The "Inside" Story on Tumor-Expressed PD-L1

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While the extracellular domain of PD-L1 is well-recognized for playing a critical role in immune evasion by suppressing $CD8^+$ T-cell activity through direct PD-1 interactions, a series of studies has evolved highlighting important functional roles for the PD-L1 cytoplasmic domain in supporting various aspects of tumorigenesis. Kornepati and colleagues contribute to our overall understanding of PD-L1 in tumor biology by describing a link between tumor PD-L1 expression and DNA repair. These studies demonstrate that PD-L1 promotes breast cancer type 1 (BRCA1)–mediated homologous recombination while inhibiting cytosolic DNA sensing, thus suppressing tumor immunogenicity. Notably, these effects could not be reversed with anti-PD-L1 antibodies utilized in the clinic, suggesting that pharmacologic agents promoting PD-L1 degradation may be a more effective treatment strategy for select tumors. Studies that are improving our understanding of the pathways driven by PD-L1 cytoplasmic signaling are providing increased insight into the design of next generation combinatorial immunotherapy strategies.

See related article by Kornepati et al., p. 2156

The introduction of checkpoint inhibitor immunotherapies designed to block tumor PD-L1 (B7-H1, *CD274*) interactions with the PD-1 regulatory receptor on the surface of effector T cells has revolutionized the field of oncology. As a result, the functional role of PD-L1 within the tumor microenvironment has been largely attributed to its extracellular domain while relatively few studies have focused on the tumor-intrinsic effects of this immune inhibitory receptor. However, there is now an evolving realization that the cytoplasmic domain of PD-L1 can mediate important biological effects on tumor behavior. Interestingly, this is consistent with prior observations of other members of the B7 family including B7.1 (CD80) that can serve as a receptor for the T-cell–expressed cytotoxic T lymphocyte antigen-4 (CTLA4) ligand in a 'reverse signaling' mechanism in dendritic cells (1). These findings are now influencing a growing number of scientific studies that are raising important questions regarding how existing PD-1/PD-L1 checkpoint inhibitors impact tumor biology while also providing insight into the design of next-generation PD-L1 inhibitors and future immunotherapy regimens.

As part of this growing focus on tumor-intrinsic PD-L1 functionality, Kornepati and colleagues report in the current issue of *Cancer Research* that PD-L1 regulates the process of homologous recombination (HR) DNA repair in tumor cells in a manner dependent upon breast cancer type 1 (BRCA1; ref. 2). The authors demonstrate that genetic silencing of PD-L1 promotes DNA damage accumulation in tumors while promoting responsiveness to PARP inhibitor therapies in BRCA1-proficient tumors. Further studies reveal this phenomenon to be accompanied by PD-L1–dependent accumulation of BRCA1 nuclear foci in response to DNA damage and demonstrate that PD-L1 directly interacts with the BRCA1-binding protein, BARD1. Consistent with its role in promoting DNA repair, this work further reveals that PD-L1 inhibits cytosolic DNA sensing based on an increase in the downstream mediators of the cGAS/STING pathway, TBK1 phosphorylation, and *CCL5* expression in tumor cells genetically silenced for PD-L1. The authors therefore suggest that loss of PD-L1 by tumors may resemble *BRCA1* mutated cancers in that they should be more sensitive to PARP inhibitors, a finding that was ultimately found to be tumor cell line dependent. Notably, these observations could not be recapitulated by surface PD-L1 blockade. Together, this work implies that tumor-intrinsic PD-L1 signaling promotes double-stranded DNA (dsDNA) break repair via BRCA1-dependent HR and indicates that this process is not susceptible to inhibition by checkpoint inhibitors that interfere with PD-1/PD-L1 interactions. It is interesting to note that these findings align closely with the elegant work of Tu and colleagues who in 2019 demonstrated that the intracellular cytoplasmic domain of PD-L1 competes with the RNA exosome by binding RNAs enriched in the DNA damage response (3). This study showed that the cytoplasmic domain of PD-L1 binds and stabilizes BRCA1 mRNA, thereby supporting the DNA damage response pathway in tumors. Antibodies capable of destabilizing PD-L1 and leading to its degradation were found to suppress this DNA repair pathway and to increase the sensitivity of tumors to radiotherapy. This finding is consistent with their observation that PD-L1 knockout mice are significantly more radiosensitive and exhibit diminished survival following whole body irradiation relative to wild-type controls. The findings from each of these studies are in-line with prior work reporting an upregulation in PD-L1 expression in response to DNA damage.

In addition to the regulation of DNA repair in tumor cells, there has been a series of previous studies describing an intrinsic role for PD-L1 in the process of tumorigenesis (**Table 1**). Indeed, in 2008, Azuma and colleagues reported that the cytoplasmic domain of PD-L1 promotes antiapoptotic signals in tumor cells including protection from cytotoxic T lymphocyte–mediated killing (4). This was supported by subsequent work showing that PD-L1 also promotes resistance to chemotherapy via activation of the MAPK pathway. Additional studies have shown that the intracellular domain of PD-L1 supports tumor cell proliferation *in vitro* as well as tumor progression in immunodeficient hosts using preclinical models of melanoma and ovarian cancer (5). Interestingly, these protumorigenic properties of PD-L1 could be inhibited by delivering an anti–PD-L1 antibody in the absence of an intact immune system. This study further demonstrated that the intracellular domain of PD-L1 triggers mTOR signaling, a serine/threonine kinase known to regulate both cellular growth and metabolism. Interestingly, this connection between tumor PD-L1 and

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Table 1. Tumor-intrinsic PD-L1 signaling pathways.

