



# Multifactorial regulators of tumor programmed death-ligand 1 (PD-L1) response

R. Dixon Dorand<sup>1</sup>, Agne Petrosiute<sup>1,2,3</sup>, Alex Y. Huang<sup>1,2,3</sup>

<sup>1</sup>Departments of Pathology, <sup>2</sup>Departments of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, OH, USA; <sup>3</sup>Angie Fowler AYA Cancer Institute/UH Rainbow Babies & Children's Hospital, Cleveland, OH, USA

Correspondence to: Alex Y. Huang, MD, PhD. Departments of Pathology/Departments of Pediatrics, Case Western Reserve University School of Medicine; Angie Fowler AYA Cancer Institute/UH Rainbow Babies & Children's Hospital, Cleveland, OH 44106, USA. Email: Ayh3@case.edu.

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Tumor cells hijack physiological mechanisms to create favorable conditions that allow them to survive and thrive within the hostile tissue and immune microenvironments. The identification and subsequent therapeutic blockade against immune checkpoint molecules including cytotoxic T lymphocyte associated antigen 4 (CTLA-4; CD152), programmed cell death protein 1 (PD-1; CD279) and its ligand programmed death-ligand 1 (PD-L1; CD274; B7-H1) have evoked much excitement in cancer immunotherapy against a variety of chemo-refractory cancers (1-4). Since the initial characterization of the PD-1/PD-L1 axis over 2 decades ago (5-7), over 4,000 articles have been published exploring how this immune checkpoint receptor-ligand pair influence tumor development, survival, and metastasis (2,8). Surprisingly, however, only a handful of studies have described how tumor PD-L1 is regulated at the transcriptional and post-translational levels. Recent studies by Mezzadra *et al.* (9), and Burr *et al.* (10) describe a novel post-translational mechanism by which PD-L1 is regulated within primary human dendritic cells and a variety of human tumor cell types, adding to our understanding of how this critical immune regulatory axis is regulated.

Using human chronic myelogenous leukemia (CML)-derived HAP1 cells, Mezzadra and colleagues (9) identified chemokine-like factor-like MARVEL transmembrane domain containing family member 6 (CMTM6) as associated with IFN $\gamma$ -induced PD-L1 expression using *in vitro* genetic screens. The correlation between CMTM6 and PD-L1 co-expression was observed in 30 human cancers in available TCGA datasets. Short hairpin depletion of CMTM6 in melanoma, colon, and non-small

cell lung cancer lines resulted in blunted surface PD-L1 protein expression without affecting PD-L1 mRNA levels following IFN $\gamma$  stimulation, a phenotype similarly observed in lipopolysaccharide (LPS)-stimulated dendritic cells. Additionally, CMTM4, with 55% homology to CMTM6, stabilized IFN $\gamma$ -induced PD-L1 protein expression in the absence of CMTM6. The authors performed co-immunoprecipitation analyses to show physical interactions between CMTM6 and PD-L1 involving both intracellular and transmembrane portions of PD-L1 at the plasma membrane. Finally, CMTM6 prolonged surface PD-L1 protein half-life by preventing ubiquitination by STUB1, an E3 ubiquitin ligase.

Burr *et al.* (10) independently identified and validated CMTM6 as an important regulator of PD-L1 through a whole-genome CRISPR-Cas9 deletion library screen in pancreatic cancer cell line, BxPC-3. As reported by Mezzadra *et al.*, the authors showed CMTM6 depletion reduced PD-L1 protein expression without altering mRNA abundance. The authors also observed plasma membrane co-localization between CMTM6 and PD-L1. Furthermore, they found that CMTM6 was also located within recycling endosomes and facilitated PD-L1 recycling to the cell surface by bypassing lysosomal degradation, an additional mechanism to ubiquitination by CMTM6 for prolonged PD-L1 protein half-life (9). The end result was *in vivo* efficacy of shRNA targeting of CMTM6 in enhancing survival of mice inoculated with CMTM6<sup>def</sup> B16F10 melanoma.

