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Post-translational regulations of PD-L1/PD-1: Mechanisms and opportunities for combined immunotherapy

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Abstract

Antibodies targeting programmed cell death protein 1 (PD-1) or its ligand programmed death-ligand 1 (PD-L1) are profoundly changing the methods to treat cancers with long-term clinical benefits. Unlike conventional methods that directly target tumor cells, PD-L1/PD-1 blockade exerts anti-tumor effects largely through reactivating or normalizing cytotoxic T lymphocyte in the tumor microenvironment to combat cancer cells. However, only a small fraction of cancer patients responds well to PD-L1/PD-1 blockade and clinical outcomes have reached a bottleneck without substantial advances. Therefore, better understanding the molecular mechanisms underlying how PD-L1/PD-1 expression is regulated will provide new insights to improve the efficacy of current anti-PD-L1/PD-1 therapy. Here, we provide an update of current progress of PD-L1 and PD-1 post-translational regulations and highlight the mechanism-based combination therapy strategies for a better treatment of human cancer.

Keywords

PD-L1; PD-1; Immunotherapy; Posttranslational modification; Ubiquitination; PROTAC; Glycosylation; Phosphorylation; Acetylation; Palmitoylation

Introduction

In the last few decades, immunotherapy has become an essential part for treating various types of human cancer. The development of immune checkpoint blockade such as antibodies targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programed death 1/

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Conflict of Interest

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programmed death-ligand 1 (PD-1/PD-L1) are fundamentally reshaping the landscape of cancer therapy [1, 2]. Multiple antibodies targeting CTLA-4 or PD-1/PD-L1 have been approved by FDA to treat different types of cancer with long-term therapeutic benefits, which was rarely observed when using conventional therapies [3, 4].

PD-1 was discovered by Honjo's group in 1992 as an apoptosis-associated gene [5]. Later, PD-1 was identified as a co-inhibitory receptor expressed mainly on immune cells, with major biological functions involved in the inhibition of immune responses [6-8]. Accumulating evidence highlights the pivotal function of PD-1 and its physiological ligands PD-L1 and programmed death-ligand 2 (PD-L2) in tumor immune microenvironment as well as the opportunities for cancer therapy [3, 8, 9]. Given that PD-L1 is more widely expressed than PD-L2 in both normal and tumor cells, later studies mainly focus on exploring the immune suppressive functions and mechanisms of PD-1/PD-L1 as well as how aberrant overexpression of PD-L1 in tumor cells allows tumor cells to escape immune surveillances [7]. Mechanistically, PD-L1 expression on the surface of tumor cells binds to PD-1 receptors on T cells, blocking the T cell proliferation and cytokine production (Fig. 1) [7, 8]. As such, antibodies targeting PD-1/PD-L1 are effective for many types of tumors because they enhance the anti-tumor activity of cytotoxic T lymphocytes (CTLs) [1, 3, 8]. However, a large proportion of cancer patients do not respond well to PD-L1/PD-1 blockade and clinical outcomes have reached a plateau without substantial advances [10, 11]. Hence, more studies are warranted to identify optimal therapeutic strategies that might improve the efficacy of cancer immunotherapy.

Although initial work focused on genetic, transcriptional and post-transcriptional regulations of the PD-1/PD-L1 pathway [12-14], a large number of studies suggest that PD-L1 and PD-1 are also regulated by protein post-translational modification, which provides additional opportunities to manipulate immune system to eradicate tumors. In this review, we mainly summarize recent progressions on PD-L1/PD-1 regulations at protein levels and further emphasize the molecular mechanisms and the implicated therapeutic opportunities of these modifications for enhancing the efficacy of cancer immunotherapy.

Post-translational modifications of PD-L1 and the therapeutic opportunities

1. Ubiquitination and PROTACs of PD-L1

The ubiquitin-proteasome system is a crucial mechanism for intracellular protein degradation, which plays important roles in various cellular processes including immunity, inflammation and cancer [15-17]. The target protein can be covalently attached with polyubiquitin chain through the sequential action of three enzymes involving an E1 activating, an E2 conjugating and an E3 ligase enzyme. The polyubiquitinated protein will be subsequently recognized and degraded by the 26S proteasome complex [16, 17]. PD-L1 has been shown to be regulated by different E3 ubiquitin ligases, including but not limited to β-transducin repeat-containing protein (β-TRCP), speckle-type POZ protein (SPOP), HMG-CoA reductase degradation protein 1 (HRD1), and STIP1 homology and U-Box containing protein 1 (STUB1) (Fig. 2A). Specifically, $SCF^{\beta\text{-}TRCP}$ promotes PD-L1 polyubiquitination and degradation following GSK3β-mediated phosphorylation of PD-L1 at the T180 and S184 residues [18, 19]. Our group identified Cullin 3^{SPOP} as a physiological

