

REVIEW

Landscape of PD-1/PD-L1 Regulation and Targeted Immunotherapy

Jieming Ni¹, Anping Ni^{2*}

¹Seven Year Program of China Medical University, Shenyang 110122, China

²Department of Clinical Laboratories, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China

Key words: programmed cell death protein 1/programmed death ligand 1; immunotherapy; regulation; clinical trial; inhibitors

Abstract Programmed cell death protein 1 (PD-1)/programmed death ligand 1 (PD-L1) is a significant immune checkpoint, and the dysfunction of this axis contributes to tumor metastasis and immune escape. PI3K/Akt/mTOR and MAPK signal network induces PD-1/PD-L1 expression and facilitates tumor progression. Transcriptional factors such as hypoxia induced factors, PTEN, p53, CDK5, BRD4, STAT modulate PD-1/PD-L1 expression. PD-1/PD-L1 level is also regulated *via* epigenetic and post-translational manner. The underlying mechanisms mentioned above may provide potential targets for tumor treatment. At present, the combination therapy of PD-1/PD-L1 monoclonal antibodies plus small molecular inhibitors has achieved good outcomes in tumor treatment.

PROGRAMMED cell death protein 1 (PD-1), a member of B7 family, is a transmembrane protein mainly expressed on T cells and tumor-infiltrating lymphocytes (TILs). Programmed death ligand 1 (PD-L1), shown to be a survivor factor, is a transmembrane protein intensively distributed in tumor cells, and its level is increased by pro-inflammation and administering interferon- γ (IFN- γ) during tumor treatment. The binding of PD-1 and PD-L1 maintains self-immune tolerance, induces

T cell apoptosis, and contributes to tumor metastasis. ITSM and ITIM at the C- and N-terminals of PD-1, respectively, act as docking sites for both SHP-2 and SHP-1, through which PD-1 recruits tyrosine phosphatase, thus impeding T cell immune signaling and inducing immune suppression under the favor of PKC θ .¹ PD-1 blocks the phosphorylation of Erk, Syk, and PLC, thereby inhibiting cytotoxic lymphocyte (CTL) activity and B cell function in peripheral blood.

The PD-1/PD-L1 axis is an important immune checkpoint in T cell exhaustion and interleukin-2 (IL-2) downregulation. PD-1/PD-L1 regulation is based on complex signaling networks, such as PI3K/Akt/mTOR and MAPK. By inhibiting ZAP70 recruitment, Lck activation, and CD3 ζ phosphorylation, PD-1/PD-L1 blocks PI3K/Akt activation and induces Gads/SOS/VAV expression, consequently leading to immune suppression.¹ SHP2 participates in the MAPK network,

Received for publication January 17, 2018. Published online July 17, 2018.

*Corresponding author Tel: 86-13311360359, E-mail: niap@pumch.cn

Cited this article as: Jieming Ni, Anping Ni. Landscape of PD-1/PD-L1 regulation and targeted immunotherapy. Chin Med Sci J 2018; 33(3): 174-182.

which subsequently induces PD-1 expression and up-regulates PD-L1 level. Furthermore, MEK inhibitors efficiently synergize with BRAF inhibitors during PD-1 inhibition. Besides, PD-1/PD-L1 level is also associated with gene expression modification, both epigenetically and post-translationally. Tumor cells remarkably induce PD-L1 expression and reduce CpG islands of immune checkpoint PD-L1. PD-1/PD-L1 high expression mimics endogenous checkpoints and downregulates E-cadherin, which facilitates tumor escape and cell proliferation.² In contrast, CD80-Fc/PD-L1 overcomes immune suppression, and PD-1 binding to immunoglobins not only increases IFN- γ and IL-12 production, but also reverses PD-1/PD-L1-induced IL-5 and IL-13 reduction.³ In summary, the PD-1/PD-L1 axis can be treated as a target for tumor immunotherapy, and many crucial components of this axis, such as CDK5 and BRD4, could become promising PD-1/PD-L1 inhibitors in the future.

PD-1 and Tim-3 are overly expressed in tumor cells, and PD-1+Tim-3+ is associated with poor survival and shorter time to transformation, indicating rapid progression, refractoriness to treatment, and an overall dismal prognosis.⁴ In addition, a solid tumor with PD-L1 expression demonstrated a decrease in tumor immune surveillance. PD-L1 blockade inhibits the shift of Tregs toward Th17 cells and inhibits alloreactive T cell apoptosis. Actually, PD-1/PD-L1 is specifically regarded as a biomarker for tumor diagnosis and is a poor prognostic indicator of solid tumors.

Currently, PD-1/PD-L1 inhibitors, comprising monoclonal antibodies and small-molecule inhibitors, have been successfully applied in clinical trials to arrest tumor progression and prevent immune escape, with high stability and low adverse effects. This review aimed to summarize the main regulatory mechanisms of PD-1/PD-L1, current clinical applications of PD-1/PD-L1 inhibitors, and combination therapy of PD-1/PD-L1 inhibitors, in order to discover potential targets for tumor treatment.

