Glutamine dependent regulation of PD-L1 expression in macrophages and non-small cell lung cancer

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Abstract

Immunosurveillance by cytotoxic T-cells is critical for prevention of tumor formation and growth. The PD-1/PD-L1 checkpoint is a mechanism by which T-cells differentiate between healthy and neoplastic cells. Cancer cells are capable of evading the immune system by bypassing this checkpoint. T-cells express programmed death protein 1 (PD-1) on their membrane in order to probe other cells for programmed death-ligand 1 (PD-L1). Cancer cells and macrophages express PD-L1 on their membrane to bind with the PD-1 receptor present on T-cells. The binding of PD-1 and PD-L1 inactivates the T-cell, and the cancer cell escapes apoptosis. PD-L1 expression is induced by cytokines such as interferon gamma (IFNγ) and interleukin 4 (IL-4), which are released by T-cells upon activation via antigen presenting cells. Understanding what conditions induce PD-L1 expression in cancer cells and macrophages is important because there are immunotherapies capable of targeting the PD-1 and PD-L1 interaction; specifically, human-derived antibodies can bind to PD-L1 to prevent PD-1 binding, which causes the activated T-cells to induce tumor apoptosis. In this study, we measure PD-L1 expression upon exposure to varying concentrations of glutamine. We hypothesize that low glutamine exposure will increase PD-L1 expression because tumors have poor vasculature which causes the core of these tumors to be glutamine-depleted. After treating cells for either 24 or 48 hours, PD-L1 protein expression was measured using Western Blots, and PD-L1 gene expression was determined using RT-PCR.

Research Question

In this study, we measure PD-L1 expression in macrophages and non-small cell lung cancer cell lines treated with varying concentrations of glutamine. Glutamine has been previously shown to have epigenetic effects on tumor cells. Due to the poor vasculature of tumors, resulting in a glutamine-depleted core, we hypothesize that glutamine decreases PD-L1 expression in tumor cells and macrophages.

Background

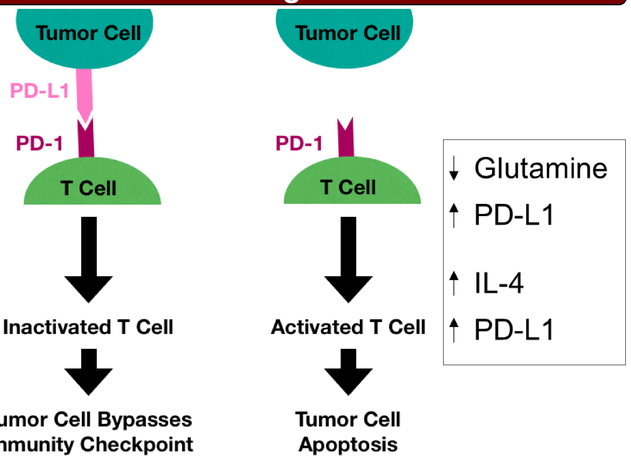
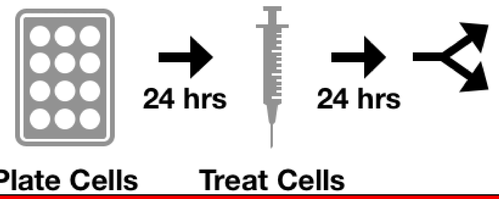


Figure 1. Immunosurveillance via the PD-1/ PD-L1 checkpoint and immune system evasion. T-cells express PD-1 on their membranes and probe tumor cells for PD-L1. If the tumor cell presents PD-L1, the T-cell is inactivated, and the tumor cell escapes apoptosis. When the tumor cell does not express PD-L1, the T-cell induces apoptosis. IL-4 increases the expression of PD-L1, and glutamine decreases the expression of PD-L1.

Methods



Results

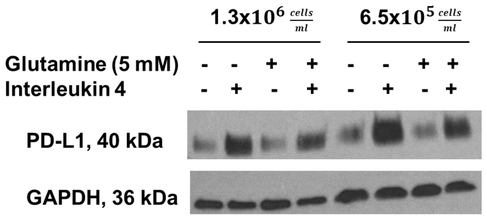


Figure 2. Expression of PD-L1 protein in bone marrow-derived macrophages. Macrophage colony-stimulating factor (M-CSF) was added to RPMI media to derive macrophages. These macrophages were plated at two different densities in a 12-well plate and treated for 48 hours. The data suggests that PD-L1 expression is increased with the addition of IL-4 and decreased with the addition of glutamine.

