1 Protein contains an intrinsic domain that could link to other signal pathways, but the mechanism has not yet been fully revealed. The present review mainly discussed the non-immune checkpoint functions of PD-L1, such as its role in regulating cell proliferation, cell metabolism, drug resistance and maintaining epithelial-mesenchymal transition and stemness.

Contents

1. Introduction

Programmed cell death ligand 1 (PD-L1), an essential member of the B7 protein family, is well known to bind to programmed cell death 1 (PD-1) to make tumor cells evade death from the immune system (1). A number of types of cancers (renal cell carcinoma (RCC), breast cancer, colorectal cancer (CRC), stomach cancer, non-small cell lung cancer (NSCLC), papillary thyroid cancer and testicular cancer) exhibit high expression of PD-L1, which is correlated with poor prognosis (2-8). At present, antibodies targeting the PD-1/PD-L1 axis have been approved to be effective in some types of cancers such as melanoma, NSCLC, RCC, Hodgkin's lymphoma, bladder cancer, head and neck squamous cell carcinoma (HNSCC), Merkel-cell carcinoma and microsatellite instable-high (MSI-H) or mismatch repair-deficient (dMMR) solid tumors (9). Although PD-1/PD-L1 blockade therapy has shown significant clinical benefits, its efficiency is only ≤40% across multiple cancer types (10,11). Until now, most studies of PD-L1 in tumors have focused on its role as an immune checkpoint. However, PD-L1 has a number of non-immune functions in tumor cells. Several studies have also demonstrated that PD-L1 possesses some intrinsic regulatory functions and can play an important role in promoting tumorigenesis and progression (12-15).

In recent years, the inherent function of PD-L1 mediated in tumor cells and the interaction with other carcinogenic pathways has attracted more and more attention. PD-L1 is a transmembrane protein that contains extracellular IgV and IgC domains, a transmembrane domain (TM) and a short intracellular domain

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Abbreviations: AML, acute myelocytic leukemia; CDR, complementary determining-like regions; CRC, colorectal cancer; CSC, cell stem cell; DFS, disease free survival; dMMR, mismatch repair-deficient; EMT, epithelial-mesenchymal transition; GC, gastric cancer; GSDMC, transcription of the gasdermin C; HNSCC, head and neck squamous cell carcinoma; ICD, intracellular domain; ICPIs, immune checkpoint inhibitors; JAK, Janus kinase; MSI-H, microsatellite instable-high; NDAT, Nano-diamino-tetras; nPD-L1, nuclear PD-L1; NSCLC, non-small cell lung cancer; OS, overall survival; PD-L1, programmed death ligand 1; PD-1, programmed cell death 1; PFS, progression-free survival; PTM, post-transcriptional modification; RCC, renal cell carcinoma; SIG, signal sequence; TCR, T cell receptor; TKIs, tyrosine kinase inhibitors; TM, transmembrane domain; TNBC, triple-negative breast cancer; WAPL, wing apart-like

Key words: programmed death ligand 1, immune checkpoint, tumor proliferation, epithelial-mesenchymal transition, stemness, metabolism, drug resistance

(ICD) (16). The extracellular domain is well known for binding with PD-1 to inhibit T cell immune killing. However, there are few studies on the ICD of PD-L1. For example, one study showed that PD-L1 can counteract the cytotoxicity caused by IFNβ through its ICD and accelerate tumor progression (17).

The immune function of PD-L1 has been well demonstrated since it has been proved effective in a number of tumors treatment. However, the therapeutic effect is still not ideal, with an efficiency rate of ≤40%, which indicates that there are still some mechanisms that have not been explored, such as whether PD-L1 has a non-immune checkpoint function. The non-immune functions of PD-L1 mainly include regulating tumor proliferation, epithelial-mesenchymal transition (EMT), cell stem cells (CSCs), cell metabolism, genome stability and drug resistance. It is of great significance to study the intrinsic function of PD-L1 to improve the antitumor therapeutic effect of the PD-L1 antibody. Therefore, the present review mainly focused on the non-immune functions of PD-L1.

2. Molecular structure of PD-L1

The *CD274* gene, located on human chromosome 9, encodes PD-L1 protein. PD-L1 belongs to a typical immunoglobulin superfamily, similar to other B7 molecules. It is a type I transmembrane glycoprotein with an immunoglobulin structure of IgV-like and IgC-like domains. The V sequence presents a standard Ig-like part with complementary determining-like regions (CDR), which forms a domain that binds to PD-1 with a stoichiometric ratio of 1:1. It is similar to the recognition of antigens with antibodies (18,19). *CD274* contains seven exons (20), the first of which is a non-coding sequence with 5'UTR. The next three exons are the signal sequence (SIG), IgV-like and IgC-like domain. The TM and ICD are contained in the subsequent two exons (exon5 and 6). The last exon is the 3' UTR region which includes an ICD (Fig. 1). PD-L1 is anchored to the cell membrane through a hydrophobic TM, followed by a short intracellular part similar to other B7 molecules. This domain is short, with only 30 amino acids and highly conserved in all reported species (19). A total of three conserved sequences in the intracellular region are identified as functional regions, including RMLDVEKC, DTSSK and QFEET motifs. Azuma *et al* (21) showed that this intracellular region can transmit survival signals, likely to be mediated by the RMLDVEKC and DTSSK motifs.

The *CD274* gene occasionally shows mutated status. For this reason, one study (22) detected the *CD274* mutations by comprehensive genomic profiling (CGP) and found the prevalence of *CD274* SV mutations was low (0.3%, 1081/314,631) with 577 unique variants. The most common *CD274* SV mutations were *R260H*, *R260C, R125Q, C272fs*13, R86W* and *R113H*. Detection of CD274 mutations in a large cohort of different tumor types can help to clarify the reasons for resistance or ineffectiveness of immune checkpoint inhibitors (ICPIs) and help to make more precise decisions when using ICPIs.