EPIGENETIC AND POST-TRANSLATIONAL REGULATION OF PD-L1

PD-L1 level is closely related to epigenetic modification, which influences tumor-associated antigens expression and the related gene copy number. Tumor cells exhibit remarkably induced PD-L1 expression and reduced CpG islands of immune checkpoint PD-L1,

subsequently suppressing Th2 cytokine production and increasing Th1 function. CpG-DNA strongly induces PD-L1 expression *via* NF- κ B signaling and reduces level of inducible costimulator ligand.⁵ DNA modification may potentially help to downregulate PD-L1 expression and reverse immune suppression *via* methyltransferase and histone deacetylase inhibitors. Decitabine, a methylation inhibitor, has been shown to promote T cell activation with cytotoxic capability and to block persistent T cell exhaustion within successive T-cell generations.⁶ MicroRNAs, such as miR-570, miR-34a, and miR-200, bind to specific mRNAs to prevent PD-L1 translation, participate in PD-L1 expression regulation, and induce epithelial-mesenchymal transition to promote metastasis.⁷

At the post-translational level, matrix metalloproteinases (MMPs), especially MMP-13, which is involved in extracellular matrix (ECM) degradation and tumor metastasis, modulates PD-L1 expression by cleaving the binding site of PD-1. Tumor-associated fibroblasts prevent PD-1 ligand depletion *via* MMP activity, possibly reversing apoptotic signals of mitogen-activated CD3⁺ T cells, while interfering exhausted memory T cells depletion.⁸ Glycogen synthase kinase 3 β (GSK3 β) binds to PD-L1 to mediate its phosphorylation, subsequently leading to PD-L1 ubiquitination and degradation in proteasomes. Besides, 17 β -estradiol-induced binding of membrane estrogen receptor α (ER α) to the p85 α regulatory subunit of PI3K activates the PI3K/Akt signaling pathway and increases PD-L1 mRNA stability and expression level.⁹

SIGNAL NETWORK OF PD-1 REGULATION

PI3K/Akt/mTOR

PD-1 impedes CD28-mediated ITSM phosphorylation, and CTLA-4 terminates Akt induction *via* PP2A, both of which block PI3K/Akt/mTOR pathway and inhibit the expression of anti-apoptotic genes such as Bcl (Fig. 1).¹⁰ PI3K inhibitors diminish resistance to BRAF inhibitors and induce PD-1 downregulation, suggesting that PD-1 positively correlate with PI3K. Moreover, many PI3K antibodies have currently been applied in clinical trials to downregulate PD-1 level. A pan-PI3K δ inhibitor reportedly inhibited PD-1 and BKM120 significantly to facilitate interferon- γ secretion, with CD4⁺ T cell proliferation.¹¹ Additionally, after administering Buparlisib (PI3K inhibitor), progressive free survival (PFS) among 63 patients with squamous carcinoma evidently

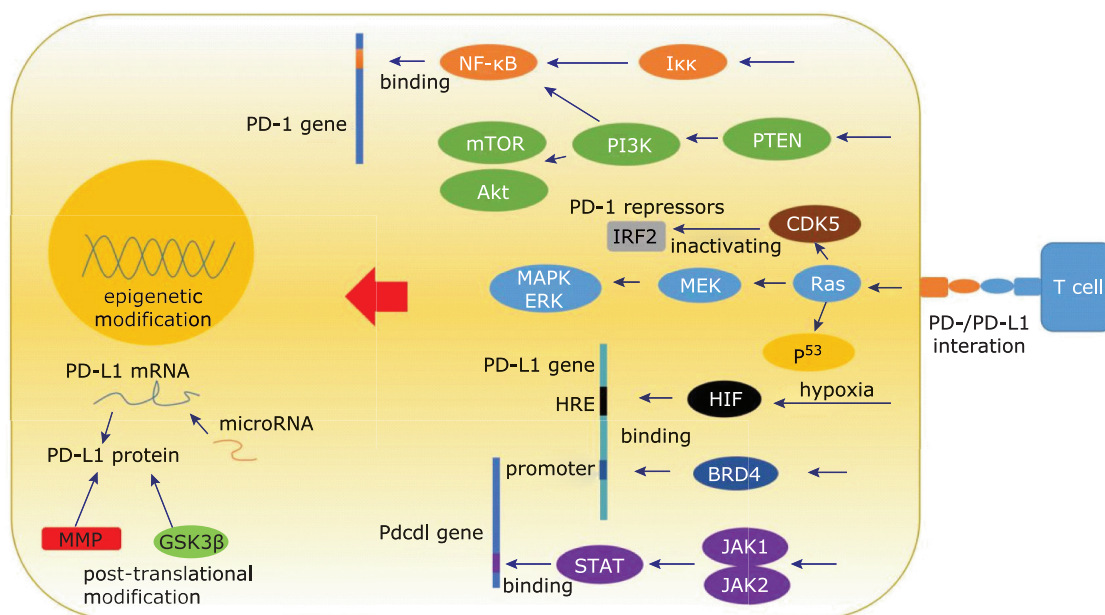


Figure 1. The regulation network of PD-1/PD-L1.

PI3K/Akt/mTOR and MAPK signal pathway induces PD-1/PD-L1 expression, and transcriptional factors HIF, PTEN, p53, CDK5, BRD4, STAT *etc* modulate PD-1/PD-L1 expression. PD-1/PD-L1 level is also regulated *via* epigenetical and post-translational manner. GSK: glycogen synthase kinase; HRE: hypoxia response element; MMP: matrix metalloprotein.

improved (NCT01820325).

