Single Cell Biology
ISSN: 2168-9431

Yang, et al., Single Cell Biol 2016, 5:3
DOI: 10.4172/2168-9431.1000146

Commentary Open Access

Interferon-Related Secretome Plays a Vital Role in PD-L1 Expression in the Tumor Microenvironment after Direct Interaction between Immune Cells and Tumor Cells

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Rec date: Jun 27, 2016; **Acc date:** Jul 16, 2016; **Pub date:** Jul 18, 2016

Commentary

PD-L1, also known as CD274 or B7-H1, is a major immune checkpoint protein [1]. The binding of PD-L1 to its receptor PD-1 induces an inhibitory signal that suppresses the immune response by several mechanisms such as inhibiting the proliferation of CD8+ T effector cells [2]. This research has resulted in the development of promising clinical drugs such as anti-PD-1 antibodies including Keytruda (pembrolizumab, Merck), and Opdivo (nivolumab, BMS) as well as the anti-PD-L1 antibody Tecentriq (atezolizumab, Roche), and other peptides and small molecule inhibitors for cancer immunotherapy [3]. However, the mechanism by which tumor cells expressing PD-L1 regulate the function of infiltrating immune cells within the tumor microenvironment is still not well understood.

In our recent work, we studied the interactions between tumor cells and immune cells in three different co-culture models (Figure 1) [4] that aim to mimic the process of cell-cell communication in the tumor microenvironment. The expression of PD-L1 by tumor cells was found to be significantly elevated by their direct co-culture with immune cells. Then, a transwell co-culture system was used to determine whether PD-L1 expression is dependent on the direct interaction of immune cells and tumor cells.

When splenocytes separated from the tumor cells by a 1 μm transwell membrane, they did not modulate PD-L1 expression by B16F10 tumor cells. However, a direct co-culture of splenocytes and B16F10 tumor cells was able to up regulate PD-L1 expression by tumor cells separated from the co-culture by a transwell membrane. These results revealed that direct interaction of tumor cells with immune cells is necessary for the up regulation of PD-L1 expression in tumor cells. This also demonstrated that secreted factors that promote tumor cell PD-L1 expression were produced in response to direct cell-cell interaction. These secreted molecules affect the tumor cells interacting with immune cells as well as neighboring tumor cells that are not interacting with immune cells. To investigate this further, we obtained the supernatant from mono-cultured B16F10 cells as well as B16F10 cells co-cultured with immune cells and added these two supernatants to B16F10 cell mono-cultures. We found that only the supernatant from the co-cultured cells induced up regulation of PD-L1 expression in tumor cells.

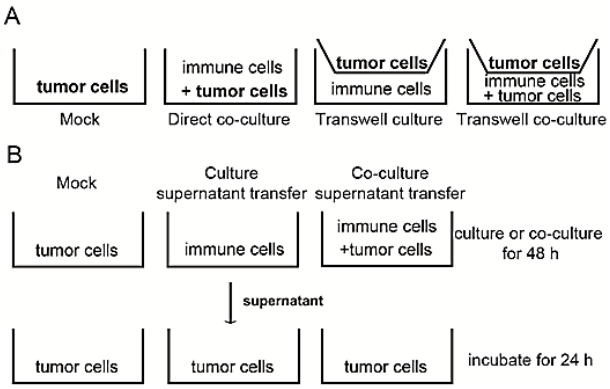

Figure 1: Three different *in vitro* co-cultured models for interactions between immune cells and tumor cells. (A) Models of the different co-cultured system for tumor cells and immune cells. Mono-cultured tumor cells (Mock) or Tumor cells co-cultured with immune cells (Direct co-culture). Brief description for the transwell co-culture system, tumor cells in upper insert and corresponding immune cells (Transwell culture) or mixture of immune and tumor cells (Transwell co-culture) in the lower plate, they were separated by transwell membrane, respectively. (B) Models of the different treatments on tumor cells by tumor cells supernatant (Mock), immune cells supernatant (Culture supernatant transfer) or tumor cells and immune cells co-cultured supernatant (Co-culture supernatant transfer).

In addition, it was observed that IFN-α and IFN-β levels significantly increased in the supernatants from these co-culture systems. Consistent with this, the levels of IRF7 and ISG15 in the tumor cells were significantly increased after treatment with the co-culture supernatant. This data suggested that type I interferon signaling might be involved in positive regulation of PD-L1 expression on tumor cell surface. Experiments utilizing a type I interferon receptor 1 neutralizing antibody as well as the Ifnar2-/- cell line U5A confirmed that PD-L1 expression in tumor cells is positively regulated by type I interferon.

We noted that while antibody-mediated neutralization of IFNAR1 fully blocks up regulation of PD-L1 in our co-culture model, it only partially inhibits the up regulation of PD-L1 in splenocytes and lymph

node cells. This suggested that other secreted factors also contribute to PD-L1 up regulation. Analysis of the components of the co-culture supernatant by both cytokine/chemokine microarray and ELISA revealed high levels of type I IFNs (IFN-α, β), type II IFN (IFN-γ) and IL-6, which have previously been shown to promote PD-L1 expression. We also discovered high expression of leptin, G-CSF, MCP-5, MIG, and MIP-1a. Further studies will determine whether these molecules are directly or indirectly involved in the expression of PD-L1. In particular, leptin [**5**,**6**] is a protein hormone usually secreted by fat cells, and it will be instructive to determine which cell types present in the tumor microenvironment secreted it and whether leptin regulates PD-L1 expression in tumor cells. The above findings can be summarized in a working model as shown in Figure 2 [**4**].

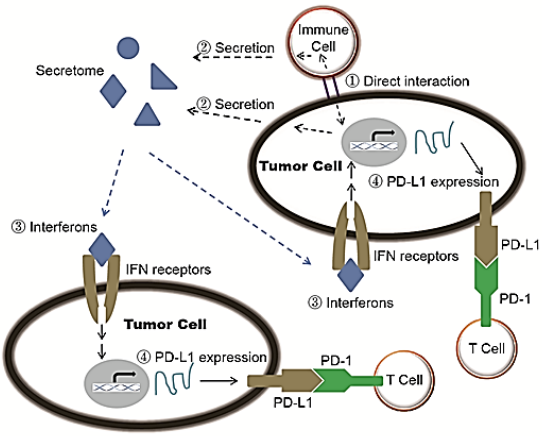

Figure 2: Schematic model for PD-L1 up regulation of tumor cells in tumor microenvironment. Briefly, tumor cells directly interacted with infiltrating immune cells, then some active factors including interferons and other several cytokines or novel protein molecules were secreted in the tumor microenvironment. Further the secreted factors such as IFNs positively regulate PD-L1 via their corresponding receptors in tumor cells or neighboring tumor cells. Finally, unregulated PD-L1 protein exerts its immunosuppressive effects by inhibiting the proliferation of targeting T cells.

Monoclonal antibodies targeting PD-1/PD-L1 signaling are a promising new immunotherapeutic. Our studies suggest that blockade of cytokines that drive PD-L1 expression can effectively reduce the expression of PD-L1 by tumor cells. Conversely, our findings can also be applied to cancer immunotherapies involving the design and administration of interferon mutants to augment the anti-tumor response [**7**]. The ability of Type I IFN to up regulate the expression of PD-L1 might limit the efficacy of these therapies. Administration of neutralizing anti-PD-1 antibody during IFN-based therapies may therefore amplify the efficacy of these therapies due to the ability of anti-PD-1 antibody to block the activity of PD-L1 in the tumor microenvironment.

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