3. Expression of PD-L1 in cancer and its potential clinical relevance

PD-L1 is aberrantly highly expressed in a number of types of human tumors and often high PD-L1 expression is associated with poor patient prognosis. A meta-analysis of included studies showed that high PD-L1 expression is associated with shorter overall survival (OS) time and poorer prognosis in patients with NSCLC (23). In an analysis of a database containing 305 curatively resected esophageal cancers, it was found that PD-L1$^+$ cases have significantly poorer OS compared with PD-L1$^-$ cases (24). In a study that included 94 patients with glioblastoma (GBM), researchers using immunohistochemistry analysis measurements found a high incidence of PD-L1 expression in patients with GBM, but only in a small subgroup, and higher PD-L1 expression was associated with poorer long-term outcomes (5). In a meta-analysis of 8,419 patients with gastric cancer (GC), researchers found that PD-L1 positivity in patients with GC is associated with poor prognosis and poor OS; however, there were no significant differences between PD-L1 expression and lymph node metastasis and overall TNM stage (25). A systematic review study including 13 clinical studies with 1,422 patients with cervical cancer found that high PD-L1 expression is associated with the poor OS but not with progression-free survival (PFS); overexpression of PD-L1 in tumor cells and tumor-infiltrating immune cells predict poor OS (26). In a meta-analysis on RCC that included six studies and 1,323 cases, it was found that higher levels of PD-L1 expression in RCC increases the risk of death by 81%, representing a poor prognosis (27). A meta-analysis included 14,367 patients in 47 studies that focused on the relationship between PD-L1 expression in primary breast cancer (PBC) and found that PD-L1 expression in tumors is correlated with higher clinical risk pathological parameters and poor prognosis in patients with PBC and that patients with PD-L1$^+$ tumors are significantly associated with shorter disease-free survival (DFS) and OS (28). In a meta-analysis of PD-L1 expression and CRC prognosis, which included clinical data from 4,344 patients in 12 studies, the results showed that PD-L1 overexpression is correlated with shorter OS and RFS/DFS ratios; the study concludes that PD-L1 can be an effective biomarker for negative prognosis and poor clinicopathological characteristics of CRC (29). In a meta-analysis of the association between PD-L1 expression and melanoma, including 13 articles with a total of 1,062 enrolled patients with melanoma, the analysis revealed that high PD-L1 expression is not associated with patient OS or PFS; however, PD-L1 overexpression is negatively related to lymph node metastasis; this study suggests that PD-L1 expression cannot be used as a marker of prognosis in melanoma patients (30).

Although PD-L1 expression is associated with poor prognosis in patients with tumors in most cases, it must be noted that the results are inconsistent in a number of studies. Therefore, one needs to be aware that PD-L1 expression is diverse in different types of tumors, PD-L1 expression is also diverse in the population and the relationship between PD-L1 and clinical tumor cases and patient prognosis is also variable. The role of PD-L1 in different types of tumors may also be inconsistent and its mechanism of action may be affected by a number of factors. Therefore, more detailed studies are needed to elucidate the mechanism of PD-L1 action in different tumors, so we can achieve better results for immunotherapy and target therapy more effectively.

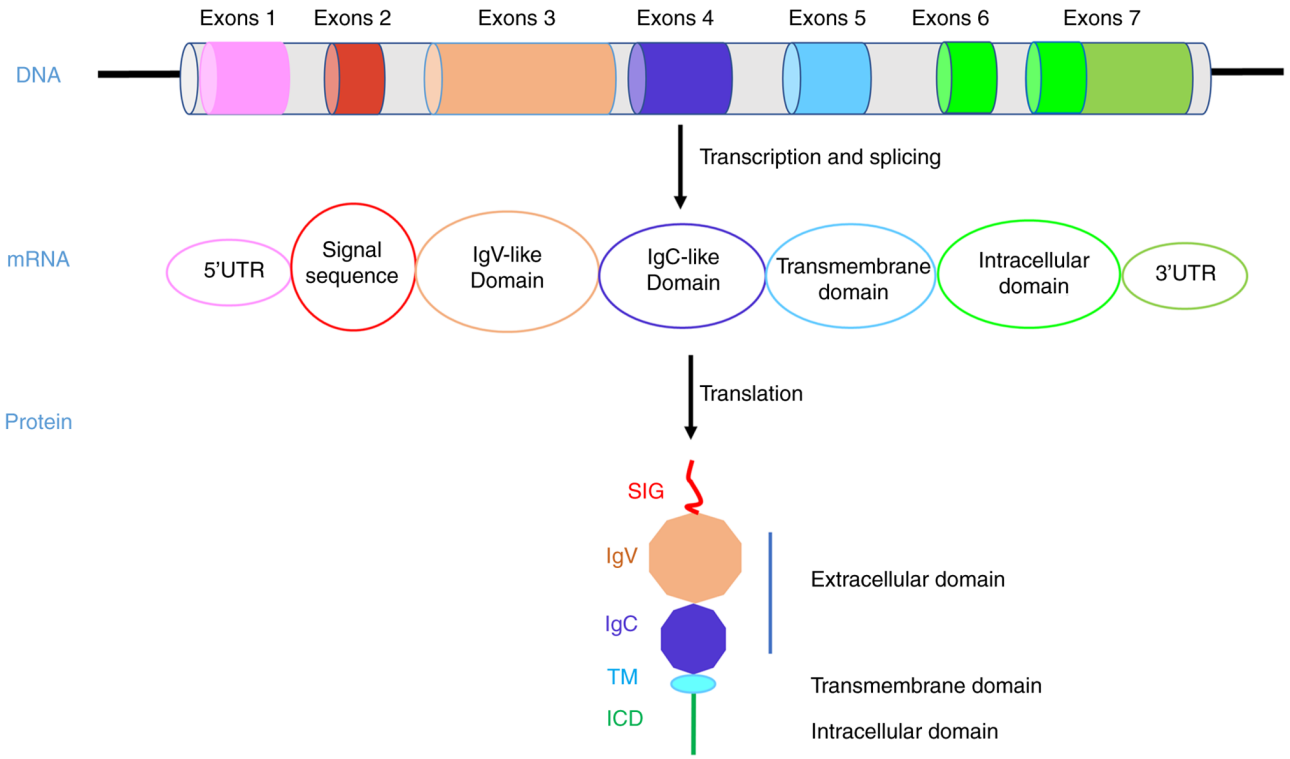

Figure 1. Structures of PD-L1 DNA, mRNA and protein. The DNA of PD-L1 consists of seven exons. The mRNA is divided into 5'UTR, signal sequence, IgV like domain, IgC-like domain, transmembrane domain, intracellular domain and 3'UTR while, correspondingly, the protein of PD-L1 contains IgV, IgC, transmembrane and intracellular domain parts (20). PD-L1, programmed death ligand 1; SIG, signal sequence; TM, transmembrane domain; ICD, intracellular domain.