PD-1-induced immune suppression downregulates Akt level, thereby contributing to T-cell receptor (TCR) normal functioning. Furthermore, an Akt inhibitor could accentuate PTEN loss with PD-1 upregulation. Clinically, an AKT inhibitor, MK-2206, induced apoptosis and estrogen deprivation, leading to an overall response rate of 15.4% among 13 patients with breast cancer.¹² However, PD-1 inhibitors may upregulate Tim-3 level through Akt/PI3K, and Tim-3 cooperates with PD-1 in a synchronized manner, resembling a compensatory loop, in tumor models,¹³ indicating that Akt inhibitor monotherapy is not that effective; hence, a combination therapy involving multiple PD-1 agents should be adopted.

Mammalian target of rapamycin (mTOR) plays a crucial role in metabolism and contributes to anti-tumor effect. Raptor and rictor are the main components and regulators of the mTOR complex, and PD-1/PD-L1 binding may activate the Akt/mTOR signaling pathway in tumor cells to promote tumor progression. Furthermore, mTOR inhibitors synergize with PD-1 blockade during the clinical treatment of cancers; however, mTOR inhibitors may lead to side effects that enhance Tregs activity and subsequently reduce anti-tumor immunity. Temsirolimus (mTOR inhibitor) forms complexes with FKBP12 and inhibits its interaction with

mTOR,¹⁴ and patients receiving temsirolimus showed partial response and prolonged disease stabilization.

MAPK

MAPK, characterized by its cascade reactions, is involved in tumor progression. SHP2 is a tyrosine phosphatase that participates in MAPK-induced PD-1 regulation and is likely to be a potential target. Furthermore, MEK inhibitors or knockdown of ERK1/2 efficiently synergizes with BRAF inhibitors in PD-1 inhibition and T cell infiltration, indicating that PD-1 is tightly related to the MAPK pathway, which activates epidermal growth factor receptor (EGFR) and upregulates PD-L1 level.⁷ Besides, combining IL-15 and a p38 MAPK inhibitor blocks redirection toward CD4⁺ Th17 cells, intensifies cytotoxic effect, and downregulates PD-1/PD-L1, leading to a positive prognosis of ovarian tumors, indicating that MAPK associated tumor vaccine therapy is beneficial.¹⁵ Patients receiving a MEK inhibitor, selumetinib, showed well-tolerance, stable disease, and prolonged survival. However, innately resistant tumors display a transcriptional signature, with a high load of BRCA2 mutation in MAPK inhibitor therapy. The innate anti-PD-1 resistance and MAPK inhibition may establish a crosstalk in epithelial-mesenchymal transition, cell adhesion, and extracellular matrix (ECM) remodeling.¹⁶

TRANSCRIPTIONAL FACTORS OF PD-1/ PD-L1 REGULATION

NF- κ B

NF- κ B, an important nuclear transcriptional factor and inflammatory mediator, is involved in stress and inflammation. NF- κ B-related gene elements undergo degradation in tumor cells, making anti-tumor effects less efficient. Regarding NF- κ B mediated PD-1 regulation, NF- κ B binding sites exist in conserved region C and p65, which lay the foundation for PD-1 initiation in macrophages under the guidance of T-cell receptor (TCR) activation and Toll-like receptor (TLR) ligands. Moreover, NFAT blockade by cyclosporin inhibits PD-1 production, indicating that NFAT is a critical inducer of PD-1 expression.¹⁷ PD-1 has been shown to inactivate NF- κ B and plays an inhibitory role in an SH2-independent manner, thereby promoting immune evasion and inhibiting the function of tumor-infiltrating dendritic cells. Further experiments using gefitinib, an EGFR-TKI inhibitor, or siRNA to knockdown p65, indicate that PD-L1 expression relies on NF- κ B *via* IFN secretion.¹⁸ Regarding the clinical use of NF- κ B inhibitors, caffeic acid phenethyl ester with tetrodotoxin could remarkably reduce lateral motility and invasion of tumors by inhibiting voltage-gated sodium channels activity.¹⁹ Stimulator of interferon genes (STING) activates NF- κ B and STAT pathways to facilitate proinflammation, and STING vaccine plus NF- κ B blockade can improve tumor regression and induce a potent anti-tumor effect.