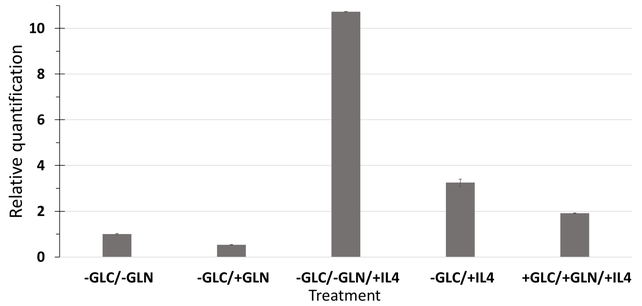


Figure 3. Gene expression of PD-L1 in bone marrow-derived macrophages. Macrophage colony-stimulating factor (M-CSF) was added to RPMI media to derive macrophages. These macrophages were plated at two different densities in a 12-well plate and treated for 48 hours. The significant finding of this data is that while (+) IL-4 and (-) glutamine increase PD-L1 expression individually, they also coordinately increase PD-L1 expression in macrophages.

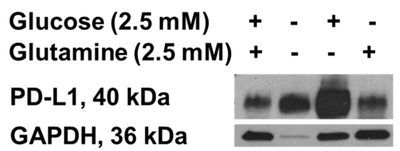


Figure 4. The expression of PD-L1 protein in A549 non-small cell lung cancer cells. Cells were plated in complete DMEM media in 12-well plate and treated 24 hours later for 24 hours. This data supports the assertion that the addition of glutamine reduces the expression of PD-L1. The GAPDH bands show that the cells treated with (-) glucose and (-) glutamine expressed a lot less protein, so assertions about the effect of glucose on PD-L1 expression were tested using RT-PCR.

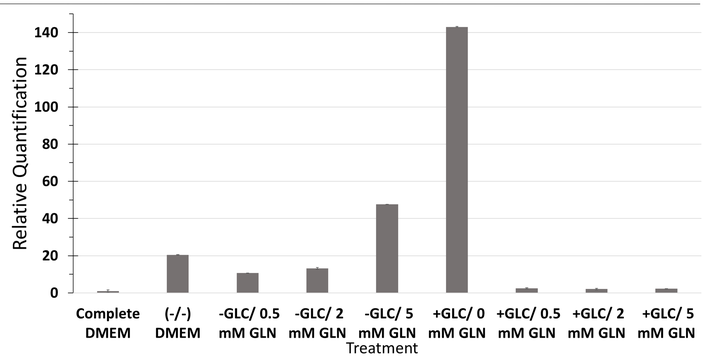


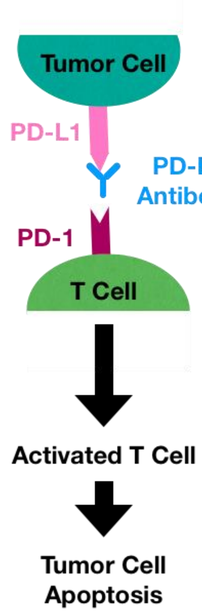
Figure 5. Gene expression of PD-L1 in A549 non-small cell lung cancer cells. Cells were plated in complete DMEM media in 12-well plate and treated 24 hours later for 24 hours. As seen in the macrophages, this data suggests that PD-L1 expression is decreased with the addition of glutamine. The addition of glucose to the media without glutamine significantly increases the expression of PD-L1.

***Data from figures 3 and 4 is supplementary– From Beatriz Rendon

Conclusion

- Treatments with no glutamine and IL-4 both increase PD-L1 expression independently of each other; however, they also display a coordinated effect on tumor and macrophage PD-L1 expression. That is, treatments with IL-4 and no glutamine have the highest PD-L1 expression.
- It is important to note that the effects of glutamine and IL-4 on PD-L1 expression occurs in both macrophages and tumor cells.

Significance/Clinical Application



Activation of anti-tumor T-cells promotes cancer cell apoptosis and limits tumor progression. Expression of PD-L1 on cancer cells allows the tumor to evade this immune response. As glutamine depletion increases PD-L1 expression, this may drive the evasion of anti-tumor immune responses and impact the survival of patients with cancer. However, tumor immunotherapies utilize the immune system to target and kill cancer cells by blocking the PD-1/PD-L1 pathway. The limitation of this therapeutic strategy is that it only works in a small percentage of cancer patients. As the efficacy of anti-PD-1/PD-L1 antibodies relies on the expression of these cell surface markers, pharmaceutical targeting of glutamine metabolism may enhance the efficacy of these immunotherapies.

Figure 6. Antibody suppression of immune system evasion by tumor cells. Human-derived PD-L1 antibodies can bind to PD-L1 to prevent PD-1 binding. The T-cell remains active and induces apoptosis of the tumor cell.

Future Work

- Determine how glucose effects PD-L1 expression in macrophages and cancer cells
- Test the effect of glutaminase inhibitors on the expression of PD-L1
- Determine if glutaminase inhibitors with PD-L1 antibodies increases the effectiveness of the immunotherapy
- Validate all findings in animal models

Acknowledgements

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