4. Intrinsic non-immune function of PD-L1

Based on the published studies, the non-immune checkpoint functions of PD-L1 are mainly: Promoting tumor proliferation, promoting EMT and stemness, regulating drug resistance, regulating tumor metabolism, maintaining genomic stability and entering the nucleus to perform functions. These are described separately in this section. (Fig. 2).

Functions of PD-L1 in tumor proliferation. The interaction between PD-L1 and PD1 has been widely reported to interfere with the T cell receptor (TCR) signaling transduction of T cells. PD-L1 is vital in inhibiting T-cell-mediated immune response in cytotoxic T cells, leading to immune killing escape and tumor progression in several malignancies (31).

Studies have shown that PD-L1 can regulate cancer cell growth, proliferation and suppress apoptosis without PD-1 involvement (12,32-39). A study showed that the knockdown of PD-L1 expression in GC cells can significantly suppress cell proliferation, migration, invasion, apoptosis, cell cycle, tumorigenicity and cytotoxic sensitivity to CIK therapy (32). Lotfinejad *et al* (33) demonstrate that PD-L1 knockdown can reduce triple-negative breast cancer (TNBC) cell proliferation and induce apoptosis via intrinsic and extrinsic apoptosis pathways. In a mouse sarcoma model, blocking PD-L1 on tumors could interrupt tumor progression and cell glycolysis; the mechanism is to suppress mTOR signals and decrease the expression of some glycolytic enzymes (34). In ovarian cancer and melanoma, Clark *et al* (35) observed that PD-L1^{low} cells proliferate more weakly than control cells *in vitro* and PD-L1 attenuation also reduces mTORC1 activity. Fan *et al* (36) found that Cbl-b could interact with STAT5a and cause its ubiquitination, which downregulates PD-L1 expression and inhibits cell proliferation, but miR-940 could target Cbl-b and then upregulate PD-L1 expression and promote gastric cancer cell proliferation. A study found that in TNBC and NSCLC, the cell surface adhesion receptor CD44 was a critical positive regulator of PD-L1; CD44 could bind to the regulatory region of PD-L1, which contains the CD44-ICD binding site and activates PD-L1 transcription through its ICD; the activated PD-L1 could promote tumor cell proliferation independent of T cell response (37). During cell division, PD-L1 is a subunit of the adhesin complex: PD-L1 could compensate for the loss of Sororin and compete with Wing Apart-Like (WAPL) for binding to PDS5B, which secures proper sister chromatid cohesion and segregation; depleting PD-L1 leads to multinuclear cells and suppresses cell proliferation *in vitro* and tumor growth *in vivo* in immunodeficient NSG mice (38). In NSCLC cells, activation of EGFR could upregulate the expression of PD-L1 through IL-6/Janus kinase (JAK)/STAT3 signal pathway and promote NSCLC cell proliferation (39). Yang *et al* found that PIM2-mediated phosphorylation of heat shock factor 1 (HSF1) at Thr120 enhanced the stability of HSF1 protein and phosphorylation of HSF1 could bind to the promoter of PD-L1, which strengthened PD-L1 expression and promoted breast cancer cells proliferation (12).

Although a number of basic studies have demonstrated the ability of PD-L1 to promote tumor proliferation and progression (Table I), clinicopathological data have also confirmed that high PD-L1 expression is associated with poor prognosis

