

Review of: "PD-L1 degradation is regulated by electrostatic membrane association of its cytoplasmic domain"

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Targeting electrostatic membrane association in cytoplasmic domain to induce PD-L1 degradation is a potential therapeutic strategy for cancers

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Abstract

Cancer cells inhibit anti-tumor immunity by increasing the expression level of PD-L1, which interacts with PD-1 to suppress the activity and proliferation of T cells. PD-L1 has become as a promising therapeutic target. Although previous studies have revealed the regulatory mechanism of PD-L1 protein level, most of them are reported in its extracellular domain, which may account for the low clinical response rate and the acquired resistance of cancers. In this study, the investigators found that electrostatic membrane interaction between acidic phospholipids and basic residues in the cytoplasmic domain of PD-L1 (PD-L1-

CD) regulates the cellular level of PD-L1 using nuclear magnetic resonance (NMR) technology. Moreover, the three positively charged residues R260/R262/R265 within PD-L1-CD combine with negatively charged acidic phospholipids to promote the cytoplasmic domain fusing into the cell membrane. Collectively, this study highlights the significance of PD-L1-CD in regulating PD-L1 stability and suggests an alternative/synergistic strategy to overcome the acquired resistance and promote immunotherapeutic effect in cancers.

The extracellular interaction between PD-L1 (programmed death ligand-1) and PD-1 (programmed death-1) inhibits T cell activity and proliferation. PD-L1 has emerged as a remarkable therapeutic target for cancers due to its immunosuppressive role in tumor microenvironment¹. As a direct treatment strategy, blocking the binding of PD-1 to PD-L1 using monoclonal antibodies has shown significant therapeutic effects in a variety of cancers, including melanoma, non-small-cell lung cancer, gastric cancer and breast cancer². Even though, the low clinical response rate still remains the main obstacle preventing most cancer patients benefiting from PD-1/PD-L1 blockage treatment. In addition, PD-1 or PD-L1 monoclonal antibodies or inhibitors only target and bind to the extracellular domain of PD-L1.

As a result, downregulating the protein level of PD-L1 that highly expressed on the surface of cancer cells has gradually become an additive or synergistic strategy for cancer immunotherapy³. An in-depth exploration of the regulation mechanism for PD-L1 protein level could contribute to improve the clinical effect in PD-1/PD-L1 blockade therapy. There are usually two ways to downregulate the protein level, reducing production at the transcriptional level or increasing degradation at the post-translational modification level. Recent studies have revealed that several signaling pathways such as MAPK, JAK/STAT3 and PI3K/AKT play important roles in the increased expression of PD-L1 in cancer cells⁴.

On the other hand, increasing evidence suggests that PD-L1 is degraded through multiple ways, including proteasome, lysosome and autophagy, which significantly enhanced T cell activity and immunotherapeutic effect for cancers^{5,6}. It has been reported that PD-L1 undergoes proteasomal degradation that promoted by β -TrCP dependent ubiquitination followed with glycogen synthase kinase 3 β (GSK3 β) mediated phosphorylation of PD-L1 at T180 and S184 residues⁷. It is noteworthy that the dynamic phosphorylation and ubiquitination modification should mostly occur in the intracellular segment of PD-L1, which highlights the need to further explore the regulation function of PD-L1 level in the cytoplasmic domain. The newly report shows that dynamic phosphorylation of Ser279 and Ser283 located in the cytoplasmic domain of PD-L1 (PD-L1-CD) drive PD-L1 degradation, which provides the evidence that phosphorylation and following ubiquitination modification in PD-L1-CD should be responsible for PD-L1 degradation⁸.

In this study, the investigators found that electrostatic membrane interaction between acidic phospholipids and basic residues in PD-L1-CD is responsible for PD-L1 degradation. Using nuclear magnetic resonance (NMR) and fluorescence energy resonance transfer (FRET) analysis, the investigators found that

N-terminal of PD-L1-CD adheres to the cell membrane through electrostatic action, which indicates that the acidic phospholipids in the cells have a significant effect on the binding affinity of PD-L1-CD to the cell membrane. In addition, the three arginine residues R260/R262/R265 within PD-L1-CD play a key role in the process of binding as these positively charged residues exhibit the function to combine with acidic phospholipids (negatively charged), which makes PD-L1-CD embedding into the cell membrane. Further experiments show that PD-L1-CD affects the ubiquitination and de-ubiquitination modification to reduce the intracellular lysosomal degradation of PD-L1, so as to sustain a more stable PD-L1 expression level. It has been reported that metformin, an oral medication widely used to treat type 2 diabetes (T2D), enhances the efficacy of immunotherapy through endoplasmic-reticulum-associated degradation of PD-L1⁹. This study delivers a new mechanism describing PD-L1 degradation in metformin-associated way. Specifically, metformin competitively binds to the acidic phospholipids in extracellular and intracellular tests, so as to affect the binding efficacy of PD-L1-CD to cell membrane and reduce the protein level of PD-L1, which exhibits a potential synergy for immunotherapy. However, the investigators also pointed out that the significance of metformin should be discounted, because only high-dose metformin exhibits strong anti-tumor effect.

The exploration of PD-L1 function in cytoplasmic domain (CD) shows great significance for the clinical treatment of cancers as multiple cancer-derived mutations (R260C, R262K, I274V, D276Y/H, T277K/S, K280N and T290M) were found within this domain¹⁰. These mutations may be responsible for the acquired resistance of cancers. This discovery emphasizes the importance of PD-L1-CD in regulating PD-L1 degradation and provides a promising strategy to improve the current immunotherapies and overcome the acquired resistance in multiple kinds of cancers.

The above discovery has led the destruction of PD-L1-CD-membrane interactions as a new strategy for cancer therapeutics. To this end, more specific inhibitors and molecules targeting to PD-L1-CD-membrane association should be screened and reformed to enhance the efficacy of PD-1/PD-L1 blockade in cancer immunotherapy. More broadly, the protein levels of other transmembrane proteins, such as EGFR, CD28 and CD3, may also exhibit the possibility to be affected by the asymmetric lipid distribution induced by excessive negative charges accumulation on the plasma membrane.

Together, the findings in this study suggest that combination treatments that targeting electrostatic membrane interaction of cytoplasmic domain have potentially alternative/combined effects on PD-L1 degradation, allowing a stronger promotion of anti-tumor immunity in various types of cancers.

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