Biologic function	Regulation by PD-L1	Signaling pathway	PD-L1 CD binding protein	References
DNA repair	Promotes	BRCA1 HR/inhibition of RNA exosome	BARD1/BRCA1 mRNA	(2, 3)
Apoptosis	Inhibits	MAPK	Unknown	(4)
Cellular proliferation and metastasis	Promotes	mTOR	Unknown	(5)
Glycolysis	Promotes	Akt/mTOR	Unknown	(6)
EMT	Promotes	p38-MAPK/GSK3β/Snail	PTP1B	(7, 8)
Immune evasion	Promotes	STAT3/caspase-7/type-I IFN signaling	Unknown	(9)
		STAT3/PKR/NLRP3/HSP70	Unknown	(10)

Abbreviation: CD, cytoplasmic domain.

activation of the mTOR pathway was also described in another study that demonstrated that PD-L1 promotes tumor glycolysis through the Akt/mTOR signaling pathway (6). These authors concluded that enhanced tumor glycolysis lowers glucose levels and restricts the function of infiltrating T-cell populations, an effect that was reversed by treatment with an anti–PD-L1 antibody. Finally, a recent report has revealed that tumor PD-L1 can drive epithelial–mesenchymal transition (EMT) in triple-negative breast cancer by stabilizing the transcription factor Snail (7). Further studies showed that this process involves the initial binding of the tyrosine phosphatase PTP1B to the PD-L1 cytoplasmic domain, which prompts p38-MAPK phosphorylation and inhibition of glycogen synthase kinase 3β, thus preventing Snail ubiquitination and degradation. This finding represents a mechanistic explanation for previous work that described a positive relationship between tumor PD-L1 expression levels and EMT in breast cancer (8).

Additional studies have also provided insight into how tumor-intrinsic PD-L1 signaling can regulate interactions with the immune microenvironment. Type I IFNs have been shown to exhibit several antitumor properties that can control tumor progression. Gato-Cañas and colleagues demonstrated that nonclassical signal transduction motifs in the PD-L1 cytoplasmic domain protect tumor cells from IFN-mediated cytotoxicity by blocking caspase-7 activation via the inhibition of STAT3 signaling (9). This protective mechanism was found to be suppressed with anti–PD-L1 antibody treatment. Interestingly, these authors also found evidence of somatic mutations involving an inhibitory motif within the PD-L1 cytoplasmic domain in human malignancies capable of enhancing protection from IFN-mediated cytotoxicity. Further studies have characterized an alternative pathway by which tumor-intrinsic PD-L1 signaling can potently suppress the generation of a cytolytic T-cell response (10). In this pathway, the PD-L1 cytoplasmic domain triggers activation of the tumor NLRP3 inflammasome, resulting in the release of heat shock protein-70 and generation of a CXCR2 chemokine gradient that promotes recruitment of granulocytic myeloid–derived suppressor cells into the tumor where $CD8^+$ T-cell function is potently suppressed. Importantly, this signaling axis is induced in response to PD-1/PD-L1 blockade and is dependent on effector T-cell activation, indicating that this pathway represents a mechanism of adaptive resistance to checkpoint inhibitor immunotherapy. Similar to the findings of Gato-Cañas and colleagues, additional studies demonstrated that PD-L1 signaling inhibits STAT3, which, in turn, inhibits dsDNA-dependent protein kinase-R (PKR)–mediated suppression of NLRP3 inflammasome activation, ultimately generating an immunotolerant tumor microenvironment.

An overall picture is now emerging, indicating that tumor PD-L1 simultaneously promotes immune evasion while also supporting several fundamental aspects of tumorigenesis. Studies highlighting the tumor intrinsic signaling pathways induced by PD-L1 support the need to reimagine future generation pharmacologic inhibitors of this pathway, as PD-1/PD-L1 blocking antibodies failed to inhibit many of these PD-L1–dependent signaling activities. Thus, agents capable of also promoting PD-L1 internalization and degradation would be necessary to effectively ablate all protumorigenic properties of PD-L1 and would be expected to be more effective at treating certain solid tumors, particularly those noted to exhibit higher PD-L1 expression coupled with low-levels of tumor-infiltrating T cells. Alternatively, these data imply that tumors with lower levels of PD-L1 expression may be more susceptible to treatment with PARP inhibitors or other chemotherapeutic agents. Indeed, the work now presented by Kornepati and colleagues provides a potential explanation for the modest responses seen in combination PARP inhibitor/anti–PD-L1 immunotherapy regimens already tested in clinical trials. Future work defining which PD-L1–dependent pathways are most critical for the development and therapeutic responsiveness of different tumor types will be important necessary steps for clinical translation. Taken together, these studies emphasize the importance of understanding both the "outside" and "inside" story of any receptor-ligand pair selected for therapeutic targeting.

Authors' Disclosures

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