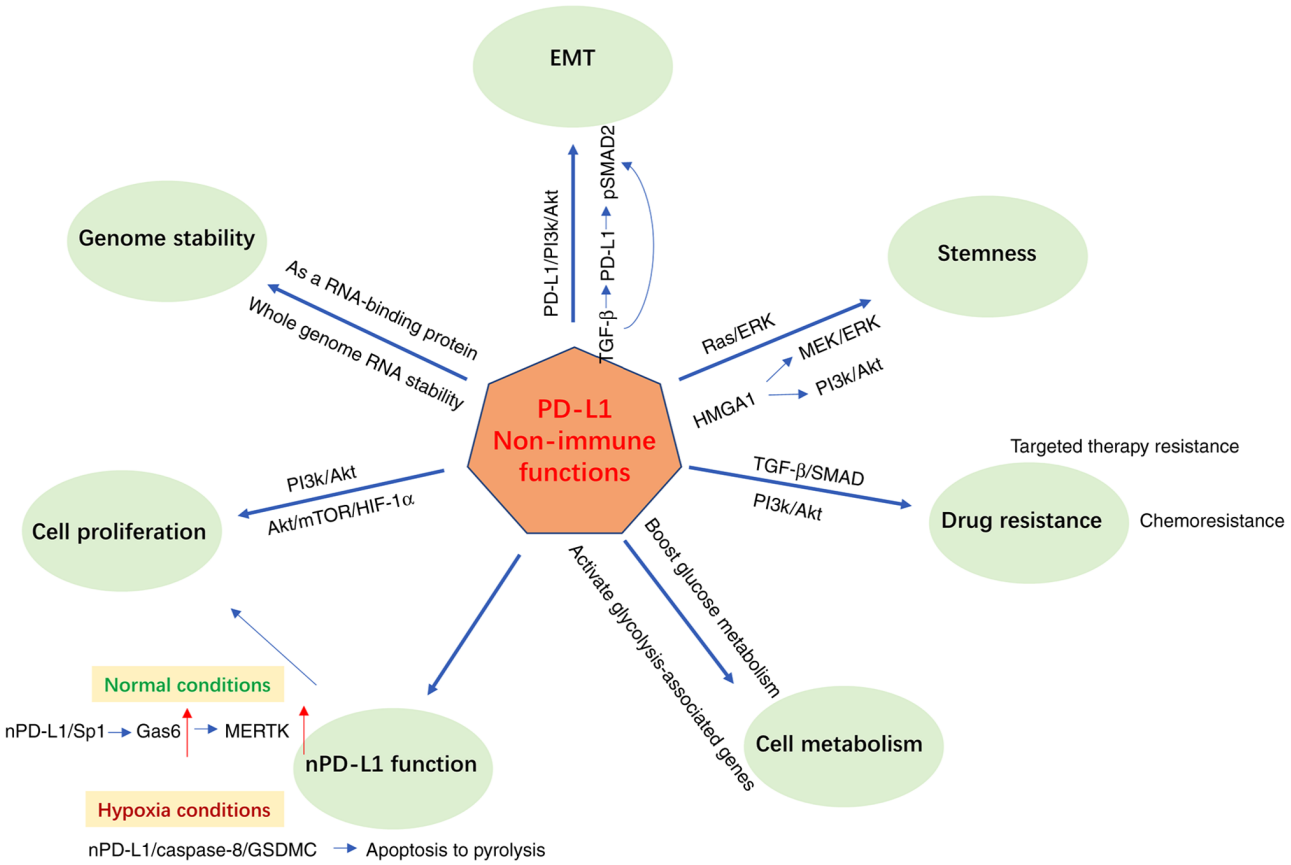

Figure 2. The non-immune functions of PD-L1. As shown in the figure, the available studies have shown that the non-immune checkpoint functions of PD-L1 in tumor cells are: Regulation of proliferation, EMT, CSCs, metabolism, drug resistance, genomic stability and entry into the nucleus to perform functions. However, there are more phenotypic studies and the related mechanisms and signaling pathways are less studied. PD-L1, programmed death ligand 1; EMT, epithelial-mesenchymal transition; CSCs, cell stem cells; p, phosphorylated; HMGA1, high mobility group AT-hook 1; HIF-1α, hypoxia-inducible factor 1α; MERTK, MER proto-oncogene, tyrosine kinase; GSDMC, transcription of the gasdermin C.

in most cases. However, the mechanism of PD-L1 promoting tumor proliferation and progression is not well studied. Most of the studies found that PD-L1 high expression can show the proliferative phenotypes of tumor cells, but how does PD-L1 promote tumor proliferation? Does it promote the activation of transcription factors and participate in post-transcriptional modification (PTM) of certain oncogenes or tumor suppressors? These may be the next breakthroughs for PD-L1 research.

Functions of PD-L1 in drug resistance. The most common and effective cancer treatment methods include surgery, chemotherapy and radiation therapy. Chemotherapy is quite important in cancer treatment and can extend the survival time of patients with a number of cancers. Advances in biotechnology and intensive research on signaling pathways have led to the rapid development of targeted therapy, which has also become an important option for tumor treatment.

Although chemotherapy has a noticeable effect in the early period of advanced tumor treatment, but, after a while, a large proportion of chemoresistance might develop, which leads to treatment failure and metastasis occurrence (40). Studies have shown that the high expression of PD-L1 in cancer cells could cause chemotherapy resistance in cancer therapy (Table II). Following doxorubicin treatment, PD-L1 was observed to transfer from membrane to nuclear concomitant with the translocation of phosphorylated Akt and promote doxorubicin-induced drug resistance (41). The mechanism was that doxorubicin-dependent downregulation of cell surface PD-L1 was accompanied by upregulation of PD-L1 in the nucleus and this redistribution of PD-L1 occurred with a similar translocation of phosphorylated Akt to the nucleus. PD-L1 was considered an independent prognostic risk factor for osteosarcoma as patients with high PD-L1 expression were observed to have a lower five-year survival rate and knocking out PD-L1 in osteosarcoma cells could increase doxorubicin and paclitaxel sensitivities (42). A pair of studies reported that PD-L1 could bind to NBS1 to form a complex and lead to cisplatin resistance in HNSCC and knockdown of PD-L1 or NBS1 could reverse this drug resistance. PD-L1 and IL-6 were over-expressed on cisplatin-resistant HNSCC cells (43,44). In cisplatin resistant NSCLC, researchers found that decreased COP1 could promote c-Jun accumulation, inhibit HDAC3 expression and enhance PD-L1 acetylation, which would mediate or maintain the drug resistance of cancer cells (45). In ovarian cancer cells, $Sp17^{high}$ ($PD\text{-}L1^{+}MHC\text{-}II^{-}$) cells showed enhanced resistance to paclitaxel-induced cell death compared with $Sp17^{low}$ ($PD\text{-}L1^{-}MHC\text{-}II^{+}$) cells (46), which means Sp17 and PD-L1 are related to paclitaxel resistance. lncRNA FGD5-antisense 1 (FGD5-AS1) could negatively regulate miR-142 and promote cisplatin resistance through miR-142-5p/PD-L1 axis (47). miR3609 could specifically bind to the 3' UTR

Table I. PD-L1 regulates tumor cell proliferation.

First author, year	Tumor type	Upstream regulator	Downstream signal pathway	Mechanism	(Refs.)
Yang *et al*, 2019	Breast cancer	HSF1	-	HSF1 Thr120 phosphorylation induced HSF1 binding to PD-L1 promoter and enhanced PD-L1 expression and promote tumor growth	(12)
Yu *et al*, 2020	Breast cancer	-	PDS5B/Sororin/WAPL signal	PD-L1 compensates for the loss of Sororin, PD-L1 competes with WAPL for binding to PDS5B and secures proper sister chromatid cohesion and segregation	(38)
Lotfinejad *et al*, 2021	Breast cancer TNBC	-	caspase 3/caspase 9 apoptotic signal	PD-L1 knockdown reduced cancer cell proliferation and induced apoptosis via intrinsic and extrinsic apoptosis pathways	(33)
Kong *et al*, 2020	Breast and Lung Cancers	CD44	PI3K/Akt/mTOR signal	CD44 activated PD-L1 transcription through its cleaved ICD	(37)
Li *et al*, 2017	Gastric cancer	-	-	Knockdown PD-L1 in gastric cancer cells could suppress cell proliferation, migration, invasion, tumorigenicity and cytotoxic sensitivity to CIK	(32)
Fan *et al*, 2018	Gastric cancer	miR-940	-	miR-940/Cbl-b/STAT5a axis regulated expression of PD-L1, promoted cancer cell proliferation and migration	(36)
Zhang *et al*, 2016	Lung cancer NSCLC	EGFR	IL-6/JAK/ STAT 3 signal	EGFR involved in the regulation of PD-L1 expression and cell proliferation via the IL-6/JAK/STAT3 signal	(39)
Chang *et al*, 2015	Mouse sarcoma model	-	Akt/mTOR signal	Blocking PD-L1 on tumors dampens cell proliferation and glycolysis, the mechanism is mTOR activity suppressed and glycolysis enzymes down-expression	(34)
Clark *et al*, 2016	Ovarian cancer and melanoma	-	Akt/mTOR signal	PD-L1^{low} cells proliferated more weakly than control, PD-L1 attenuation destroyed mTORC1 activity	(35)

HSF1, heat shock factor 1; PD-L1, programmed death ligand 1; JAK, Janus kinase.

region of PD-L1 and suppress PD-L1 expression to sensitize breast cancer cells to doxorubicin (48).