HIF

Hypoxia induced factor (HIF) is a heterodimer, which is constituted of α subunit and β subunit. HIF cooperates with CBP/p300 and binds to hypoxia response element (HRE) on PD-L1 promoter to initiate adaptation to hypoxia. PD-1 enhances HIF-1 α level and inhibits ubiquitin mediated HIF-1 degradation to make HIF more stable. ERO1- α is the oxidase of endoplasmic reticulum and acts as an indicator of hypoxia. ERO1- α gives rise to PD-1 production and promotes oxidative protein folding.²⁰ HIF induces PD-1 upregulation, which promotes resistance to cytolysis effect and contributes to T cell dysfunction. And PD-1 blockade delays tumor progression without interfering TILs function, which enhances PPAR- α level and fatty acid catabolism in hypoxic cells. PD-1 mediated angiogenesis with HIF collaboration manifests poor prognosis and HIF can be treated as direct target for PD-1 inhibition. HIF inhib-

itor (LW6) is benzimidazole analogue without severe side effects and reduces tumor lesion by 58.6% *via* Hsp90-Akt pathway.²¹ Applying nitric oxide (NO) signaling agonist glyceryl trinitrate also blocks HIF-1 α accumulation and mitigates immune escape with reduced level of IL-10 and diminished resistance to cytolysis.²²

CDK5

The CDK family generally regulate the cell cycle, and CDK5, a unique member of the family, binds to p35 or p39 to maintain activation of the central nervous system (CNS). CDK5 determines PARP activity and associates with STAT3 at the C-terminal to participate in DNA repair and replicative stress response, indicating its direct role in cell cycle checkpoint.²³ K-Ras mutation-induced CDK5-p35 complex is overly amplified in pancreatic carcinoma, which is associated with angiogenesis and chemotherapy resistance.²⁴ Conversely, knockdown of CDK5 *via* siRNA arrests cell cycle and enhances TP53 level. CDK5 deficiency contributes to persistent hyperphosphorylation of PD-1 transcriptional repressors (IRF2BP2 and IRF2), which depress PD-L1 level and enhance anti-tumor immunity of CD4⁺ T cells. In addition, c-myc-mediated mutant expression of EGFR and β -catenin triggers PD-L1 expression, with the absence of T cell signature; conversely, CDK5 disruption reduces PD-L1 level. CDK5 serves as a potential target in inhibiting the PD-1/PD-L1 axis and anti-tumor treatment. Dinaciclib is a novel inhibitor of CDK1/2/5/9, and after administrating dinaciclib, partial response rate in patients with relapsed multiple myeloma reached 11% (NCT01096342).

PTEN

PTEN is an important tumor-suppressor gene and acts as a negative regulator of the PI3K/Akt/mTOR pathway. Conventionally, PI3K/Akt activation in tumors is closely accompanied with PTEN loss, which contributes to a high PD-L1 expression level and constraints T cell infiltration. PI3K blockade (rapamycin), which decreases PD-L1 expression, indicates a tight link between PTEN and PD-L1. PTEN, phosphorylated by CK2 at the C-terminal regulatory domain, could be more stable; however, PD-1 recruits PTEN phosphatase and accelerates ubiquitin-dependent degradation of PTEN.²⁵ Therefore, CK2 may be considered as a potential target of PD-1. PTEN loss is a novel player in PD-L1 regulation, and thus can be used as a biomarker for tumor diagnosis and as a treatment target to ameliorate PD-

L1 expression from PTEN perspective. PTEN may also act as a potential candidate target for blocking the PD-L1 axis in gastric cancer using antibodies.

TP53/p53

The wild-type of p53 cooperates with p21 to arrest the cell cycle and is regulated by post-translational modifications, while the abnormal phosphorylation of p53 disrupts p53-Mdm2 interaction, with p53 stabilization and accumulation. p53 is closely related to PD-L1 level in tumors, and PD-L1 expression is positively related to p53 aberrant expression in lung adenocarcinoma. IFN induces TP53, K-RAS, and epidermal growth factor receptor (EGFR)-vIII mutations to jointly upregulate PD-L1 level and alter the expression of genes involved in the cell cycle. p53 also modulates PD-L1 expression through miR-200 and suppresses the effect of PD-L1 in NK cell and T cell recognition. Besides, *via* miR-34, p53 modulates transforming growth factor (TGF- β), CCL22 (chemokine C-C motif ligand 22), DGK ζ (diacylglycerol kinase ζ), and Tregs activity.²⁶ Furthermore, after administering PD-1 inhibitors, the pathological status of patients showed a remarkable progress in limiting cancer metastasis.

BRD4

BRD4, a member of the BEF family, binds to an epigenetic reader and is involved in autophagy, inflammation, and lysosomal function. BRD4 localizes to P-TEFb complexes consisting of Cdk9 and cyclin, which play a central role in RNA polymerase II promoter-proximal pause release and phosphorylation under the favor of acetylated histones.²⁷ C-myc binds to CD47 (PD-1) promoter and PD-L1 to facilitate downstream molecules production. JQ1, a co-inhibitor of BRD4 and CHK1, mediates c-myc functional suppression and reduces PD-1/PD-L1 level. In contrast, JQ1 affects specific lineage genes, such as CD4⁺ T cell promoter and LAG3 expressed on TIL, which hinder BRD4 from recognizing histones, to suppress PD-1/PD-L1 expression.²⁸ Apart from the aforementioned, BRD4, epigenetic readers, and super-enhancers have been confirmed to have a strong association with gene copy number and high level of single nucleotide polymorphisms (SNPs) in breast cancer. In summary, BRD4 is a significant factor in tumor progression and is a direct target for blocking PD-1/PD-L1 axis transcription. Due to the enriched BRD4 at the CD274 gene promoter, the active expression of BRD4 in tumors contributes to high levels of

CD274 and IFN- γ induced PD-1.²⁹ Besides, bromodomain and external domain (BET) inhibitors could suppress c-myc gene expression, leading to PD-1/PD-L1 and CCR4 expression blocking. Therefore, a combination therapy of BET bromodomain kinase inhibitors can potentially help to control PD-1/PD-L1 level and to treat tumors.

