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RESEARCH HIGHLIGHT

Caught in the middle: MARCH5 mediates PD-1-induced IL-2R degradation

Xinghao Wang [1,2,3] and Weiping Zou[1,2,3,4,5,6 ✉]

Blockade of the PD-L1/PD-1 pathway in combination of IL-2 therapy appears to yield synergistic anti-tumor immunity in the treatment of various cancers. In a recent paper published in *Cell Research*, Liu et al. uncover previously unknown synergistic mechanisms and identify FDA-approved pitavastatin as a potential drug to facilitate such synergy.

The tumor microenvironment (TME) is a complex, heterogeneous structure which hosts tumor–immune cell interactions. Activated T cells, both anti-tumor effector $CD8^+$ T cells and pro-tumor regulatory T cells (Tregs), express PD-1 which can interact with PD-L1 on tumor cells and antigen presenting cells (APCs).[1–3] This interaction mediates $CD8^+$ T cell dysfunction in the TME, and antibodies targeting the PD-L1/PD-1 pathway have boosted anti-tumor immunity and provided long-term remission for a fraction of patients with a variety of tumors.[1,4] Despite this, most patients do not respond to therapy, for reasons still poorly understood, prompting the consideration of combination therapy.[4,5]

One proposed combination includes the use of IL-2.[6–8] Unfortunately, IL-2 requires high doses that lead to serious toxicities.[6] However, combination therapy with PD-L1/PD-1 blockade allows for low doses of IL-2.[9] Recently, PD-L1/PD-1 blockade in combination with IL-2 has shown initial promise in animal models[6–8] and is now in clinical trials.[10] The mechanisms underlying this may be related to upregulation of IL-2Rα$_c$ (CD25).[10] However, it is unknown whether PD-L1/PD-1 blockade regulates IL-2R level.

New data in a recent paper of *Cell Research* from Liu et al. reveal that PD-1 signaling regulates IL-2Rγ$_c$ (CD132) level via induction of MARCH5, an E3 ubiquitin ligase that targets γ$_c$ for lysosomal degradation.[11] They also find that γ$_c$ can be inactivated through SHP2 dephosphorylation induced by PD-1 signaling. Overall, these data uncover new mechanisms of how PD-L1/PD-1 blockade synergizes with IL-2 and provides both justification and potential new targets to exploit this sensitization (Fig. 1).

To explore whether PD-1 signaling regulates γ$_c$ level, Liu et al. used immunochemistry, showing an inverse correlation between PD-L1 and γ$_c$ protein level in vivo in human non-small cell lung cancer tissues. In vitro, ex vivo, and in vivo studies demonstrated that PD-L1 and PD-1 engagement resulted in decreased γ$_c$ protein levels with no effect on γ$_c$ mRNA levels in T cells, suggesting post-transcriptional regulation of γ$_c$.

The authors set out to understand how PD-1 regulates γ$_c$ level post-transcriptionally. They reveal that PD-1 signaling promotes γ$_c$ degradation via lysosome, but not proteasome or autophagosome pathways, in vitro. Additionally, they find evidence that γ$_c$ is polyubiquitinated after PD-L1 engagement. After screening 196 ubiquitin-related proteins, MARCH5 was identified as the E3 ubiqituin ligase responsible for K27-linked polyubiquitination and degradation of γ$_c$. MARCH5 was associated with γ$_c$ at baseline, though PD-1 signaling further promotes their association. Furthermore, MARCH5's activity was opposed by USP5, a deubiquitin enzyme that interacts with γ$_c$ in resting conditions. PD-1 signaling upregulates BATF, which targets the MARCH5 promoter to induce its transcription. These findings uncover the dynamic regulatory pathways mediated by PD-1 signaling in T cells.

To investigate the role of MARCH5 in vivo with the constraints that homozygous *MARCH5* deletion is embryonic lethal, the authors generated mice with heterozygous deletion of *MARCH5* in hematopoietic cells (*March5*^f/f:Vav1-Cre^). Upon challenge with MC38 colon adenocarcinoma and B16F10 melanoma in *March5*^+/f:Vav1-Cre^ and *March5*^+/f^ mouse models, they observed that MARCH5 knockdown enhances anti-tumor immunity and inhibits tumor growth. This is accompanied by increased γ$_c$ protein level in T cells. Furthermore, MARCH5 knockdown enhances the efficacies of both IL-2 alone and combination therapy.

There are no known drugs targeting MARCH5. The authors designed a dual plasmid reporter system encoding both MARCH5 and γ$_c$-luciferase to screen a compound library of 2571 FDA-approved drugs for its inhibition of MARCH5-mediated degradation of γ$_c$. The most potent MARCH5 inhibitor was found to be pitavastatin, an HMG-CoA reductase inhibitor used clinically to lower cholesterol and triacylglycerol levels. They confirmed its specificity, as its induction of γ$_c$-luciferase activity was lost in the absence of MARCH5. They then recapitulated this finding in vivo, as demonstrated by the inhibitory role of pitavastatin treatment on tumor growth in MC38 colon cancer and B16F10 melanoma models. Additionally, the authors find that pitavastatin further enhances combination therapy with PD-1 blockade and IL-2 therapy, providing greater evidence of its anti-tumor effects. However, the specificity of pitavastatin's anti-tumor effect was not tested in MARCH5-knockdown mice.