Although targeted therapy is developing rapidly, the problem of rapid drug resistance is a key obstacle to its further development. PD-L1 has also been found to play a role in the resistance of some targeted therapies. In *EGFR*-mutated NSCLC cells, PD-L1 was correlated with the sensitivity of tyrosine kinase inhibitors (TKIs) and PD-L1 could induce EMT by activating the TGF-β/Smad signal pathway, leading to primary resistance to gefitinib (49). PD-L1 expression was found to be increased in gefitinib-resistant CRC cells, but nano-diamino-tetras (NDAT)-induced low PD-L1 expression could reverse tumor gefitinib resistance (50). In sorafenib-resistant hepatoma cells, nuclear factor erythroid 2-related factor 2 inhibited the expression of miR-1 and loss of miR-1 contributed to the PD-L1 upregulation and drug resistance (51).

Most of these studies on the role of PD-L1 in drug resistance have also focused on the description of the phenotype, while the underlying mechanisms have not been much studied. It was hypothesized that PD-L1 may be involved in regulating the expression or PTM of certain drug resistance-related genes to cause the development of drug resistance. If the mechanism can be found, it will help find a targeted drug resistance solution.

Functions of PD-L1 in EMT and maintaining stemness. Studies have shown that PD-L1 plays a vital role in promoting EMT and maintaining the stemness of cancer stem cells (52-57) (Table III).

PD-1 fusion protein-mediated stimulation of PD-L1 and the cytoplasmic domain of PD-L1 play a critical role in promoting the EMT phenotype of Eca-109 cells (52). Upregulation of PD-L1 in skin epithelial cells promotes EMT and accelerates carcinogenesis in squamous cell carcinoma (53). The significant association between PD-L1 expression and EMT phenotype was maintained in *EGFR*-mutated pADCs in

Table II. PD-L1 regulates tumor cell drug resistance.

Author, year	Tumor type	Resistant drugs	Downstream or upstream signal pathway	Mechanism	(Refs.)
Ghebeh *et al*, 2010	Breast cancer	Doxorubicin	PI3K/Akt signal	Doxorubicin-dependent cell surface downregulation of PD-L1 accompanied with an upregulation of nucleus PD-L1. It was concurrent with a similar translocation of phosphorylated Akt to the nucleus	(41)
Li *et al*, 2019	Breast cancer	Adriamycin	-	Knockdown of PD-L1 by siRNA restored the sensitivity of MCF7/ADR cells to Adriamycin	(48)
Huang *et al*, 2020	CRC	Gefitinib	PI3K/Akt signal	Gefitinib suppress PD-L1 expression but did not inhibit proliferation via PI3K in gefitinib-resistant cells	(50)
Li *et al*, 2020	Hepatoma cells	Sorafenib	NRF2/microRNA-1 (upstream)	NRF2 was induced in sorafenib-resistant hepatoma cells and inhibited miR-1 expression. Loss of miR-1 contributed to PD-L1 upregulation	(51)
Shen *et al*, 2020	HNSCC	Cisplatin	NBS1/MRN complex	Knockdown of either PD-L1 or NBS1 re-sensitized the chemoresistant cell line to cisplatin	(43)
Zhang *et al*, 2018	HNSCC	Cisplatin	LfcinB/IL-6 signal	LfcinB displayed a direct cytotoxic effect on cisplatin-resistant cells, increase of IL-6 and PD-L1 in cisplatin resistant cells was abolished *in vitro* by LfcinB	(44)
Zhu *et al*, 2021	Lung cancer	Cisplatin	FGD5-AS1/miR-142 (upstream)	PD-L1 was a key effector of FGD5-AS1/miR-142 axis to regulate chemoresistance of DDP-resistant LAD cells	(47)
Wang *et al*, 2020	NSCLC	Cisplatin	COP1/c-Jun/HDAC3 axis (upstream)	Enhanced histone H3 acetylation of the PD-L1 promoter via the COP1/c-Jun/HDAC3 axis was crucial for the PD-L1 increase in drug-resistant cancer cells	(45)
Zhang *et al*, 2019	Lung cancer NSCLC	Gefitinib	TGF-β/Smad signal	PD-L1 contributes to resistance to EGFR-TKI in EGFR-mutant NSCLC cells, mediated through the induction of EMT via activation of the TGF-β/Smad signal	(49)
Liao *et al*, 2017	Osteosarcoma	Doxorubicin and Paclitaxel	PI3K/Akt/mTOR signal	PD-L1 knockdown increased drug sensitivities for doxorubicin and paclitaxel	(42)
Gao *et al*, 2018	Ovarian Cancer	Paclitaxel	-	$Sp17^{high}$ ($PD\text{-}L1^{+}MHCII^{-}$) cells showed enhanced resistance to Paclitaxel-induced cell death compared with $Sp17^{low}$ ($PD\text{-}L1^{-}MHCII^{+}$) cells	(46)

COP1, constitutively photomorphogenic 1; CRC, colorectal cancer; FGD5, FVVE, RhoGEF and ph domain-containing protein 5; HNSCC, head and neck squamous cell carcinoma; MHC II, major compatibility complex II; NSCLC, non-small cell lung cancer; NRF2, nuclear factor erythroid 2-related factor 2.

lung cancer cells (54). A study found that CRC characterized by a lack of CDX2 and prominent expression of ALCAM frequently (71%) showed PD-L1 positivity, representing the relations between PD-L1 and EMT (55). It was also found

Table III. PD-L1 regulates EMT and maintain stemness.