STAT

Pdcd1 gene locus contains STAT/NFAT binding sites. PD-1 expression is associated with IL-6 and IL-12 levels, which are induced by STAT3 and STAT4 respectively. IFN sensitive response element leads to upregulation of PD-1/PD-L1 expression through the effect of STAT1/2, which lays foundation for STAT inhibitor (Ruxolitinib) induced anti-tumor immune response. JAK2 inhibitor or Ruxolitinib can enhance adaptive immune response, and selectively inhibit STAT1 and STAT3.^{15, 30} PD-1 blocks localization and polarization of M1 type macrophage *via* reducing STAT1 molecule, however, PD-1 enhances M2 type macrophage activity through STAT6.³¹ Besides, IFN- γ produced by NK cell activates STAT1 and increases resistance of PD-L1 to NK cell lysis. This indicates JAK/STAT inhibitor may cooperate with PD-1/PD-L1 monoclonal antibody to prevent tumor cytotoxicity, which might cause serious immune disorder. In addition, phosphorylation of STAT pathway is associated with PD-L1 transcription, which is stimulated by chemotherapy agent like 5-fluorouracil in dosage dependent manner.³²

IMMUNOTHERAPY WITH PD-1/PD-L1 INHIBITOR IN CLINICAL TUMOR TREATMENT

In recent years, cancer researches on PD-1/PD-L1 and its related inhibitors have gradually become the new focuses, and most of inhibitors have been reported with clinical efficacy as well as potential adverse effects. Long-term survival rate only occurred in small scale of patients who received ipilimumab and the overall response rate is still concerned. Regarding immune suppressive microenvironment induced by PD-1/PD-L1, therefore we recommend combination use of PD-1/PD-L1 agents instead of monotherapy.

Pembrolizumab

Pembrolizumab, the first-line PD-1 inhibitor agent, is a highly selective and humanized monoclonal antibody,

which binds to PD-1 receptor to prevent interaction of PD-1 with PD-L1, and stimulates anti-tumor immune response. Pembrolizumab has been administrated in melanoma treatment with a progression free survival rate of 47.3% in 6 months (NCT01866319). A total of 23 patients with colorectal cancer were selected for pembrolizumab trial in 2014. Overall response rate was 4%, 1 patient in this cohort experienced partial response, and 4 patients had stable diseases (NCT02054806). However, the adverse effects could not be ignored with rate of 70.9%, which manifest as fatigue, decreased appetite, and hypothyroidism. These adverse effects could be ameliorated *via* managing dosage with cautious consideration and administering corticosteroids to reduce inflammation.

Nivolumab

Nivolumab is also a humanized IgG4 monoclonal antibody of PD-1. It is deemed safe in dosage use and observed with median overall survival for 12.2 months among 292 patients in non-small cell lung cancer (NSCLC) treatment.³³ Compared to other anti-PD1 agents like docetaxel, nivolumab administration prolongs overall survival rate and shows less severity including fatigue, nausea, and asthenia, which could be possibly reversed by immune modulators like glucocorticoids.

Atezolizumab

Atezolizumab is anti-PD-L1 antibody, and has been clinically applied in renal carcinoma and NSCLC treatment, which presents superiority to docetaxel concerning death rate and adverse effects. Atezolizumab obviously improves IFN- γ expression in TILs and increases overall survival rate among patients with PD-L1 positive tumor. In atezolizumab group, overall survival of 63 patients with renal clear cell carcinoma reached 28.9 months, objective response rate reached 15%, and progression free survival lasted for 5.6 months (NCT01375842).

Ipilimumab

Ipilimumab is a kind of anti-CTLA4 antibody, has become a standard practice in treatment of metastatic melanoma, prostate carcinoma, and small cell lung cancer, which improves overall survival. In phase 3 of anti-melanoma clinical trial, ipilimumab monotherapy plus dacarbazine improved the overall survival, however, clinical response rate was still low and only small scale of patients achieved the long-term sur-

vival rates.³⁴ Ipilimumab commonly coordinates with nivolumab in clinical trials and has achieved success. In PD-L1 negative patients, the combination therapy of nivolumab plus ipilimumab demonstrates superiority in higher progressive free survival to monotherapy, while PD-L1 positive patients would not be sensitive to this. Complete response rate reached 22%, objective response attained 61% in the combination group, and no complete responses were demonstrated in patients of the ipilimumab monotherapy group (NCT01927419).