E3 ubiquitin ligase promoting PD-L1 polyubiquitination and degradation in a cell cycle dependent manner [20]. Mechanistically, cyclin-D-CDK4 stabilizes SPOP in part through phosphorylating SPOP at the S6 residue, leading to the inhibition of APC/Cdh1-mediated SPOP degradation. Furthermore, the combination of CDK4/6 kinase inhibitor palbociclib with anti-PD-1 immunotherapy dramatically enhanced the therapeutic efficacy in part by enhancing tumor regression and improving overall survival rates [20]. PD-L1 can also be targeted by the E3 ligase HRD1 through the ER-associated degradation (ERAD) pathway [21]. The phosphorylation of PD-L1 by AMPK at S195 blocks its ER-to-Golgi translocation, leading to PD-L1 degradation by the ERAD system [21]. As such, combination of AMPK agonist metformin with CTLA-4 blockade significantly enhances T lymphocyte infiltration and suppresses tumor growth in syngeneic mouse models [21]. Furthermore, STUB1 has been shown to polyubiquitinate and down-regulate PD-L1, which can be blocked by MARVEL transmembrane domain containing 4/6 (CMTM4/6) [22, 23]. However, whether targeting STUB1 or CMTM4/6 will pave a way to strength the immunotherapy is important to investigate in the future.

Proteolysis-targeting chimeras (PROTACs) have recently arisen as novel therapeutic modalities to target traditionally “undruggable” proteins by hijacking the endogenous ubiquitin-proteasome system (UPS) to specifically degrade the protein of interest (POI) [24, 25]. PROTACs are ternary chemical complexes that usually consist of three functional parts, an E3 ligase-recruiting chemical ligand, a POI-binding chemical ligand and a linker [25, 26]. Typically, von Hippel–Lindau disease tumor suppressor (VHL) and Cereblon (CRBN) are the most commonly used endogenous E3 ligases in the PROTAC field. Chen *et al.* synthesized P22, a novel resorcinol diphenyl ether-based PROTAC molecule targeting the PD-1/PD-L1 pathway (Fig. 2B) [27]. The resorcinol diphenyl ether-based PROTAC molecule P22 uses pomalidomide as the chemical ligand of the Cullin 4^{CRBN} E3 ubiquitin ligase [27]. P22 on one hand can inhibit the PD-1/ PD-L1 interaction, on the other hand, P22 can moderately reduce the protein levels of PD-L1 likely in a lysosome-dependent manner [27]. Besides the traditional inhibitor based PROTAC of PD-L1, Cotton and colleagues developed first antibody-based PROTACs (AbTACs) inducing the degradation of PD-L1 [28]. AbTACs are recombinant bispecific antibodies that recruit membrane-bound E3 ligases for the degradation of cell-surface proteins based on E3 ligase RNF43, a single-pass transmembrane E3 ligase. Cotton *et al.* synthesized a bispecific antibody which can target both PD-L1 and the E3 ligase RNF43 to induce the lysosomal degradation of PD-L1 (Fig. 2B) [28]. Moreover, the Bertozzi laboratory developed lysosome-targeting chimeras, termed LYTACs, which are composed of an antibody specific to the targeted protein conjugated to a synthetic oligoglycopeptide ligand that binds the cation-independent mannose-6-phosphate receptor (CI-M6PR), a transmembrane glycoprotein responsible for trafficking proteins to lysosomes for degradation [29]. Using this platform, Banik *et al.* successfully targeted PD-L1 for lysosomal degradation (Fig. 2B) [29]. Although several PROTACs have been developed for targeting degradation, they are only validated in cells. Thus, how these PROTACs work *in vivo* to retard tumorigenesis warrant further investigation.

2. Deubiquitination of PD-L1

As ubiquitination is a reversible process, the ubiquitination of PD-L1 has been reported to be reversed by several deubiquitinating enzymes (DUBs), namely COP9 signalosome 5 (CSN5), Ubiquitin Specific Peptidase 22 (USP22), OTU domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) and Ubiquitin Specific Peptidase 9 X-Linked (USP9X) (Fig. 2A). Lim and colleagues identified CSN5 as a DUB for PD-L1 deubiquitination. CSN5 removes the poly-ubiquitin chain on PD-L1, leading to the stabilization of PD-L1. Moreover, CSN5 natural inhibitor curcumin [30] destabilizes PD-L1 and enhances the therapeutic efficacy of CTLA-4 blockade therapy [31]. Except for CSN5, USP22 also can directly deubiquitinate PD-L1 and inhibit its proteasome degradation [32, 33]. Interestingly, USP22 also regulates PD-L1 protein level through modulating the CSN5-PD-L1 axis. Mechanistically USP22 interacts with CSN5 and stabilizes CSN5 in part through deubiquitination, which further inhibits the degradation of PD-L1 in cells [33]. USP9X deubiquitinates and stabilizes PD-L1 in oral squamous cell carcinoma (OSCC) [34]. On the other hand, OTUB1 was identified as another DUB for PD-L1 [35]. Mechanistically, OTUB1 removes K48-linked ubiquitin chains from the PD-L1 and inhibits the degradation of PD-L1 through the ERAD pathway [35]. Although multiple DUBs have been identified for antagonizing PD-L1 ubiquitination process, it remains largely unknown which DUB is more physiological involved in regulating PD-L1 ubiquitination and whether there is cellular context or tissue context dependent regulation of PD-L1 ubiquitination by different DUBs in different cancer types. More importantly, as the specific chemical inhibitors targeting DUBs are becoming a new method for cancer treatment [36], it will be very interesting in the future to evaluate the potential of DUBs inhibitor in combination with PD-1/PD-L1 blockade as more effective anti-cancer therapies.