These two recent studies add to a growing repertoire of mechanisms by which tumors regulate PD-L1 expression

upon sensing immune pressure. PD-L1 is known to be induced on the surface of cancers and immune cells in response to IFN $\gamma$ , which signals in a JAK/STAT-dependent way to promote PD-L1 gene transcription via interferon regulatory factor 1 (IRF1) (11). In multiple myeloma (MM), IFN $\gamma$ -induced PD-L1 was abrogated by UO126, a potent MEK inhibitor, suggesting MEK/ERK signaling pathway contributed to this signaling axis (12). The same study found that surface PD-L1 on MM could be further driven by the toll-like receptor (TLR) ligands LPS, peptidoglycan, and CpG oligonucleotide in a MyD88-dependent manner (12). Lastly, in myelodysplastic syndrome both IFN $\gamma$  and TNF $\alpha$  were capable of inducing PD-L1 on blast cell surface via NF- $\kappa$ B (13). In addition to induction by cytokines, mutations within tumor cells can drive PD-L1 expression. For example, loss of function mutations of PTEN combined with activation of PI3K led to increased PD-L1 gene expression in human glioblastoma multiforme (14). Additionally, activating EGFR mutations have been shown to upregulate PD-L1 in an ERK-dependent mechanism (15). Micro-RNAs (miRs) have also been shown to alter PD-L1 expression via binding the 3' UTR region of *PD-L1* (15,16). Recently miR-34, which itself is regulated by p53, was shown to inhibit PD-L1 in murine models of non-small cell lung cancer (16). Delivery of miR-34 via liposomal nanoparticles increased the percentage of CD8<sup>+</sup> cells and abundance of IFN $\gamma$  and TNF $\alpha$  in the tumor microenvironment, and slowed tumor growth. These results and others studies were recently reviewed (17).

A recent study by Dorand *et al.* (18) demonstrated that the serine-threonine kinase, cyclin dependent kinase 5 (Cdk5), critically regulates IFN $\gamma$ -induced PD-L1 gene transcription in multiple tumor types. CRISPR-Cas9 disruption of Cdk5 in a murine medulloblastoma model resulted in CD4<sup>+</sup> T cell-dependent rejection. The authors proposed a mechanism by which Cdk5 regulates the stability of IRF2—an antagonist of IRF1-mediated IFN $\gamma$  signaling (19)—via phosphorylation of a co-repressor IRF2 binding protein 2 (IRF2BP2) (20) to alter PD-L1 gene transcription. Another elegant study by Casey *et al.* (21) described an additional mechanism by which MYC controls PD-L1 and CD-47 transcriptions. Using human and murine MYC-driven T cell acute lymphoblastic leukemia (T-ALL), they showed that MYC inhibition led to decreased PD-L1 gene transcription and resulted in immune-mediated rejection of established tumors. Chromatin immunoprecipitation (ChIP) analysis revealed direct physical interaction between

MYC and the *PD-L1* promoter. These two studies provide alternative mechanisms of tumor PD-L1 control at the transcriptional promoter/enhancer level.

Additional tumor PD-L1 control exists at the gene transcript level. Kataoka *et al.* (22) identified structural variants in the 3' UTR region of *PD-L1* leading to persistence of *PD-L1* mRNA. Such 3' UTR structural variants were found in human T cell leukemia and lymphoma, diffuse large B cell lymphoma, and gastric adenocarcinoma. Structural PD-L1 variants containing functional extracellular and intracellular domains resulted in immune escape. Mice bearing EG-7 tumors with forced overexpression of 3' UTR variants had sustained tumor growth compared to control tumors following treatment with poly I:C treatment. While these 3' UTR variants were not commonly found among tumor types, they nevertheless offer an alternative mechanism for tumor PD-L1 regulation.

Post-translational PD-L1 modifications represent yet another level of control. Two studies demonstrated different mechanisms affecting the PD-L1 protein stability. Li *et al.* (23) revealed that phosphorylation of PD-L1 by glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) resulted in proteasomal degradation, which can be inhibited by epidermal growth factor (EGF). EGF increases PD-L1 glycosylation to inhibit GSK3 $\beta$  interaction, leading to sustained PD-L1 expression. In this regard, gefitinib, an EGF inhibitor, synergizes with anti-PD-1 antibodies *in vitro* and *in vivo* in multiple murine cancers. Lim *et al.* (24) showed that TNF $\alpha$  signaling resulted in increased expression of COP9 signalosome 5 (CSN5), a deubiquitinating enzyme, to enhance PD-L1 protein expression. CSN5 associated kinase activity could be inhibited by curcumin, the treatment with which in mice bearing 4T1 tumors slowed tumor growth, increased tumor free survival, and increased IFN $\gamma$ <sup>+</sup> CD8<sup>+</sup> T cells when combined with CTLA-4 blockade.