Combination therapy

It has been proved combining several blockades simultaneously can achieve higher response rate and mitigate tumor escape, therefore combination therapy is gaining prominence in tumor treatment. (1) Combination therapy of first-line and second-line PD-1/PD-L1 inhibitors simultaneously can increase tolerance of patients and decrease toxicity. Pembrolizumab plus ipilimumab combination therapy has been practiced among 153 patients, PFS was 69% in 1 year and overall survival was 89% (NCT02089685). (2) Combining more than two checkpoint monoclonal antibodies, like ipilimumab/nivolumab, would provide a novel therapeutic approach to increase efficacy and decrease toxicity. The overall survival rate reached 58% in nivolumab-ipilimumab combination cohort, as compared with 34% in the ipilimumab mono-group and 52% in the nivolumab mono-group (NCT01844505). (3) PD-1/PD-L1 monoclonal antibody in combination with tumor vaccine also offers a fresh outlook for melanoma treatment. The median relapse-free survival among patients who received both nivolumab and tumor vaccine was 47.1 months, and overall survival rate reached more than 80%.³⁵ (4) There also emerges a trend that redirected T cell (CAR-T) plus PD-1 blockade would favor tumor treatment. Recently, anti-GD2 specific CAR plus pembrolizumab therapy has been observed with long persistence and limited immune escape (NCT01822652). (5) Indoleamine 2, 3-dioxygenase 1 (IDO1) involves in tryptophan catabolism and facilitates immunosuppressive microenvironment. Erk/p38/MAPK signaling in dendritic cells is associated with PD-L1 reduction and IDO inactivation. IDO1 overexpression accompanies with immune escape, and IDO1 inhibition plays a crucial role in anti-tumor surveillance and prognosis. At present, PD-1/PD-L1 and IDO1 double inhibition indicates a higher benefit and a potential trend for tumor treatment. Among

19 patients who received combination therapy of IDO inhibitor (epacadostat) plus pembrolizumab, 4 were observed with complete responses, 7 with partial responses, and 3 with stable diseases (NCT02178722). (6) Apart from specific PD-1/PD-L1 monoclonal antibodies (mAbs), small molecule inhibitors of PD-1/PD-L1 undergo slow but emerging preclinical development, which incorporate multiple disciplines. *Via* binding to PD-1/PD-L1, small molecule inhibitors present variable structures and better tumor infiltration capability. Based on data of compounds like BMS-202 applied in clinical treatment, small molecule inhibitors have been structurally and biochemically designed to induce PD-L1 dimerization, which subsequently impede PD-1 pathway.³⁶ Additionally, small molecule GSK-3 α/β inactivation by siRNA may increase NFAT in nucleolus, which contains Pdc1 transcription regulator and consequently inhibits PD-1 expression without influencing CTL killing effect.³⁷ Besides, BET plays a crucial role in PD-1/PD-L1 expression, thus may serve as a novel clinical target for PD-1/PD-L1+ tumor treatment. The BET inhibitor (FT-1101) has been testing its clinical efficacy among patients with relapsed/refractory myeloid leukemia at present (NCT02543879). (7) Modification of current monoclonal antibodies would be plausible for PD-1/PD-L1+ tumor treatment. MPDL-3280A is an engineered humanized anti-PD-L1 IgG1 mAb and the function of IgG1 Fc domain completely abolishes the antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Patients with advanced cutaneous melanoma who received MPDL-3280A had a response rate of 29%. In addition, BMS-936559 is a fully humanized anti-PD-L1 IgG4 mAb, which inhibits PD-1 and CD80/B7 binding, and 16% patients with melanoma had objective responses.³⁸

To sum up, PD-1/PD-L1 serves as an important immune checkpoint and is regulated *via* PI3K/Akt/mTOR and MAPK signal network. Transcriptional factors including NF- κ B, HIF, PTEN, p53, CDK5, BRD4, STAT are critical factors influencing PD-1/PD-L1 expression. Besides, epigenetic and post-translational modification also participates in PD-1/PD-L1 regulation. All these could serve as potential targets, some of which have made clinical progress. One thing must be noted that seeking for managements of toxicity and adverse effects is imperative. Recently, PD-1/PD-L1 mAbs applied in treatment showed high efficacy and combination therapy would provide bright future for PD-1/PD-L1

immunotherapy.

Conflicts of interest statement

The authors declare that they have no competing interests.

REFERENCES

1. Sheppard KA, Fitz LJ, Lee JM, et al. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3 signalosome and downstream signaling to PKC theta. *FEBS Letters* 2004; 574(1-3):37-41. doi: 10.1016/j.febslet.2004.07.083.
2. Zhang B, Yu W, Feng X, et al. Prognostic significance of PD-L1 expression on tumor cells and tumor-infiltrating mononuclear cells in upper tract urothelial carcinoma. *Med Oncol* 2017; 34(5):1-9. doi: 10.1007/s12032-017-0941-2.
3. Waldmann TA. The shared and contrasting roles of IL2 and IL15 in the life and death of normal and neoplastic lymphocytes: implications for cancer therapy. *Cancer Immunol Res* 2015; 3(3):219-27. doi: 10.1158/2326-6066.CIR-15-0009.
4. Smeltzer JP, Jones JM, Ziesmer SC, et al. Pattern of CD14⁺ follicular dendritic cells and PD1⁺ T cells independently predicts time to transformation in follicular lymphoma. *Clin Cancer Res* 2014; 20(11):2862-72. doi: 10.1158/1078-0432.CCR-13-2367.
5. Kubo S, Yamada T, Osawa Y, et al. Cytosine-phosphate-guanosine-DNA induces CD274 expression in human B cells and suppresses T helper type 2 cytokine production in pollen antigen-stimulated CD4-positive cells. *Clin Exp Immunol* 2012; 169(1):1-9. doi: 10.1111/j.1365-2249.2012.04585.x.
6. Bai J, Gao Z, Li X, et al. Regulation of PD-1/PD-L1 pathway and resistance to PD-1/PD-L1 blockade. *Oncotarget* 2017; 8(66):110693-707. doi: 10.18632/oncotarget.22690.
7. Chen J, Jiang CC, Jin L, et al. Regulation of PD-L1: a novel role of pro-survival signalling in cancer. *Ann Oncol* 2016; 27(3):409-16. doi: 10.1093/annonc/mdv615.
8. Dezutter-Dambuyant C, Durand I, Alberti L, et al. A novel regulation of PD-1 ligands on mesenchymal stromal cells through MMP-mediated proteolytic cleavage. *Oncoimmunology* 2015; 5(3):e1091146. doi: 10.1080/2162402X.2015.1091146.
9. Yang L, Huang F, Mei J, et al. Posttranscriptional control of PD-L1 expression by 17 β -estradiol *via* PI3K/Akt