3. N-glycosylation of PD-L1

Glycosylation including N-linked glycosylation and O-linked glycosylation is an enzyme-directed site-specific process of glycoconjugate formation, which plays important roles in regulating various human diseases [37, 38]. Accumulating evidence indicates that PD-L1 is glycosylated with heavy N-linked glycan moieties that regulates its protein stability and interaction with cognate receptor PD-1, thereby affecting anticancer immunotherapy (Fig. 3) [18, 39]. Although PD-L1 is N-linked glycosylated on N35/192/200/219, the N192/200/219 residues' glycosylation regulates PD-L1 protein stability in part through suppressing GSK3β-β-TRCP-mediated PD-L1 polyubiquitination [18]. Moreover, proper glycosylation of PD-L1 is essential for its recognition by PD-1 in cells. Hence, B3GNT3-mediated poly-N-acetyllactosamine (poly-LacNAc) is required for PD-L1/PD-1 interaction [39]. Li and colleagues also generated glycosylation-specific PD-L1 antibodies (gPD-L1), which can efficiently block PD-L1/PD-1 interaction and subsequently promote PD-L1 internalization and degradation [39]. Furthermore, gPD-L1-ADC (antibody-drug conjugate) has potent anti-tumor activities in triple-negative breast cancer models [39]. STT3, the catalytically active subunit of oligosaccharyltransferase, increases PD-L1 glycosylation in cancer stem cells, leading to PD-L1 upregulation [40]. Etoposide, which can suppress the epithelial-mesenchymal transition (EMT) induced STT3 expression, enhances the therapeutic efficacy of T cell immunoglobulin mucin-3 (TIM-3) blockade therapy [40]. Chaperone Sigma1 and FKBP51s have also been reported to promote PD-L1 glycosylation and protein stability

with unclear mechanisms [41, 42]. Pharmacologic inhibition of Sigma 1 with IPAG (1-(4-Iodophenyl)-3-(2-adamantyl) guanidine) reduced PD-L1 expression and activated T cells *in vitro* in prostate and triple-negative breast cancer models [42]. Inhibition of FKBP51s using selective inhibitor SAFit [43] reduced PD-L1 protein levels and subsequently induced peripheral blood mononuclear cells (PBMCs) death cocultured with glioma cells [41].

4. Phosphorylation of PD-L1

Protein phosphorylation is a ubiquitous post-translational modification of proteins in which an amino acid residue is phosphorylated by a protein kinase by the addition of a covalently bound phosphate group [44]. Emerging evidence has shown that PD-L1 is subjected to phosphorylation at different Ser/Thr/Tyr residues that impact its protein stability and functions. Specifically, glycogen synthase kinase 3β (GSK3β) directly phosphorylates PD-L1 at the T180 and S184 sites, leading to the poly-ubiquitination by β-TRCP (Fig. 4) [18]. Phosphorylation of PD-L1 by GSK3β can be inactivated by the epidermal growth factor (EGF) signaling pathway, leading to inhibition of PD-L1's degradation [18]. Based on this mechanism, EGFR inhibitor gefitinib destabilizes PD-L1 through activating GSK3β and enhances the therapeutic efficacy of PD-1 blockade in syngeneic mouse models [18]. In studies of liver cancer mice with orthotopic tumors grown from Hepa 1-6 cells, MET-mediated phosphorylation and activated GSK3β at the Y56 residue, leading to decreased expression of PD-L1 [45]. The combination of MET inhibitor with anti-PD-1 blockade significantly suppresses tumor growth and prolongs survival in hepatocellular carcinoma (HCC) mouse models [45]. Moreover, AMP activated kinase (AMPK) as an energy sensor directly phosphorylates PD-L1 at the S195 residue, resulting PD-L1 degradation in part through an ERAD pathway (Fig. 4) [21]. Based on these results, metformin that can activate AMPK elevates the efficacy of CTLA-4 blockade in different syngeneic mouse models [21]. Besides the Ser/Thr phosphorylation, PD-L1 can be phosphorylated on tyrosine [46]. Chan *et al.* reported that IL-6-activated Janus Kinase 1 (JAK1) phosphorylates PD-L1 at Y112 (Fig. 4) [46]. The phosphorylation of PD-L1 at Y112 recruits STT3 to promote PD-L1 glycosylation, which protects PD-L1 from proteolytic degradation [46]. Blocking IL-6/JAK1-mediated PD-L1 protein stability through targeting IL-6 by an IL-6 antibody induces synergistic T cell killing effects when combined with anti-TIM-3 therapy in a Hepa 1–6 liver cancer immunocompetent mouse model [46].