The PD-1/PD-L1 axis is being thoroughly investigated for clinical applications as potent mediators of anti-tumor immunity. Current emphasis in the field focuses on characterizing which tumor subsets will respond to such immunotherapeutic approaches (25,26). While antibodies targeting cell surface PD-L1 expression provide one such method for overcoming immune checkpoints, growing mechanistic studies on the regulatory pathways of tumor PD-L1 expression have the potential to uncover additional tumor-specific therapeutic targets while avoiding adverse side effects of autoimmunity due to non-tumor specific

nature of global PD-1/PDL1 blockade approach. The exciting discovery of CMTM6 and CMTM4 in PD-L1 protein regulation further enhances our basic knowledge of PD-L1 regulation, significantly contributes to our basic understanding of cancer immunotherapy, and offers a new exciting venue for future immunotherapeutic development.

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### References

- Iwai Y, Hamanishi J, Chamoto K, et al. Cancer immunotherapies targeting the PD-1 signaling pathway. *J Biomed Sci* 2017;24:26.
- Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;12:252-64.
- Postow MA, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. *J Clin Oncol* 2015;33:1974-82.
- Topalian SL, Taube JM, Anders RA, et al. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat Rev Cancer* 2016;16:275-87.
- Dong H, Zhu G, Tamada K, et al. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med* 1999;5:1365-9.
- Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000;192:1027-34.
- Ishida Y, Agata Y, Shibahara K, et al. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* 1992;11:3887-95.
- Koebel CM, Vermi W, Swann JB, et al. Adaptive immunity maintains occult cancer in an equilibrium state. *Nature* 2007;450:903-7.
- Mezzadra R, Sun C, Jae LT, et al. Identification of CMTM6 and CMTM4 as PD-L1 protein regulators. *Nature* 2017;549:106-10.
- Burr ML, Sparbier CE, Chan YC, et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. *Nature* 2017;549:101-5.
- Lee SJ, Jang BC, Lee SW, et al. Interferon regulatory factor-1 is prerequisite to the constitutive expression and IFN-gamma-induced upregulation of B7-H1 (CD274). *FEBS Lett* 2006;580:755-62.
- Liu J, Hamrouni A, Wolowicz D, et al. Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN- $\gamma$  and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway. *Blood* 2007;110:296-304.
- Kondo A, Yamashita T, Tamura H, et al. Interferon-gamma and tumor necrosis factor-alpha induce an immunoinhibitory molecule, B7-H1, via nuclear factor-kappaB activation in blasts in myelodysplastic syndromes. *Blood* 2010;116:1124-31.
- Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat Med* 2007;13:84-8.
- Chen L, Gibbons DL, Goswami S, et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral

- immunosuppression. *Nat Commun* 2014;5:5241.
16. Cortez MA, Ivan C, Valdecanas D, et al. PDL1 Regulation by p53 via miR-34. *J Natl Cancer Inst* 2015;108. pii: djv303.
  17. Smolle MA, Calin HN, Pichler M, et al. Noncoding RNAs and immune checkpoints-clinical implications as cancer therapeutics. *FEBS J* 2017;284:1952-66.
  18. Dorand RD, Nthale J, Myers JT, et al. Cdk5 disruption attenuates tumor PD-L1 expression and promotes antitumor immunity. *Science* 2016;353:399-403.
  19. Harada H, Fujita T, Miyamoto M, et al. Structurally similar but functionally distinct factors, IRF-1 and IRF-2, bind to the same regulatory elements of IFN and IFN-inducible genes. *Cell* 1989;58:729-39.
  20. Childs KS, Goodbourn S. Identification of novel co-repressor molecules for Interferon Regulatory Factor-2. *Nucleic Acids Res* 2003;31:3016-26.
  21. Casey SC, Tong L, Li Y, et al. MYC regulates the antitumor immune response through CD47 and PD-L1. *Science* 2016;352:227-31.
  22. Kataoka K, Shiraishi Y, Takeda Y, et al. Aberrant PD-L1 expression through 3'-UTR disruption in multiple cancers. *Nature* 2016;534:402-6.
  23. Li CW, Lim SO, Xia W, et al. Glycosylation and stabilization of programmed death ligand-1 suppresses T-cell activity. *Nat Commun* 2016;7:12632.
  24. Lim SO, Li CW, Xia W, et al. Deubiquitination and Stabilization of PD-L1 by CSN5. *Cancer Cell* 2016;30:925-39.
  25. Lipson EJ, Forde PM, Hammers HJ, et al. Antagonists of PD-1 and PD-L1 in Cancer Treatment. *Semin Oncol* 2015;42:587-600.
  26. Taube JM, Young GD, McMiller TL, et al. Differential Expression of Immune-Regulatory Genes Associated with PD-L1 Display in Melanoma: Implications for PD-1 Pathway Blockade. *Clin Cancer Res* 2015;21:3969-76.

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