Editorial

Commentary on "The MLL1-H3K4me3 axis-mediated PD-L1 expression and pancreatic cancer immune evasion"

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Pancreatic cancer is a lethal disease, with most patients presenting at late stages. This leads to a mortality rate that is very close to the incidence rate (1). The current 5-year survival rate is 8% and the American Cancer Society estimates that 40,000 Americans will die from the disease in 2017. Current treatments, including chemotherapy, and radiation therapy, are insufficient and efforts have been turned to newer options such as immune checkpoint blockade therapy.

Immune checkpoints are critical for self-tolerance and to minimize collateral tissue damage from immune responses. An important immune checkpoint in cancer is programmed death-ligand 1 (PD-L1), and its inhibitory receptor PD-1 found on T cells. PD-L1 is often upregulated in tumor cells, resulting in significant immune suppression and increased survival. For this reason, blocking the interaction between PD-L1 and PD-1 can induce tumor suppression in cancer patients, and multiple PD-L1 and PD-1 antibodies are approved for therapeutic use (2). While antibodies targeting PD-1/PD-L1 have been successful in many cancers including advanced melanoma and non-small cell lung cancer (3,4), pancreatic cancer is one of few cancers that have not responded to these therapies (5). The mechanistic basis for this resistance to PD-1/PD-L1 antibodies is unknown, and understanding this process is critical if anti-PD-1/PD-L1 immunotherapy is to succeed in the treatment of pancreatic cancer.

Many studies have suggested tumor PD-L1 levels play a role in the efficacy of blockade therapy. A recent study by Lu *et al*. aimed to elucidate whether an epigenetic mechanism was involved in PD-L1 expression in cancer cells and whether targeting this mechanism would help enhance PD-L1/PD-1 blockade therapy (6). There have been reports that expression of PD-L1 on pancreatic tumor cells is relatively low (7). In this study, the authors show that while mouse pancreatic cancer cell lines do express low levels of PD-L1 *in vitro*, the expression is significantly higher in the orthotopic tumor cells *in vivo*. Human pancreatic carcinoma patient specimens also showed abundant PD-L1 expression. As a further confirmation, flow cytometry of ten human pancreatic cancer cell lines showed high PD-L1 expression in nine of the ten cell lines.

When assessing for epigenetic regulation of PD-L1 expression, Lu *et al*. show an enrichment in H3K4me3 levels in the *cd274* promoter region in mouse pancreatic cancer cell lines when compared to normal pancreatic cells. They attribute this methylation to MLL1, a specific H3K4 histone methyltransferase that was first identified in leukemia patients (8). Expression of MLL1 is elevated in both murine tumor cell lines and human pancreatic carcinoma patient specimens. The authors also show direct association of the methyltransferase with the *cd274* promoter both *in vitro* and *in vivo* through chromatin immunoprecipitation analysis. Blocking MLL1 activity with two histone methyltransferase inhibitors, verticillin A and chaetocin, decreased H3K4me3 levels, and accordingly,

PD-L1 mRNA and protein expression in human and mouse pancreatic cancer cell lines, as well as in orthotopic tumors. Interestingly, another study showed that MLL1 associates with the transcription factor NF-κB and regulates transcription of NF-κB dependent genes (9). PD-L1 is known to be regulated by NF-κB (10) and it would be interesting to see if this association is occurring at the PD-L1 transcription start site.

The authors suggest that verticillin A increases the efficacy of immunotherapy by reducing PD-L1 expression and thus the threshold of anti-PD-L1 antibody needed for an effective response. Mice treated with a combination of verticillin A and an anti PD-L1/PD-1 monoclonal antibody had reduced tumor growth, decreased cancer cell proliferation and increased apoptosis when compared to each treatment on its own. This contradicts studies suggesting that low PD-L1 expression has been correlated with lower efficacy of anti PD-1/PD-L1 therapy. However, it is important to note that this is not consistent for all cancer types (11,12). An important consideration is that verticillin A inhibits other histone methyltransferases and may have other off-target effects. Moreover, the study could not demonstrate a synergistic effect of verticillin A and anti-PD-L1 therapy on tumor growth. Therefore, it is possible that some of the observed effects of verticillin A are independent of decreased PD-L1 expression due to inhibition of histone H3 methylation.

Verticillin A also plays a role in Fas expression in colon cancer cells by regulating H3K9me3. Fas is an apoptosis pathway that is known to play a role in immune surveillance, and expression of Fas has been associated with positive prognosis and improved disease-free survival in pancreatic cancer (13). The authors found that cancer cells in syngeneic orthotopic tumors had high expression of Fas and that FasL was expressed on tumor infiltrating lymphocytes (TILs). This is consistent with other findings showing that Fas and FasL have increased expression levels in pancreatic cancer (14,15). When pancreatic cancer cell lines are transplanted into FasL knockout mice, tumor growth is faster than in wild-type controls. This attributes Fas/FasL as a key suppressor of pancreatic cancer development.

To determine whether the effects of verticillin A and anti-PD-L1 on tumor growth were dependent on FasL, the authors treated FasL deficient mice harboring pancreatic tumors with verticillin A and anti-PD-L1 antibody. As expected, tumor growth was suppressed more effectively in the wild-type mice, compared to the FasL deficient mice. To further test whether these effects were mediated by cytotoxic T lymphocytes (CTLs), tumor-bearing mice were treated with a CD8 neutralizing antibody, with or without verticillin A and anti-PD-1 combination therapy, as well as just the combination therapy. They found that depletion of CD8+ T cells reduced the efficacy of the therapies and increased tumor growth. Interestingly, verticillin A increased CTL infiltration and activation induced by anti-PD-L1 therapy.

Lu *et al*. show that pancreatic tumor growth and the efficacy of anti-PD-L1 immunotherapy is dependent on FasL and CTLs. However, although Fas/FasL is a mechanism of immune surveillance, most cancer cells are resistant to Fas-mediated apoptosis (16). It has widely been shown that once cancer cells acquire resistance to Fas-mediated apoptosis, increased expression of Fas is tumorigenic, resulting in higher invasive potential and tumor cell survival (17,18). This suggests that the role of Fas and FasL expression on the efficacy of anti-PD-1/PD-L1 therapy may be dependent on the stage of pancreatic tumor progression, and the resulting resistance to apoptosis.

The study by Lu *et al*. supports new approaches of combining epigenetic therapies and immunotherapies in cancer treatment. There are currently at least seven clinical trials testing this combination therapy (19) and it will be interesting to see how these treatments develop in the future.

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Footnote

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