5. Acetylation of PD-L1

Non-histone protein acetylation has been demonstrated to play pivotal roles in affecting various physiological and pathological processes through modulating protein stability, protein-protein interaction, subcellular localization, and functional activity [47]. While an unbiased snapshot of key post-translational modification profiles for PD-L1 reported that PD-L1 could be acetylated and EGF treatment increased acetylation level of PD-L1, the detailed molecular mechanisms as well as the biological function of PD-L1 acetylation remains unknown [48]. Recently, our group uncovered that PD-L1 is acetylated on the K263 residual of the cytoplasmic domain, which is dynamically regulated by acetyltransferase p300 and deacetylase HDAC2 (Fig. 4) [49]. Furthermore, we revealed an unexpected PD-L1 translocation from membrane to the nucleus, a process that is largely dependent on the acetylation status of PD-L1 [49]. Through high throughput RNA-seq and ChIP-seq

methods, we showed that PD-L1 *per se*, is essential for the expression of the immune-related genes that governs the anticancer immune response [49]. Moreover, we demonstrated that genetically or pharmacologically modulating PD-L1 acetylation blocks its nuclear translocation and consequently enhances the anti-tumor efficacy of the PD-1 blockade [49]. Interestingly, almost at the same time, several other groups also independently reported nuclear PD-L1 as emerging factor to affect cancer cell proliferation and necrosis [50-52]. However, whether and how PD-L1 acetylation plays important roles in these broad processes warrants further investigation. As the predictive marker for immunotherapy is urgently needed in clinic and high PD-L1 expression is widely used as a maker for patient selection, we also speculate whether nuclear PD-L1 or acetylation status of PD-L1 could serve as a useful biomarker for cancer immunotherapy in the future [53].

6. Palmitoylation of PD-L1

Palmitoylation is the covalent attachment of fatty acids to proteins which is generally done by proteins with the DHHC domain [54, 55]. Recently, three independent groups found that palmitoyltransferases ZDHHC3 or ZDHHC9 could induce PD-L1 palmitoylation at the cystine-272 site, which increases PD-L1 cell surface distribution by preventing its ubiquitination and degradation (Fig. 4) [56-58]. Genetically knockdown the palmitoyltransferase for PD-L1, or using a palmitoylation deficient C272A mutant version of PD-L1, remarkably reduced membrane PD-L1 levels in cancer cells, resulting in increased sensitivity to T-cell mediated cancer cell killing in an *in vitro* co-culture assay [56, 57]. Notably, Yao *et al.* designed a competitive inhibitor PD-PALM, which is a chimeric peptide comprising a cell-penetrating peptide and a peptide fragment from PD-L1 encompassing the Cys272 residue [57]. Treatment of cancer cells with PD-PALM significantly decreased the palmitoylation and expression of PD-L1 and suppressed tumorigenesis [57]. More importantly, in 4T1 syngeneic mouse breast cancer model, *Zdhhc9* knockout potently inhibited 4T1 tumor growth and improved anti-mPD-1 therapeutic efficacy [56]. These results thus support the notion of interrupting PD-L1 palmitoylation to enhance the immunotherapy response.

Regulations of PD-L1 functions via exosomal secretion

Exosomes are small extracellular vesicles (EVs) that are membrane-enveloped particles produced by most cell types [59, 60]. Various protein post-translational modifications including ubiquitination, palmitoylation and acetylation have been previously suggested to be involved in regulation of exosome formation and protein transportation to the exosome [61-64]. Although PD-L1 can be primarily expressed on the surface of tumor cells, it is also found to exist in exosomes of various cancer types [65-68]. Exosomal PD-L1 displays the same extracellular domain topology as its cell surface counterpart, hence circulating PD-L1-positive exosomes can systemically inhibit anti-tumor immunity [65, 67]. Genetic ablation of exosomal PD-L1 or blockade of exosome secretion with inhibitors suppressed tumor growth via anti-tumor immunity in different cancer models [65-67, 69]. Immunotherapy requires biomarkers for providing personalized precision treatment and predicting tumor progression. Hence, exosomal PD-L1 is emerging as a non-invasive and readily available biomarker. In support of this point, the levels of exosomal PD-L1 in the

blood are found to be relatively higher in different tumors such as melanoma, non-small cell lung cancer (NSCLC) [65, 70-72]. However, whether and how exosomal PD-L1 is regulated by post-translational modifications in a similar fashion as acetylation does in regulating PD-L1 nuclear translocation [49] warrants further investigation. Hence, therapeutic agents targeting the related protein modification and exosome pathway are attractive candidates for developing new combined immunotherapy.

Post-translational modifications of PD-1

Different from PD-L1, PD-1 mainly expresses on activated T and B cells [7]. Although the regulation of PD-1 on transcriptional levels has been extensively studied [12, 73], recent studies suggest that post-translational modifications also play important roles in the regulation of PD-1 expression as well as anti-cancer immunity [74, 75].

