Editorial

Another important step towards understanding tumor immune evasion—novel mechanisms of PD-L1 overexpression

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Overview

Recent advancements in the search for new treatment strategies for cancer have led to the development of therapeutic agents that target specific molecules that are critical to cancer development and/or expansion. The inhibition of the programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) pathway is one of such upcoming strategies that is being extensively explored in the field of oncology. Immunotherapy agents targeting the PD-1/PD-L1 signaling pathway have demonstrated promising anti-tumor efficacy in several malignancies, including non-small cell lung cancer (NSCLC) and melanoma, among others (1-6). Tumor cells are known to overexpress *PD-L1*, which aids in immune evasion by inducing T cell anergy and exhaustion within the tumor microenvironment (7). Several chromosomal alterations, most notably 9p24.1 amplification [identified in Hodgkin's lymphoma (HL) cell lines], are reported to result in overexpression of PD-1 ligands (8,9). In addition, endogenous molecules such as interferon-γ (IFNγ) have also been identified to induce PD-L1 overexpression (10,11). The ability of tumors to suppress the host immune response is considered to be central to the clinical benefit observed with immunotherapy agents targeting the PD-1/PD-L1 signaling pathway (8,12-17). The mechanistic relationship between evasion of endogenous immunity by cancer cells and overexpression of PD-1 ligands, however, is not fully understood.

Background

Several chromosomal alterations have been identified that result in overexpression of PD-1 ligands (*Table 1*). Analysis of HL cell lines revealed major histocompatibility complex (MHC) class II trans-activator *CIITA* gene fusions that resulted in PD-L1 and PD-L2 overexpression, along with decreased surface human leucocyte antigen (*HLA*) class II expression (20). Studies on B cell lymphoma noted a direct association between a decrease in tumor immune-surveillance and loss of MHC class II gene expression (23). The significance of chromosomal amplifications was appreciated in a study evaluating primary mediastinal large B cell lymphoma and nodular sclerosing Hodgkin lymphoma (NSHL) cell lines. It was observed that 9p24.1 amplification both directly and indirectly increased *PD-L1* expression, through gene copy number gain and by amplification of janus kinase 2 (*JAK2*), respectively (8,9). *PD-L1* overexpression has also been noted in Epstein Barr virus (EBV) positive HL without 9p24.1 amplification, thus suggesting an alternative pathway for *PD-L1* overexpression in a subset of cases (8,21).

In addition to chromosomal aberrations, innate mechanisms have also been implicated in influencing *PD-L1* expression in tumor cells (*Table 1*). According to the adaptive immune resistance theory, tumor cells adapt to the endogenous anti-tumor immune response, specifically the IFNγ cytokine, with increased PD-L1 expression (10,11). A study evaluating melanoma cells noted an association

Table 1 Molecular mechanisms for *PD-L1* overexpression

Primary mechanism	Description	Associated tumor histologies	Notes	Reference
Interferon mediated	Interferons mediate an increase in *PD-L1* expression in both normal and tumor cells, that in-turn inhibits the activity of PD-1 positive T cells	Melanoma	IFNγ primarily implicated	(10,11,18,19)
Gene fusions	MHC class II trans-activator *CIITA* gene fusions lead to *PD-L1* and *PD-L2* overexpression, along with decreased surface HLA class II expression	HL	–	(20)
Gene amplifications	Chromosome 9p24.1 amplification directly increases *PD-L1* gene expression and also enhances *PD-L1* transcription through *JAK2* amplification	Primary mediastinal large B cell lymphoma and NSHL	–	(8,9)
3'-UTR disruption	Viral genome integrates into the *PD-L1* locus, causing 3'-UTR sequence disruption which leads to increased gene expression and transcript stability	HPV 16: cervical squamous cell carcinoma and HNSC; EBV: HL, stomach adenocarcinoma	No data on the HTLV-1 induced genomic alterations leading to ATL	(8,21,22)

PD-1, programmed cell death-1; *PD-L1*, programmed cell death ligand-1; IFNγ, interferon-γ; MHC, major histocompatibility complex; HLA, human leucocyte antigen; HL, Hodgkin's lymphoma; NSHL, nodular sclerosing Hodgkin lymphoma; *JAK2*, janus kinase 2; HPV, human papilloma virus; HNSC, head and neck squamous cell carcinoma; EBV, Epstein Barr virus; ATL, adult T cell leukemia/lymphoma.

between intra-tumoral levels of tumor infiltration lymphocytes (TILs) and IFNγ with PD-L1 on tumor cells. The presence of a negative feedback mechanism has been speculated that downregulates PD1 positive T cells through IFNγ mediated an increase in PD-L1 expression (18). Our knowledge of various pathways that mediate immune escape of tumor cells has provided a foundation in the formulation of treatment strategies for various malignancies.