First author, year	Tumor type	EMT or stemness	Downstream signal pathway	Mechanism	(Refs.)
Alsuliman *et al*, 2015	Breast cancer	EMT	EMT markers	Strong association between PD-L1 and claudin^{low} breast cancer subset, which had high EMT score	(59)
	Breast cancer	stemness	CSC markers PI3K/Akt signal	PD-L1 promotes OCT4 and Nanog expression in breast cancer stem cells by activating PI3K/Akt pathway	(62)
Gao *et al*, 2019	Breast cancer	stemness	CSC markers PI3K/Akt/ ERK	miR-873 inhibited PD-L1 expression through binding to its 3'-UTR and miR-873 attenuated the stemness dependent on PD-L1 and PI3K/Akt/ERK1/2 signal	(69)
Rogers *et al,* 2019	Breast cancer TNBC	EMT	EMT markers	Reversing a classic EMT signature, miR-200c repressed a number of genes encoding immunosuppressive factors including *PD-L1/CD273, HMOX-1* and *GDF15*	(68)
Zhi *et al*, 2015	CRC	EMT and stemness	EMT markers CSC markers	CD133^{+} cells expressed high level of PD-L1. PD-L1^{+} cancer cells showed the characteristic of EMT	(57)
Inaguma *et al*, 2017	CRC	stemness	EMT markers	Lack of CDX2 and prominent expression of ALCAM frequently (71%) showed PD-L1 positivity	(55)
Wei *et al*, 2019	CRC	stemness	HMGA1 signal	PD-L1 promotes CRC stem cell expansion by activating HMGA1-dependent signal	(64)
Chen *et al*, 2017	Esophageal cancer	EMT	EMT markers	PD-1 fusion protein mediated stimulation of PD-L1 and the cytoplasmic domain of PD-L1 played a critical role in promoting EMT phenotype of esophageal cancer cells	(52)
Ock *et al*, 2016	HNSCC	EMT and stemness	EMT markers CSC markers	CMTM4-knockdown inhibited the expression of interferon-γ induced PD-L1, CMTM4 played an important role in regulating EMT/CSC phenotypes	(58)
Fang *et al*, 2016	Leukemia	stemness	JNK/Cyclin D2 signal	PD-L1 could promote cell cycle entry of leukemia initiating cells through JNK/ Cyclin D2 signal	(63)
Chen *et al*, 2014	Lung cancer	EMT	EMT markers	ZEB1, an EMT activator and transcriptional repressor of miR-200, relieves miR-200 repression of PD-L1	(66)
Kim *et al,* 2016	Lung cancer	EMT	EMT markers	The significant association between PD-L1 and EMT phenotype was maintained in EGFR-mutated pADCs	(54)
David *et al*, 2017	Lung cancer NSCLC	EMT	EMT markers	TGF-β1 upregulated PD-L1 gene transcription in a SMAD2-dependent manner and a positive association between PD-L1 and p-Smad2 was found in NSCLC	(61)
Tieche *et al*, 2019	Lung cancer NSCLC	EMT	EMT markers	EMT was associated with overexpression of PD-L1 in NSCLC	(56)
Hong *et al,* 2020	Lung cancer NSCLC	EMT	EMT markers	Circular RNA Circ-CPA4 could act as an RNA sponge for let-7 miRNA and inhibit cell growth, migration and EMT by down-regulating PD-L1 to promote cancer cell death	(70)

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Table III. Continued.

First author, year	Tumor type	EMT or stemness	Downstream signal pathway	Mechanism	(Refs.)
Bouillez *et al*, 2017	Lung cancer NSCLC	stemness EMT	CSC markers EMT markers	Targeting MUC1-C in NSCLC tumors suppresses PD-L1 and induces innate and adaptive immunity, linking this inflammatory response to EMT and self-renewal	(84)
Zhao *et al*, 2019	Lymphoma	EMT	EMT markers	SNHG14 sponged miR-5590-3p to upregulate ZEB1 and ZEB1 transcriptionally activated SNHG14 and PD-L1 to promote the immune evasion of DLBCL cells	(65)
Wang *et al*, 2019	Lymphoma	EMT	EMT markers Ras/ERK signal	MALAT1 sponged miR-195 to regulate the expression of PD-L1, knocking down of MALAT1 suppressed EMT-like process via Ras/ERK signaling pathway	(67)
Wang *et al*, 2015	renal cell carcinoma	EMT and stemness	EMT markers CSC markers	PD-L1 could induce EMT and enhance RCC cancer stemness through up-regulation of SREBP-1c	(60)
Cao *et al,* 2011	Squamous cell carcinoma	EMT	EMT markers	Upregulation of PD-L1 in skin epithelial cells promotes EMT and accelerates carcinogenesis	(53)

ALCAM, activated leukocyte cell adhesion molecule; CDX2, caudal-type homeobox protein 2; CMTM4, CKLF-like MARVEL transmembrane domain containing 4; CSC, cell stem cell; EMT, epithelial-mesenchymal transition; HMGA1, high mobility group AT-hook 1; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; MUC1-C, mucin 1-c; NSCLC, non-small cell lung cancer; OCT4, octamer-binding protein 4; pADCs, pulmonary adenocarcinomas; PD-L1, programmed death ligand 1; SNHG14, small nucleolar RNA host gene 14.

that EMT is associated with the overexpression of PD-L1 in NSCLC (56). One study reports that PD-L1$^+$ cancer cells show the characteristics of EMT (57). A survival analysis using The Cancer Genome Atlas database shows that PD-L1$^+$/EMT$^-$ patients have a better prognosis than PD-L1$^+$/EMT$^+$ patients in HNSCC (58). A study reports that PD-L1 expression increases in the induction of human breast EMT by activating PI3K/Akt pathway and that PD-L1 can also regulate the EMT state of breast cancer cells (59). PD-L1 can induce EMT and enhance the stemness of renal cell carcinoma by upregulating SREBP-1c (60). In NSCLC, TGF-β1 can upregulate PD-L1 expression at the transcriptional level through phosphorylation of Smad2, M7824 is a novel bifunctional agent which could target both PD-L1 and TGF-β1, using M7824 to treat NSCLC could attenuate TGF-β1 mediated EMT (61).

PD-L1 is considered essential in maintaining the stemness of breast cancer stem cells because it can upregulate the expression of Oct4 and Nanog in a PI3K/Akt-dependent pathway and directly promote BMI1 expression to affect the stemness of breast CSCs (62). CD133$^+$ cells in both cell lines and CRC tissues express a high level of PD-L1 (57). Fang *et al* (63) found that PD-L1 can promote the proliferation of leukemia-inducing cells through PD-L1/JNK/Cyclin D2 signaling pathway and prompt leukemia stem cells to enter the cell cycle. PD-L1 can induce a stem cell-like state and interact directly with high mobility group AT-hook 1 (HMGA1) to activate PI3K/Akt and MEK/ERK pathways in CRC to maintain the self-renewal of CSCs (64).