- signaling pathway in ER α -positive cancer cell lines. *Int J Gynecol Cancer* 2017; 27(2):196-205. doi: 10.1097/IGC.0000000000000875.
10. Ok CY, Young KH. Targeting the programmed death-1 pathway in lymphoid neoplasms. *Cancer Treat Rev* 2017; 54(3):99-109. doi: 10.1016/j.ctrv.2017.01.009.
 11. Sai J, Owens P, Novitskiy SV, et al. PI3K inhibition reduces mammary tumor growth and facilitates anti-tumor immunity and anti-PD1 responses. *Clin Cancer Res* 2017; 23(13):3371-84. doi: 10.1158/1078-0432.CCR-16-2142.
 12. Ma CX, Sanchez C, Gao F, et al. A phase I study of the AKT inhibitor MK-2206 in combination with hormonal therapy in postmenopausal women with estrogen receptor-positive metastatic breast cancer. *Clin Cancer Res* 2016; 22(11):2650-8. doi: 10.1158/1078-0432.CCR-15-2160.
 13. Shayan G, Srivastava R, Li J, et al. Adaptive resistance to anti-PD1 therapy by Tim-3 upregulation is mediated by the PI3K-Akt pathway in head and neck cancer. *Oncoimmunology* 2017; 6(1):e1261779. doi: 10.1080/2162402X.2016.1261779.
 14. Busaidy NL, LoRusso P, Lawhorn K, et al. The prevalence and impact of hyperglycemia and hyperlipidemia in patients with advanced cancer receiving combination treatment with the mammalian target of rapamycin inhibitor temsirolimus and insulin growth factor-receptor antibody cixutumumab. *Oncologist* 2015; 20(7):737-41. doi: 10.1634/theoncologist.2015-0065.
 15. Cannon MJ, Goyne HE, Stone PJ, et al. Modulation of p38 MAPK signaling enhances dendritic cell activation of human CD4⁺ Th17 responses to ovarian tumor antigen. *Cancer Immunol Immunother* 2013; 62(5):839-49. doi: 10.1007/s00262-013-1391-0.
 16. Hugo W, Zaretsky JM, Sun L, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* 2016; 165(1):35-44. doi: 10.1016/j.cell.2016.02.065.
 17. Bally AP, Lu P, Tang Y, et al. NF-kappaB regulates PD-1 expression in macrophages. *J Immunol* 2015; 194(9):4545-54. doi: 10.4049/jimmunol.1402550.
 18. Lin K, Cheng J, Yang T, et al. EGFR-TKI down-regulates PD-L1 in EGFR mutant NSCLC through inhibiting NF- κ B. *Biochem Biophys Res Commun* 2015; 463(1-2):95-101. doi: 10.1016/j.bbrc.2015.05.030.
 19. Fraser SP, Hemsley F, Djamgoz MB. Caffeic acid phenethyl ester: inhibition of metastatic cell behaviours *via* voltage-gated sodium channel in human breast cancer *in vitro*. *Int J Biochem Cell Biol* 2016; 71(2):111-8. doi: 10.1016/j.biocel.2015.12.012.
 20. Tanaka T, Kutomi G, Kajiwarra T, et al. Cancer-associated oxidoreductase ERO1- α promotes immune escape through up-regulation of PD-L1 in human breast cancer. *Oncotarget* 2017; 8(15):24706-18. doi: 10.18632/oncotarget.14960.
 21. Naik R, Han S, Lee K. Chemical biology approach for the development of hypoxia inducible factor (HIF) inhibitor LW6 as a potential anticancer agent. *Arch Pharm Res* 2015; 38(9):1563-74. doi: 10.1007/s12272-015-0632-5.
 22. Barsoum IB, Smallwood CA, Siemens DR, et al. A mechanism of hypoxia-mediated escape from adaptive immunity in cancer cells. *Cancer Res* 2014; 74(3):665-74. doi: 10.1158/0008-5472.CAN-13-0992.
 23. Chiker S, Pennaneach V, Loew D, et al. Cdk5 promotes DNA replication stress checkpoint activation through RPA-32 phosphorylation, and impacts on metastasis free survival in breast cancer patients. *Cell Cycle* 2015; 14(19):3066-78. doi: 10.1080/15384101.2015.1078020.
 24. Eggers JP, Grandgenett PM, Collisson EC, et al. Cyclin-dependent kinase 5 is amplified and overexpressed in pancreatic cancer and activated by mutant K-Ras. *Clin Cancer Res* 2011; 17(19):6140-50. doi: 10.1158/1078-0432.CCR-10-2288.
 25. Patsoukis N, Li L, Sari D, et al. PD-1 increases PTEN phosphatase activity while decreasing PTEN protein stability by inhibiting casein kinase 2. *Mol Cell Biol* 2013; 33(16):3091-8. doi: 10.1128/MCB.00319-13.
 26. Cortez MA, Ivan C, Valdecana D, et al. PDL1 regulation by p53 *via* miR-34. *J Natl Cancer Inst* 2015; 108(1). pii: djv303. doi: 10.1093/jnci/djv303.
 27. Liu W, Ma Q, Wong K, et al. Brd4 and JMJD6-associated anti-pause enhancers in regulation of transcriptional pause release. *Cell* 2013; 155(7):1581-95. doi: 10.1016/j.cell.2013.10.056.
 28. Melaiu O, Mina M, Chierici M, et al. PD-L1 is a therapeutic target of the bromodomain inhibitor JQ1 and, combined with HLA class I, a promising prognostic biomarker in neuroblastoma. *Clin Cancer Res* 2017; 23(15):4462-72. doi: 10.1158/1078-0432.CCR-16-2601.
 29. Zhu H, Bengsch F, Svoronos N, et al. BET bromodomain inhibition promotes anti-tumor immunity by suppressing PD-L1 expression. *Cell Rep* 2016; 16(11):2829-37. doi: 10.1016/j.celrep.2016.08.032.