Understanding the biology that influence cancer immune evasion has been pivotal in the identification of PD-L1 as a target molecule for immunotherapy (16). It has also led to the development of diagnostic tests predictive of treatment outcomes with specific immunotherapy agents, such as PD-L1 IHC for pembrolizumab therapy in NSCLC (16,24). As we continue to elucidate additional mechanisms of cancer immune escape, it may be possible to accurately assess cancer immune biology on an individual basis that would in turn allow for a personalized anti-cancer immuno-oncology treatment regimen.

New advances

Recently, Kataoka and colleagues described a novel mechanism of cancer immune escape (*Table 1*). They utilized adult T cell leukemia/lymphoma (ATL) samples to study genomic structural variations that disrupted the 3' region of *PD-L1* using a new platform (Genomon-SV) (22). These structural variations caused a significant increase in PD-L1 transcripts which were stabilized by 3'-untranslated region (3'-UTR) truncation. Further, *in vivo* experiments on murine models functionally demonstrated that PD-L1 3'-UTR disruption EG7-OVA tumor growth with increased *PD-L1* expression and decreased CD8+ TILs. Thus, it was reckoned that specific structural variations, specifically those involved in disrupting the 3'-UTR of *PD-L1*, could potentially mediate clonal selection of tumor cell populations through immune escape and serve as genetic markers for tumors to target with checkpoint inhibitors.

This study made a number of noteworthy observations. Genome-wide mapping for breakpoints associated with structural variations identified several recurrent breakpoint clusters. Many of these significant breakpoints were located in a 3.1 kilobase segment at the 3' region of the *PD-L1* locus in chromosome 9p24.1. Although several types of structural variations were noted, all of them resulted in an anomalous *PD-L1* allele. However, the aberrant PD-L1 proteins generated from these abnormal transcripts exhibited functional transmembrane and extracellular receptor binding domains. The cases with these structural variations also displayed significantly increased *PD-L1* expression and protein levels. Interestingly, these structural variations had

no effect on *PD-L2* expression. The above findings suggest that ATL cell populations with these structural variations were clonally selected through immune escape.

Encouraged by the evidence gathered in ATL, Kataoka and colleagues investigated if immune escape mediated clonal selection occurred in other tumor types (22). A total of 10,210 specimens from 33 tumor panels [RNA sequence data retrieved from The Cancer Genome Atlas (TCGA)] were evaluated for structural variations that involved the *PD-L1* 3'-UTR. Thirty-one samples from 13 types of tumors (including ATL) were reported to be positive for *PD-L1* 3'-UTR disruption and demonstrated overexpression of anomalous PD-L1 transcripts. This disruption significantly correlated with increased PD-L1 expression independent of *PD-L1* gene copy number. Unlike *PD-L1*, the expression of *JAK2* (also located on chromosome 9p24.1) showed no significant changes secondary to these structural variations. In order to assess the anti-tumor response, the samples positive for structural variation were further evaluated for cytolytic activity score, a marker of anti-tumor immune activity and cytotoxic T-cell infiltration (25). Cytolytic activity was revealed to have a prominent association with PD-L1 expression for every tumor type, and was similarly found to be elevated in cases with structural variations. Additionally, a comparison of samples with similar PD-L1 expression revealed that those positive for these structural variations had a considerably lower cytolytic activity, thereby indicating diminution of anti-tumor immunity. These findings further supported the primary hypothesis that tumor cells positive for specific structural variations undergo clonal selection attributable to PD-L1 overexpression and the resulting immune evasion.

