Letter to the Editor

Epigenetic regulation of PD-L1 expression and pancreatic cancer response to checkpoint immunotherapy

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Checkpoint blockade cancer immunotherapy, including anti-PD-1 and anti-PD-L1 antibody immunotherapy, has shown durable efficacy in many types of human cancers. However, pancreatic cancer is one of the few cancers that do not respond to anti-PD-1/PD-L1 immunotherapy (1). It has been shown that targeted therapy can increase the efficacy of checkpoint immunotherapy against pancreatic cancer (2,3), but the mechanisms underlying this non-response of pancreatic cancer is still unknown. In a recent study, we showed that PD-L1 is uniformly and abundantly expressed on the surface of 9 of the 10 human pancreatic cancer cell lines obtained from American Type Culture Collection (Manassas, VA, USA). Using a FDA-approved PD-L1-specific antibody, we also detected abundant membrane and cytoplasmic PD-L1 expression in human pancreatic cancer specimens. Furthermore, pancreatic tumor cells from tumor-bearing mice exhibited significantly higher PD-L1 on mRNA and protein levels than that *in vitro* cultured tumor cell lines. These observations suggest that the non-responsiveness of pancreatic cancer to anti-PD-1/PD-L1 immunotherapy is unlikely due to lack of PD-L1 expression in the tumor cells.

As pointed out by Minassian *et al.*, PD-L1 protein was detected in tumor cells of about 5% cases and in tumor-infiltrating immune cells of about 15% cases in human pancreatic cancer specimens (4). Other studies have detected PD-L1 protein in 43–59% human pancreatic cancer cases (5,6). Although therapies and tumor stages may affect PD-L1 expression level, the sensitivity of the antibodies used in the different studies might also be a contributing factor. In addition, our observation that the same tumor cell lines express much higher PD-L1 *in vivo* than *in vitro* suggest that the pancreatic tumor microenvironment might also dictate the expression level of PD-L1 (7,8). It has been shown that only PD-L1-positive tumors respond to anti-PD-1 immunotherapy and FDA has approved Keytruda for treatment of patients with metastatic non-small cell lung cancer (NSCLC) that highly expresses PD-L1 (9). However, the value of PD-L1 expression level as a predictor of cancer cell response to anti-PD-1/PD-L1 immunotherapy is apparently cancer type-dependent since PD-L1-negative tumor also respond to anti-PD-1 immunotherapy (10). Because pancreatic cancer generally does not respond to anti-PD-1/PD-L1 immunotherapy, the value of PD-L1 expression level as a predictor of cancer response to anti-PD-1/PD-L1 immunotherapy is currently not clear.

The physiological function of PD-L1 is to engage its receptor PD-1 on T cells to transmit a co-inhibitory signal to maintain peripheral tolerance. Under pathological conditions, tumor cells exploit this inhibitory pathway for its advantage to inhibit T cell activation to evade immune eradication. From this aspect, higher PD-L1 expression level should be functionally associated with stronger immune suppression and tumor promotion. Indeed, PD-L1-positive status was prognostic indicator of poor disease-specific survival (5) and was also significantly associated with poor tumor differentiation and advanced tumor stages (6) in pancreatic cancer patients. In the tumor microenvironment, in addition to the tumor cells, PD-L1 is also abundantly expressed in myeloid-derived suppressive cells

(MDSCs) (11). Therefore, targeting PD-L1 expression by pharmacological means should repress PD-L1 expression not only in tumor cells but also in MDSCs in the tumor microenvironment, which theoretically should not only decrease the anti-PD-1/PD-L1 antibody dose requirement during immunotherapy but also reduce MDSC-induced immune suppression, and thereby enhancing tumor-specific CTL function to suppress tumor progression. A recent study has shown that CSN5 inhibits the ubiquitination and degradation of PD-L1 to maintain PD-L1 expression level in tumor cells. Inhibition of CSN5 by curcumin diminished cancer cell PD-L1 expression level and sensitized cancer cells to anti-CTLA-4 therapy (12). This observation suggests that targeting PD-L1 expression level in tumor cells is an effective approach to overcome cancer cell resistance to checkpoint blockade immunotherapy. Our observation that verticillin A inhibits the MLL1-H3K4me3 axis to repress PD-L1 expression to increase pancreatic cancer response to anti-PD-1/PD-L1 immunotherapy is in line with this concept and finding.