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[1]Department of Surgery, University of Michigan Medical School, Ann Arbor, MI, USA. [2]Center of Excellence for Cancer Immunology and Immunotherapy, University of Michigan Rogel Cancer Center, University of Michigan Rogel Cancer Center, Ann Arbor, MI, USA. [3]Graduate Program in Immunology, University of Michigan, Ann Arbor, MI, USA. [4]Graduate Program in Cancer Biology, University of Michigan, Ann Arbor, MI, USA. [5]University of Michigan Rogel Cancer Center, University of Michigan, Ann Arbor, MI, USA. [6]Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA. ✉email: wzou@umich.edu
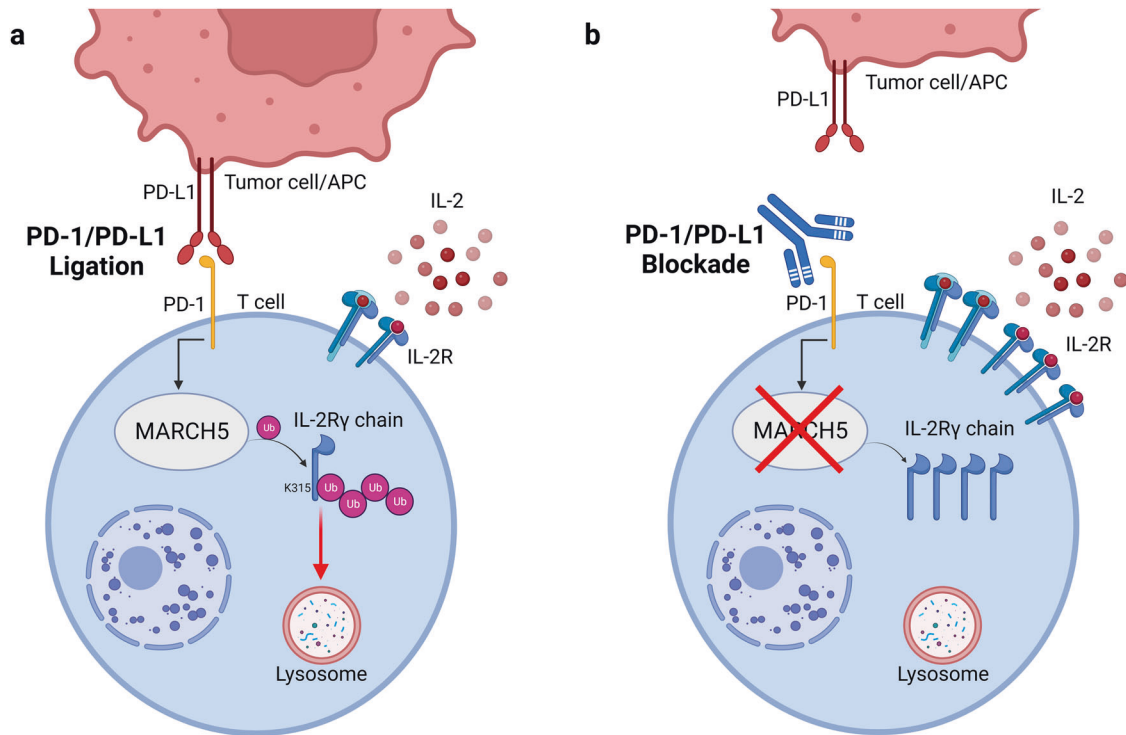
Fig. 1 PD-1/PD-L1 signaling regulates IL-2Rγ$_c$ level via MARCH5. a Tumor cell/APC interaction with T cells through PD-1/PD-L1 ligation results in induction of the E3 ubiquitin ligase MARCH5, which polyubiquitinates IL-2Rγ$_c$ for lysosomal degradation. **b** PD-1/PD-L1 blockade prevents MARCH5 induction, which results in increased IL-2Rγ$_c$ level, thereby sensitizing T cells to IL-2. Created with BioRender.com.

Aside from indirect regulation, PD-1 signaling also directly inhibits γ$_c$ signaling via SHP2. While PD-1 signaling recruits SHP2 to dephosphorylate TCR and CD28 signaling molecules,[12] whether other substrates important for T cell activation can be targeted remains unknown. Interestingly, the authors find that PD-1 signaling promotes the association of SHP2 with γ$_c$ and its dephosphorylation at γ$_c$[357], thus representing yet another mechanism by which PD-1 can propagate immunosuppressive signaling.

Overall, this study reveals invaluable insight into the mechanisms by which PD1/PD-L1 blockade synergizes with IL-2 to promote anti-tumor responses and identify new drugs to be potentially repurposed in treating cancer. These data add new players in our understanding of mediators of anti-tumor and pro-tumor immunity (Fig. 1). In the future, it is important to consider how MARCH5 inhibition and/or combination therapy might affect Treg phenotype and function. Tregs express the high-affinity IL-2R and may be preferentially targeted by low doses of IL-2.[6,8] Whether MARCH5 inhibition may potentiate immunosuppression by Tregs remains unexplored. Additionally, why there is specificity in regulating the γ$_c$ and not the other subunits of IL-2R remains unknown, as previous reports have suggested that the high-affinity IL-2R (which requires the α$_c$) mediates the synergy between PD-1 blockade and IL-2.[10] Finally, the role of tumor cell MARCH5 is not examined in their in vivo studies as pitavastatin does not discriminate between tumor and immune cells. Nonetheless, this study provides a foundational framework into

understanding mechanisms of synergy in combination therapy that will undoubtedly improve the lives of cancer patients.

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