1. Ubiquitination of PD-1

Through mass spectrometry, Meng *et al.* identified the E3 ubiquitin ligase SCF^{FBXO38} as a binding partner of PD-1 and promoted K48-linked poly-ubiquitination of PD-1 at the K233 site, leading to subsequent degradation of PD-1 through the 26S proteasome (Fig. 5) [75]. Conditional knockout *Fbxo38* in T cells led to faster tumor progression in mice owning to increased expression of PD-1 [75]. More importantly, IL-2 therapy could suppress tumor progression through rescuing *Fbxo38* transcription and downregulating PD-1 protein levels [75]. Recently, Zhou *et al.* identified KLHL22, an adaptor of the Cul3-based E3 ligase, as another PD-1-interacting protein (Fig. 5) [76]. Biochemically, KLHL22 polyubiquitinates PD-1, leading to degradation of PD-1 before its transportation to the cell surface [76]. Treatment with 5-fluorouracil (5-FU) could increase PD-1 expression by inhibiting the transcription of KLHL22, which suggests that PD-1 expression is possibly responsible for the limited efficacy of 5-FU [76]. On the other hand, Casitas B-lineage lymphoma (c-Cbl) was reported to destabilize PD-1 in part through ubiquitination-proteasomal degradation depending on c-Cbl's RING finger function (Fig. 5) [77]. In syngeneic colorectal cancer xenografts, immune cell infiltration was higher in *c-Cbl*$^{+/-}$ compared to *c-Cbl*$^{+/+}$ mice and tumor-associated $CD8^+$ T-lymphocytes and macrophages of *c-Cbl*$^{+/-}$ mice showed higher levels of PD-1 [77]. Although multiple E3 ligases have been reported to ubiquitinate PD-1, it remains unknown which E3 ligase is more physiologically relevant in governing PD-1 protein stability, aberrancy of which will lead to deregulated PD-1 signaling to impact tumorigenesis. Moreover, potential DUBs that targeting PD-1 are highly interested, which also require additional in-depth studies in the future.

2. Glycosylation of PD-1

As a common modification in eukaryotic cells, glycosylation changes, including abnormal core fucosylation and increased N-glycan branching, have been observed in tumor cells [37, 38]. Similar to PD-L1 that has been reported to be heavily glycosylated, PD-1 is also glycosylated in cells [74, 78, 79]. PD-1's molecular weight is about 14 kDa, when expressed in *E. coli* with no glycosylation, but shifts to 35–40 kDa, when expressed in 293T cells with glycosylation similar to that present in host cells [80]. Moreover, upon mutating four potential N-glycosylation sites, PD-1 exhibits substantially reduced molecular weight [80].

Notably, the binding affinity of PD-1 to Camrelizumab, a recently FDA approved PD-1-specific monoclonal antibody (mAb) [81], can be regulated by N-glycan composition of PD-1 [78]. Notably, Camrelizumab can strongly bind to glycosylated PD-1, but the binding to N58A mutant PD-1 that cannot be glycosylated on this site or non-glycosylated PD-1 proteins from *E. coli* is substantially decreased. These results suggest that glycosylation of PD-1 affects the activity of PD-1-specific mAbs [78].

Core-fucosylation is a kind of N-linked glycosylation in which an alpha-1,6 linked fucose is added to the innermost N-acetylglucosamine (GlcNAc) residue and plays important roles in tumorigenesis and immune escape [82, 83]. Through CRISPR-Cas9 screen, Okada *et al.* identified critical genes involved in the core fucosylation pathway as positive regulators of cell-surface PD-1 expression [74]. Among the genes identified, fucosyltransferase Fut8 is the only enzyme that catalyzes core fucosylation. Fut8 promotes PD-1-N-linked oligosaccharides at positions N49 and N74 to regulate cell-surface expression of PD-1 [74]. Notably, blocking the fucosylation using fucosylation inhibitor 2-fluoro-L-fucose (2F-Fuc) leads to better immune response in mouse models [74]. Mechanistically, loss of core fucosylation enhances the PD-1 ubiquitination and in turn leads to the degradation of PD-1 by the 26S proteasome [79].

Concluding Remarks

Although PD-1/PD-L1 immune checkpoint blockade exhibits promising clinical benefit, response rates are still lower than 40% in most cancer types [2-4]. The post-transcriptional regulations of PD-1/PD-L1 and the suggested combination therapy strategies thus provide new avenues to increase the efficacy of the PD-1/PD-L1 blockade. To this end, further in-depth investigation and elucidation of the new modifications will be a key step for improving the response for cancer therapy. In addition to find new regulation of PD-1/PD-L1, future work should also be put into developing preclinical/clinical models to translate the existing regulations into clinical benefit. Several novel PROTACs such as AbTACs [28] and LYTACs [29] have been developed to target PD-L1 for degradation, but their effects in preclinical models remain unknown and whether these PROTACs have better clinical outcomes than the original antibodies are needed in-depth exploration.

Compared with the post-translational modification of PD-L1, the modifications of PD-1 are also critical for anticancer immune response. Unlike PD-L1, the modulation of PD-1 at protein level is emerging and remains largely elusive. Although PD-1 can be polyubiquitinated by different E3 ligases such as FBXO38 [75], KLHL22 [76] and c-Cbl [76], it still remains unknown whether there is any DUB that can reverse PD-1's polyubiquitination. It is also important to reveal which E3 ligase plays an important role in physiologically governing PD-1 ubiquitination and which E3 ligase is pathologically dysregulated to cause immune surveillance defects. Besides ubiquitination and glycosylation, whether is PD-1 regulated by other post-translational modifications such as phosphorylation, acetylation and palmitoylation? More importantly, how to translate these regulatory mechanisms into the enhancement of immunotherapy warrants further investigation.