Some studies show that non-coding RNAs and miRNAs can regulate cancer stemness and EMT by binding with PD-L1. SNHG14 can sponge miR-5590-3p to upregulate ZEB1 and ZEB1 transcriptionally activate SNHG14 and PD-L1 to promote the immune evasion of diffuse large B cell lymphoma cells (65). ZEB1, an EMT activator and transcriptional repressor of miR-200, can relieve miR-200 repression of PD-L1, leading to lung adenocarcinoma metastasis (66). In lymphoma cells, MALAT1 can sponge miR-195 to regulate the expression of PD-L1, knocking down MALAT1 also suppresses the EMT-like process via the Ras/ERK signaling pathway (67). In TNBC, miR-200c can repress a number of genes encoding immunosuppressive factors, including *CD274*/*CD273, HMOX-1* and *GDF15* to reverse the classic EMT signature (68). miR-873 can inhibit PD-L1 expression by directly binding to the 3'-UTR of *CD274*, which attenuates the stemness and chemoresistance of breast cancer cells through the PI3K/Akt signaling pathway (69). Circular RNA circ-CPA4 can act as an RNA sponge for let-7 miRNA and inhibit cell growth, migration and EMT by downregulating PD-L1 to promote NSCLC cell death (70).

These studies have mainly focused on the role of PD-L1 in cancer cell stemness maintenance and EMT and most of them are limited to detecting the relationship between PD-L1 expression changes and markers of stemness and EMT; however, little research has been performed on the specific mechanisms. Whether PD-L1 could regulate cancer metastasis

Table IV. PD-L1 regulates cell metabolism.

First author, year	Tumor type	Downstream signal pathway	Mechanism	(Refs.)
Ma *et al*, 2020	Acute myeloid leukemia (AML)	Akt/mTOR/HIF-1α signal	Glycolysis-associated genes were highly expressed in a PD-L1 high-expressed cell line. Overexpressed PD-L1 enhanced glucose consumption and the extracellular acidification rate	(75)
Cao *et al*, 2019	Bladder cancer	ITGB6/STAT3 signal	RORC regulates bladder cancer glucose metabolism by participating in PD-L1/ITGB6/STAT3 signaling	(74)
Wang *et al*, 2018	Cervical cancer	Integrin β4/SNAI1/ SIRT3 signal	PD-L1 promotes the growth and metastasis of cervical cancer by activating the ITGB4/SNAI1/ SIRT3 signal	(73)
Takada *et al*, 2017	Lung cancer NSCLC	-	PD-L1-expressing NSCLC had a high glucose metabolism	(72)
Feng *et al*, 2017	Lung cancer	TAZ/GRP81 signal	GPR81-mediated upregulation of PD-L1 in glucose-stimulated cancer cells that recapitulates glycolysis dependent on LDHA	(76)
Cui *et al*, 2020	Lung cancer	Glycolysis pathway	Tumor PD-L1 expression was positively correlated with PET-CT SUV max, total lesion glycolysis, HK2 and GLUT-1	(77)
Chang *et al*, 2015	Squamous cell carcinoma	PI3K/Akt/mTOR signal	Blocking PD-L1 on tumors dampens glycolysis by inhibiting mTOR and decreasing glycolysis enzymes	(34)

GRP81, G protein-coupled receptor 81; HIF-1α, hypoxia-inducible factor 1α; ITGB6, integrin subunit β6; PD-L1, programmed death ligand 1; PET-CT, positron emission tomography-computed tomography; SIRT3, sirtuin-3; SNAI1, Snail family transcriptional repressor 1; SUVmax, maximum standardized uptake value; TAZ, tafazzin.

or the mechanism behind this has not been discovered, but is worth exploring, as several clinical studies (3,15,36,70) have confirmed the association between PD-L1 and lymph node metastasis and distant metastasis of tumors.

Functions of PD-L1 in cell metabolism. Tumor cells can continuously adjust the metabolic pathways to meet their energy requirements and respond to the availability of nutrients. Warburg found that despite sufficient oxygen, most solid tumor cells still choose the aerobic glycolysis pathway rather than the oxidative phosphorylation pathway to adapt to their microenvironment (71). Studies show that PD-L1 can promote tumor progression by boosting the glucose metabolism of tumor cells (Table IV). A study used ^{18}F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography (^{18}F FDG PET/CT) to evaluate the metabolic effects of PD-L1 protein on lung cancer and it was found that high PD-L1 expression can promote glucose metabolism in NSCLC (72). PD-L1 can promote tumor cell glycolysis through Akt/mTOR signal pathway and induce immune cells to consume glucose in the microenvironment. Inhibiting the expression of PD-L1 can cause mTOR activity inhibition to downregulate glycolytic enzymes, thereby inhibiting glycolysis (34). In cervical cancer, PD-L1 directly binds to integrin β4 and activates Akt/GSK3β signaling pathway and promotes glucose metabolism (73). Retinoic acid-related orphan receptor C (RORC) is found to negatively regulate the expression of PD-L1 by binding to the PD-L1 promoter region, which can inhibit the nuclear translocation of STAT3 and further inhibit the proliferation and glucose metabolism of bladder tumor cells (74). Ma *et al* (75) report that in acute myeloid leukemia (AML) cell lines, glycolysis-associated genes *ALDOA, PGK1, LDHA* and *HK2* are highly expressed in the PD-L1^{high} cell line and overexpressed PD-L1 enhances glucose consumption rate, accompanied by decreased apoptosis and S phase cells. Feng *et al* (76) report that lactate-induced PD-L1 is mediated by its receptor GPR81 and GPR81-mediated upregulation of PD-L1 in glucose-stimulated lung cancer cells that recapitulated the enhanced glycolysis is dependent on LDHA. In patients with primary lung adenocarcinoma who received ^{18}F-FDG PET/CT before treatment, PD-L1 expression in the tumor was positively correlated with ^{18}F-fluorodeoxyglucose maximum standardized uptake value (SUVmax), total lesion glycolysis (TLG), HK2 and glucose transporter 1 (GLUT-1) expression (77).

These studies initially reveal the regulatory role of PD-L1 in glucose metabolism, but a number of mechanisms remain to be elucidated. Moreover, the regulation of PD-L1 in other metabolic pathways has yet to be reported. As tumor metabolism is not limited to glucose metabolism, it is hypothesized that PD-L1 may also play an important role in regulating other metabolic pathways in tumor cells, so more in-depth and extensive research is needed.