This study has generated intriguing data on the effects of viral infection on PD-L1 expression. The authors reported on three TCGA cases in which integration of the viral genome resulted in aberrant PD-L1 transcripts. The viral DNA were identified to be from human papilloma virus (HPV) 16 in cervical squamous cell carcinoma and head and neck squamous cell carcinoma (HNSC), and EBV in stomach adenocarcinoma. In view of the above, it was argued that PD-L1 overexpression in these cases may have stemmed from the loss of 3'-UTR genome sequence. To validate this hypothesis, they utilized the clustered regulatory interspaced short palindromic repeats (CRISPR)-Cas9 system to introduce deletions and inversions in the *PD-L1* 3'-UTR sequence in several murine and human cell lines. As expected, these cells displayed increased PD-L1 levels. Additionally, these 3'-UTR truncated cells displayed delayed clearance of *PD-L1* mRNA (after inhibiting *de novo* transcription with actinomycin D), suggesting that the *PD-L1* 3'-UTR may have a negative regulatory effect on the stability of mRNA. Further, it was observed that *PD-L1* 3'-UTR truncation had a stronger impact on PD-L1 overexpression than IFNγ, yet the combination of stimulating 3'-UTR-disrupted cells with IFNγ still resulted in a synergistic increase in *PD-L1* expression.

The study assessed the outcomes of disrupting the *PD-L1* 3'-UTR sequence. Co-culturing PC-9 cells (human lung cancer cells with or without *PD-L1* 3'-UTR disruption) with Jurkat cells (PD-1 positive T cells) resulted in a significantly higher degree of T cell apoptosis when the cancer cells contained the 3'-UTR disruption. A tumor regression model (EGA-OVA cells with/without disrupted *PD-L1* 3'-UTR disruption inoculated in syngeneic C57BL/6 mice) was adopted to evaluate the effects of this structural variation on the anti-tumor immune response. Treatment with immunostimulatory RNA polyinosinic-polycytidylic acid [poly(I:C)] in mice with EGA-OVA cells with the disrupted *PD-L1* 3'-UTR sequence resulted in a weak CD8+ T cells response and, consequently, minimal tumor regression. On the other hand, mice with EGA-OVA cells bearing the intact *PD-L1* 3'-UTR sequence exhibited tumor regression with a high degree of CD8+ TILs when subjected to poly(I:C). These results demonstrate that increased activation of PD-1/PD-L1 pathway secondary to *PD-L1* 3'-UTR disruption can mediate immune escape of tumor cells from CD8+ cytotoxic T cells.

Discussion

Kataoka and colleagues have elaborated upon several significant hypotheses in this paper. Their study noted a direct association between PD-L1 overexpression and viral infections, wherein the latter was hypothesized to cause a loss of 3'-UTR sequence. Of note, the validation of this particular hypothesis was achieved using the CRISPR-Cas9 system. Additional studies are warranted in many other histologies where this novel mechanism may be relevant as well, especially in virally driven cancers. In this study, the authors have not shown a direct link between HTLV-1 infection and disruption of the 3'-UTR sequence in ATL. A study demonstrating whether or not HTLV-1 infection can cause *PD-L1* 3'-UTR sequence disruption in ATL can be considered.

The study also investigated the possibility of clonal selection of *PD-L1* overexpressing tumor cells in presence

of anti-tumor immunity. *In vivo* murine experiments demonstrated that disruption of the *PD-L1* 3'-UTR sequence induced immune escape from CD8+ cytotoxic T cells, thus highlighting the significance of certain structural variations in mediating clonal selection of tumor cells. Considering the above, it may be prudent to further investigate the disruption of the *PD-L1* 3'-UTR as a potential marker for tumors that exhibit greater clinical response to anti-PD-1/PD-L1 blocking therapies. Building upon our current knowledge, we believe that the observations made in this study will contribute towards realization of the full potential of PD-L1 targeted therapy agents in the treatment of cancer.

In summary, the study identified another novel mechanism for immune evasion by cancer cell via PD-L1 overexpression relevant in various cancer types. Furthermore, *PD-L1* 3'-UTR disruption that invariably leads to PD-L1 overexpression across different tumor types can be developed as a robust biomarker not only to identify tumors that utilize PD-L1 as a main mechanism for immune evasion but also to better predict outcomes to any T cell medicated immuno-oncology treatments including immune modulatory treatments and genetically engineered T cell therapy.

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Footnote

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