As we discussed and as well as pointed out by Minassian *et al.*, verticillin A also inhibits other histone methyltransferases such as SUV39H1 and SUV39H2 to decrease H3K9me3 level in the tumor cells. We have shown in a previous study that H3K9me3 is a key repressor of Fas expression in colon cancer cells, especially in metastatic colon cancer cells (13). Therefore, verticillin A might have off-target effect. Verticillin A may repress H3K9me3 to increase Fas expression in tumor cells to increase tumor sensitivity to $FasL^+$ CTLs.

Although it has been shown that the death receptor Fas also mediate non-apoptotic signals to promote tumor cell survival (14), the best known function of the Fas-FasL pathway is induction of apoptosis under physiological conditions. It is common that most cancer cells are resistant to Fas-mediated apoptosis, likely due to decreased Fas receptor expression level and/or deregulation of the apoptosis signaling pathway downstream of the Fas receptor. It has been shown that membrane-bound FasL induces apoptosis whereas soluble FasL promotes tumor cell survival (15). Therefore, the recombinant FasL protein used in the apoptosis assay is critical. The physiological FasL on T-cell surface are trimers and we have reproducibly shown that trimerized FasL (MegaFasL or APO010) always induce apoptosis and does not exhibit tumor promotion activity *in vitro* (13). Therefore, the apoptosis assay system is critical for determining whether Fas mediates an apoptosis or survival signal.

FasL-mediated cytotoxicity is one of the two major effector mechanisms that CTL use to kill target cells. Under pathological conditions, such as cancer, FasL is essential for host cancer immunosurveillance since a simple loss of FasL expression or function (i.e., in $Fasl^{gld}$ mouse) without any other genetic alterations leads to increased tumor incidence *in vivo* (7,13,15-17). The expression and function of Fas in pancreatic cancer are variable from study to study. In one study, it was observed that pancreatic cancers exhibits increased Fas on mRNA levels as compared to normal tissues (18). However, it was reported that Fas protein was high in the normal pancreatic ducts and in the intraductal dysplasia. Whereas in all cases of invasive ductal-type adenocarcinoma, membranous Fas could not be detected and cytoplasmic Fas protein level was reduced or completely lost (19). Furthermore, loss of Fas expression in pancreatic adenocarcinoma is significantly correlated with poorer differentiation, increased invasion and a shorter overall survival (19). Although more studies are needed to determine the expression profiles and function of Fas in pancreatic cancer, the above observation that Fas is silenced in invasive pancreatic cancer suggest that the Fas-FasL pathway might play a key role in host cancer immunosurveillance against pancreatic cancer progression and pancreatic cancer cells use silencing Fas expression as a mechanism to evade the host anti-tumor immune response to advance the disease. Therefore, the "off-target" effect of verticillin A to target H3K9me3 level to increase Fas expression might contribute to the increased efficacy of anti-PD-1/PD-L1 immunotherapy in our study. The relative and potentially contrasting roles of H3K4me3-PD-L1 axis and the H3K9me3-Fas axis in tumor-reactive CTL function and anti-PD-1/PD-L1 immunotherapy efficacy require further studies. Nevertheless, as Minassian *et al.* discussed, new approaches of combining epigenetic therapies and immunotherapies are being tested in multiple human clinical trials.

In summary, our study supports new approaches of combined epigenetic and immune checkpoint blockade immunotherapy. Particularly, it will be interesting to see whether specific histone methyltransferase small molecule inhibitors can effectively down-regulate PD-L1 to increase the efficacy of anti-PD-1/PD-L1 immunotherapy in pancreatic cancer. Conversely whether an H3K9me3-specific inhibitor can effectively re-activate Fas expression in invasive pancreatic cancer to sensitize the tumor cells to $FasL^+$ tumor-infiltrating CTLs remains to be determined.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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