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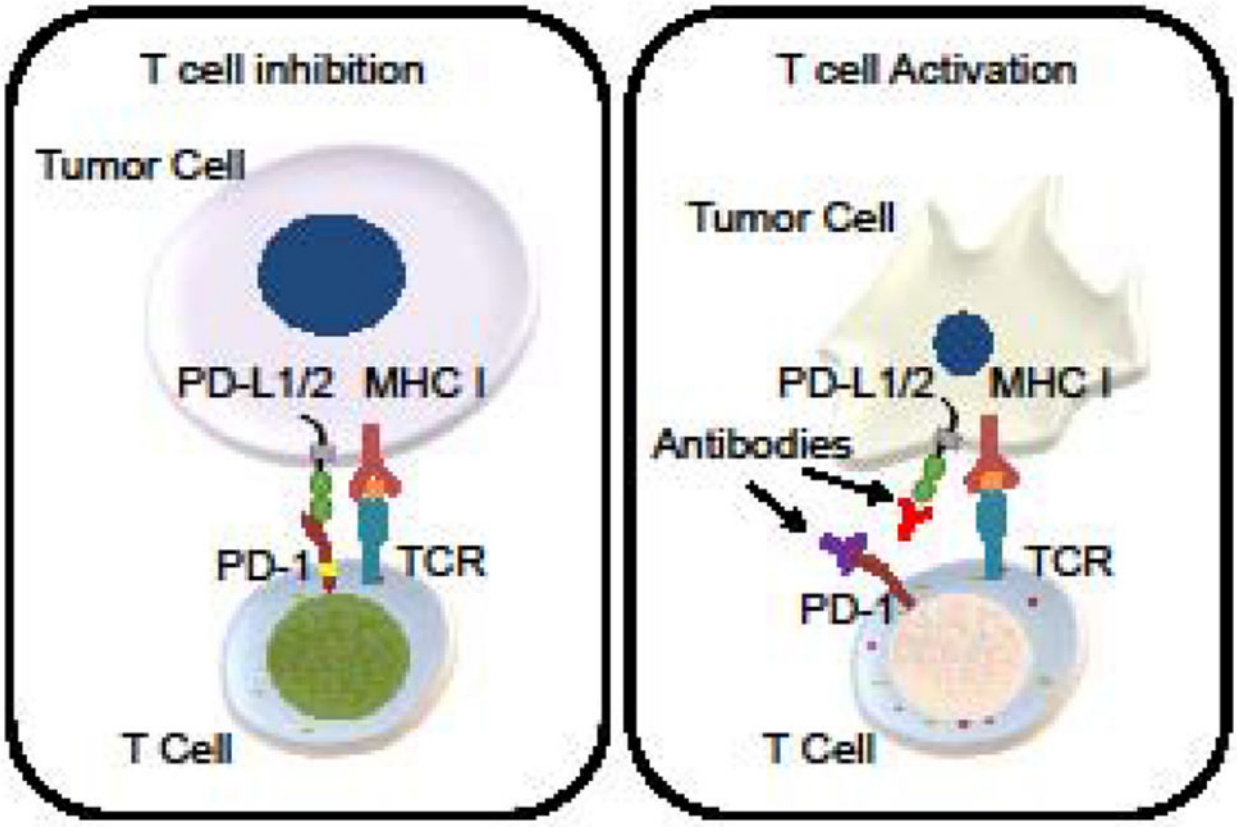

Figure 1. Mechanism of PD-1/PD-L1 blockade.

The binding of TCR and MHC activates adaptive immune response. PD-L1 expressed on tumor cells interacts with its physiological receptor, PD-1 on T cell surface, preventing the signaling transduction of T cells to inhibit the immune response. Anti-PD-1 or anti-PD-L1 antibodies block the interaction of PD-1 and PD-L1, and abolish the inhibition of $CD8^+$ T cell, thus enhancing the antitumor activity. TCR, T cell receptor; MHC, major histocompatibility complex.

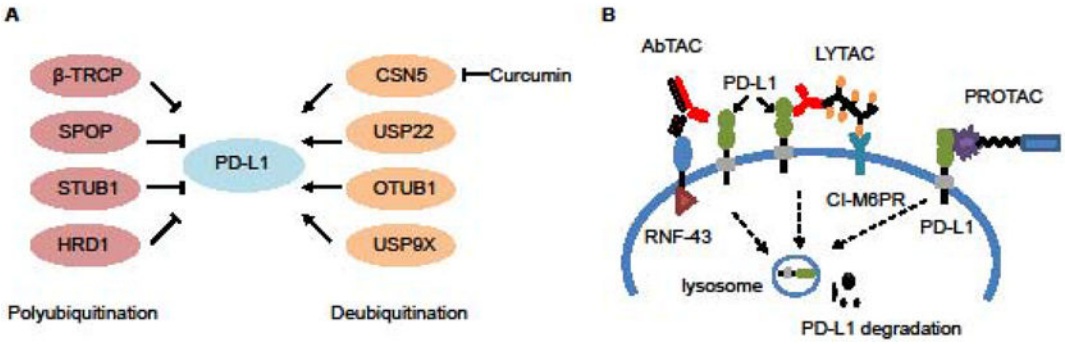

Fig. 2. Regulations of PD-L1 by polyubiquitination, deubiquitination and PROTACs.