30. Lu C, Talukder A, Savage NM, et al. JAK-STAT-mediated chronic inflammation impairs cytotoxic T lymphocyte activation to decrease anti-PD-1 immunotherapy efficacy in pancreatic cancer. *Oncoimmunology* 2017; 6(3):e1291106. doi: 10.1080/2162402X.2017.1291106.
31. Yao A, Liu F, Chen K, et al. Programmed death 1 deficiency induces the polarization of macrophages/microglia to the M1 phenotype after spinal cord injury in mice. *Neurotherapeutics* 2014; 11(3):636-50. doi: 10.1007/s13311-013-0254-x.
32. Doi T, Ishikawa T, Okayama T, et al. The JAK/STAT pathway is involved in the upregulation of PD-L1 expression in pancreatic cancer cell lines. *Oncol Rep* 2017; 37(3):1545-54. doi: 10.3892/or.2017.5399.
33. Borghaei H, Paz-Ares L, Horn L. Nivolumab *versus* docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015; 373(2):1627-39. doi: 10.1056/NEJMoa1507643.
34. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* 2011; 364(26):2517-26. doi: 10.1056/NEJMoa1104621.
35. Gibney GT, Kudchadkar RR, DeConti RC, et al. Safety, correlative markers, and clinical results of adjuvant nivolumab in combination with vaccine in resected high-risk metastatic melanoma. *Clin Cancer Res* 2015; 21(4):712-20. doi: 10.1158/1078-0432.CCR-14-2468.
36. Zak KM, Grudnik P, Magiera K, et al. Structural biology of the immune checkpoint receptor PD-1 and its ligands PD-L1/PD-L2. *Structure* 2017; 25(8):1163-74. doi: 10.1016/j.str.2017.06.011.
37. Taylor A, Harker JA, Chanthong K, et al. Glycogen synthase kinase 3 inactivation drives T-bet-mediated downregulation of co-receptor PD-1 to enhance CD8(+) cytolytic T cell responses. *Immunity* 2016; 44(2):274-86. doi: 10.1016/j.immuni.2016.01.018.
38. Mahoney KM, Freeman GJ, McDermott DF. The next immune-checkpoint inhibitors: PD-1/PD-L1 blockade in melanoma. *Clin Ther* 2015; 37(4):764-82. doi: 10.1016/j.clinthera.2015.02.018.

PD-1/PD-L1 调控及其在肿瘤靶向治疗的前景展望

倪杰明¹, 倪安平^{2*}

¹ 中国医科大学临床医学七年制, 沈阳 110122

^{2*} 中国医学科学院 北京协和医学院, 北京协和医院检验科, 北京 100730

关键词: PD-1/PD-L1; 免疫治疗; 调控机制; 临床试验; 抑制剂

摘要 PD-1/PD-L1 是重要的免疫检查点, PD-1/PD-L1 功能障碍导致肿瘤转移和免疫逃逸。PI3K/Akt/mTOR、MAPK 信号网络诱导 PD-1/PD-L1 表达并促进肿瘤发展; 转录因子 HIF、PTEN、p53、CDK5、BRD4、STAT 调节 PD-1/PD-L1 表达; PD-1/PD-L1 水平也受表观修饰和翻译后修饰调节, 以上调控机制均作为潜在的肿瘤治疗靶点。目前, PD-1/PD-L1 单克隆抗体及小分子抑制剂联合治疗肿瘤在临床上取得了良好的效果。