Function of PD-L1 in regulating mRNA stability. In addition to the numerous intrinsic functions previously described,

Table V. Function of nPD-L1.

First author, year	Tumor type	Regulator	Downstream signal pathway	Mechanism	(Refs.)
Ghebeh *et al*, 2010	Breast cancer	-	PI3K/Akt signal	Doxorubicin-dependent cell surface downregulation of PD-L1 was accompanied by an upregulation of nPD-L1. This re-distribution of PD-L1 was concurrent with a similar translocation of phosphorylated Akt to the nucleus	(41)
Hou *et al*, 2020	Breast cancer	TNFα	caspase-8/GSDMC signal	Under hypoxia, p-STAT3 interacts with PD-L1 and facilitates its nuclear translocation, enhancing the transcription of GSDMC. GSDMC is cleaved by caspase-8 with TNFα treatment, generating a GSDMC N-terminal domain that induces pyroptosis	(82)
Gao *et al*, 2020	Breast cancer	p300	NF-κB signal-related genes and MHC-I genes	PD-L1 translocated from plasma membrane into nucleus through interactions with endocytosis components and nucleocytoplasmic transport ways, regulated by p300-mediated and HDAC2-dependent deacetylation of PD-L1	(83)
Satelli *et al*, 2016	CRC and prostate cancer	-	-	nPD-L1 expression was significantly associated with short survival durations	(81)
Chen *et al*, 2014	Lung cancer NSCLC	KPNB1	Gas6/MERTK signal	PD-L1 translocated into cancer cell nucleus via binding of KPNB1, nPD-L1 coupled with Sp1, regulated Gas6 synthesis, promoted Gas6 secretion to activate MERTK signal	(80)

GSDMC, transcription of the gasdermin C; HDAC2, histone deacetylase 2; KPNB1, karyopherin subunit β1; MERTK, MER proto-oncogene, tyrosine kinase; MHC-I, major compatibility complex I; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1; p-, phosphorylated.

PD-L1 also has a role in regulating gene stability. Tu *et al* (78) demonstrate that PD-L1 can act as an RNA-binding protein in cells to regulate the mRNA stability of NBS1, BRCA1 and a number of other DNA damage-related genes; intracellular PD-L1 can prevent these target RNAs from being degraded, thus increasing the resistance of cells to DNA damage. This study also found that PD-L1 has the ability to regulate whole genome RNA stability by RNA immunoprecipitation and RNA-seq assays. Thus, it provides strong evidence that PD-L1 possesses an intrinsically powerful gene regulatory function. It also predicts that PD-L1 may become a target to interfere with tumor radiotherapy resistance.

Functions of nuclear PD-L1. PD-L1 was previously widely considered to be localized in the cytoplasm and cell membrane, but recently some studies report the nuclear localization and role of PD-L1 in tumor cells (Table V).

The distribution of PD-L1 in different tumor specimens is diverse. Nuclear PD-L1 (nPD-L1) is expressed in RCC, lung cancer and hepatocellular carcinoma tissues and nPD-L1 in human esophageal cancer tissues is significantly correlated with tumor invasion (79,80). According to some reports, the expression of nPD-L1 is associated with a poor prognosis in some tumors. Expression of nPD-L1 in cell-surface vimentin-positive circulating tumor cells is significantly associated with the short-term survival rate of CRC and prostate cancer (81). Doxorubicin treatment can redistribute PD-L1 and increase the expression of nPD-L1 through PI3K/Akt signaling pathway (41). One study has shown that in NSCLC, KPNB1 binds to PD-L1 and promotes its entry into the nucleus (79). At the same time, nPD-L1 can integrate Sp1 to regulate the synthesis of Gas6, promote the secretion of Gas6 and activate the MER proto-oncogene tyrosine kinase signaling pathway to promote cell proliferation (79). In breast cancer, under hypoxic

conditions, pSTAT3 can physically interact with PD-L1 and upgrade its nuclear translocation and enhance the transcription of the gasdermin C (GSDMC) gene, GSDMC is cleaved explicitly by caspase 8 to switch cell apoptosis to pyrolysis and induce tumor necrosis (82). This study showed a new signal pathway of nPD-L1/caspase-8/GSDMC, which is required for macrophage-derived TNFα-induced tumor necrosis. PD-L1 can be acetylated and modified by p300 acetyltransferase at Lys 263 in the cytoplasmic domain and blocking the acetylation of PD-L1 can damage its nuclear translocation, reprogram the expression of immune response-related genes and block the anti-tumor response to PD-1/PD-L1 therapy (83).

These aforementioned studies report how PD-L1 enters the nucleus and the functions it plays in the nucleus, but the exact mechanism of PD-L1 action in the nucleus remains to be elucidated. Therefore, further in-depth studies are needed to clarify the nuclear membrane transfer process and the internal effects of nPD-L1, which will help understand the non-immune checkpoint functions of PD-L1 widely.

5. Conclusions

PD-L1 has an important immune checkpoint function. Its role in tumor evasion of immune killing has been apparent, but PD-L1 is highly expressed in various types of tumor and shows some inherent non-immunological functions. PD-L1 has a number of intrinsic functions, such as promoting tumor proliferation, maintaining the stemness of cancer stem cells and EMT, regulating tumor cell metabolism and promoting drug resistance in tumors (Fig. 2). It also can perform specific functions by entering the nucleus and regulating genome stability.

At present, it is known that PD-L1 has these non-immune checkpoint functions, but not the exact mechanism. For example, the exact mechanism of PD-L1 to promote tumor progression through non-immune checkpoint-dependent pathways, the mechanism of PD-L1 to regulate the EMT process and maintain the stemness of tumor stem cells, the functions of PD-L1 in cancer metastasis, the specific role and function of PD-L1 after entering the nucleus and possible role of PD-L1 in regulating other metabolic pathways in tumors need to be explored widely and deeply. If these functions and mechanisms can be studied carefully, it will help precisely to target and intervene in the PD-L1 pathway from tumor prevention to tumor recurrence and metastasis, providing more possibilities for combining tumor immunotherapy with targeted therapy.

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JD, LL, WZ and YW wrote the manuscript, WJ and XX revised the manuscript, and XX reviewed the final version of the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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