A. PD-L1 can be polyubiquitinated by E3 ubiquitin ligases β-TRCP, SPOP, STUB1 and HRD1 and deubiquitinated by deubiquitinases CSN5, USP22, OTUB1 and USP9X. **B.** PROTACs of PD-L1. AbTAC of PD-L1 is a bispecific antibody which can target both PD-L1 and the E3 ligase RNF43 to induce the lysosomal degradation of PD-L1. LYTAC of PD-L1 is composed of an antibody specific to PD-L1 conjugated to a synthetic oligoglycopeptide ligand that binds CI-M6PR, a transmembrane glycoprotein responsible for trafficking proteins to lysosomes for degradation. PROTAC of PD-L1 is a novel resorcinol diphenyl ether-based PROTAC molecule which consists of BMS1198, a linker region and Pomalidomide.

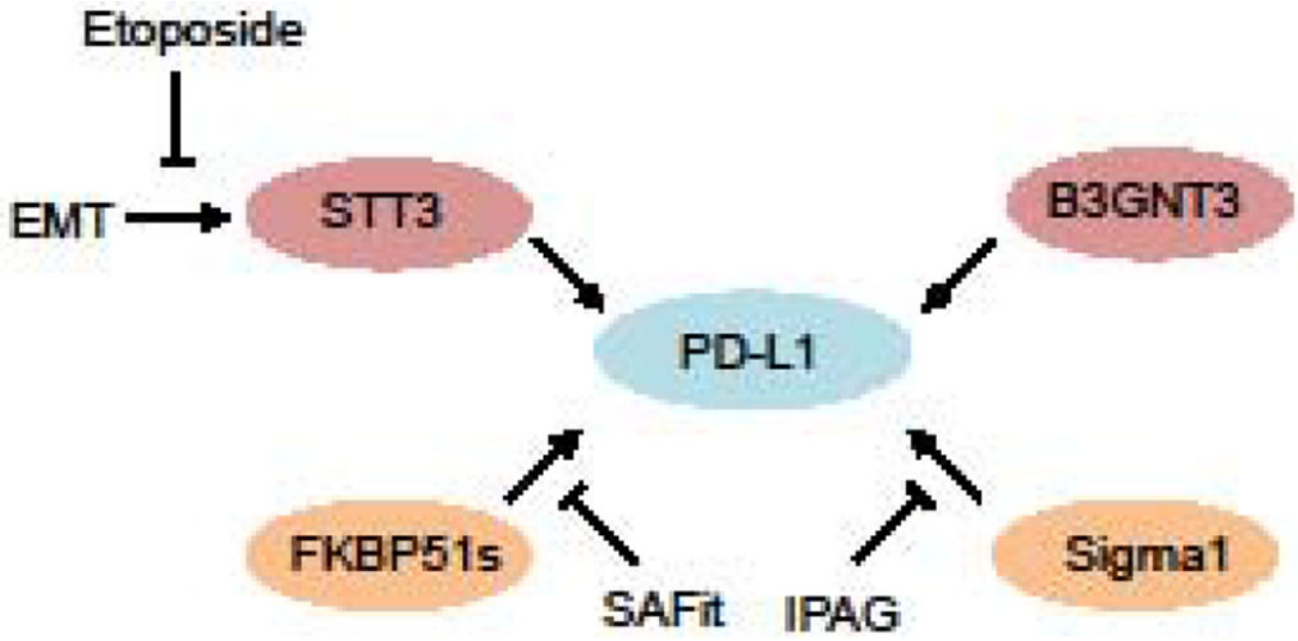

Fig 3. Regulation of PD-L1 by glycosylation.

STT3 and B3GNT3 can glycosylate PD-L1, protecting PD-L1 from degradation. Chaperone Sigma1 and FKBP51s have also been reported to promote PD-L1 glycosylation.

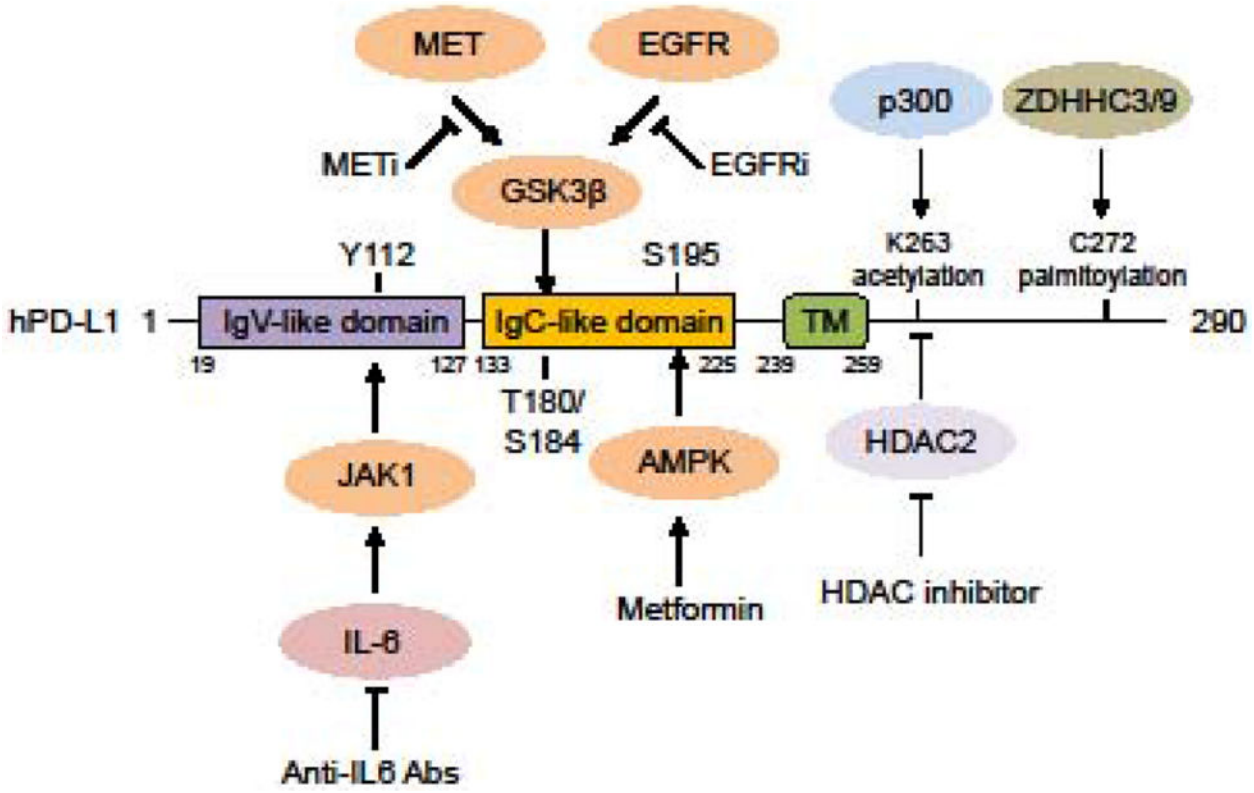

Fig. 4. Regulations of PD-L1 by phosphorylation, acetylation and palmitoylation.

PD-L1 can be phosphorylated by GSK3β, AMPK and JAK1. PD-L1 is acetylated at K263 by the acetyltransferase p300 and deacetylated by HDAC2. Moreover, ZDHHC3/9 promote the palmitoylation of PD-L1 at the C272 residue.

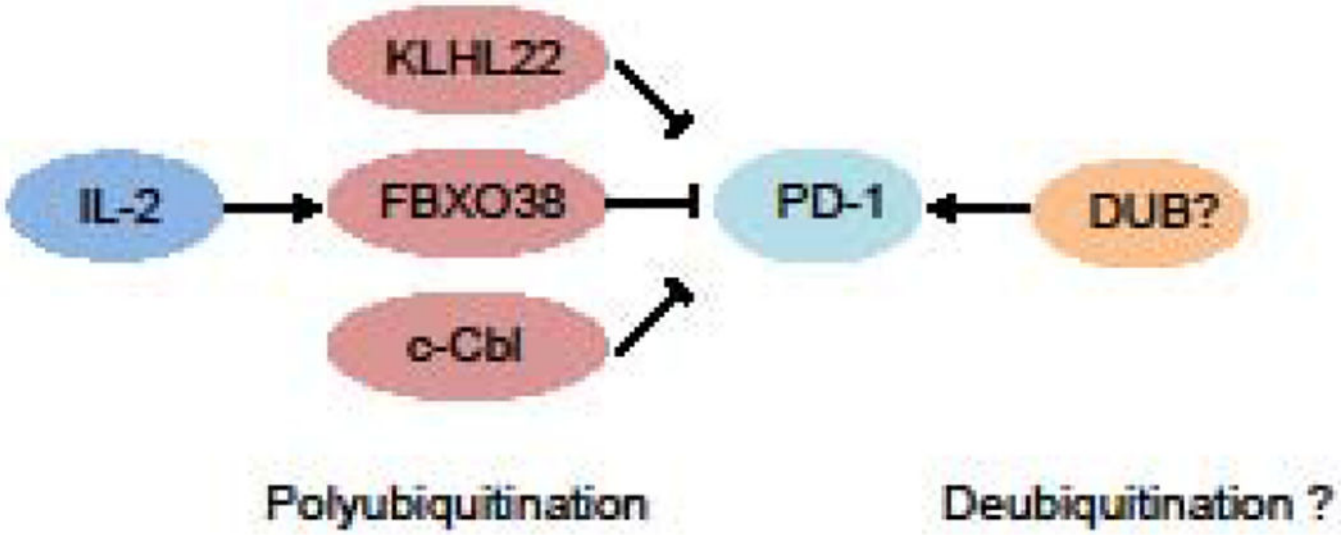

Fig 5. Regulation of PD-1 by polyubiquitination.

PD-1 can be polyubiquitinated by E3 ligases FBXO38, KLHL22, and c-Cbl, leading to PD-1 degradation through the 26S proteasome.