Effect of Anti-PD1 and Anti-LAG3 Checkpoint Inhibitors on T-cell Mediated Cancer Cell Killing

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Opportunity

Hypothesis: The use of anti-PD1 and anti-LAG3 checkpoint inhibitors will enhance the killing ability of T-lymphocytes exposed to them, resulting in a shorter time needed for killing and increased amount of cancer cells killed compared to control.

Introduction: Triple Negative Breast Cancer (TNBC) cancer does not exhibit any of the three biomarkers used to characterize breast cancer: the estrogen receptor (ER), progesterone receptor (PR), or Human Epidermal Receptor 2 (HER2). Currently, chemotherapy is the only option for treatment but recent research has been looking into using the patient’s own immune system to help fight the cancer with an approach called immunotherapy. One promising type of immunotherapy are called checkpoint inhibitors which are a class of immunotherapy drugs that are composed of different antibodies to block the interaction between T-cells and cancer cells. Many of these cancer cells are able to avoid being detected by T cells because their ability to present ligands which are similar to normal healthy cell ligands. This therapeutic approach will allow T cells to identify those cells that are able to evade identification and initiate the immune response. Programmed cell death protein 1 (PD1) is one such checkpoint receptor. PD1 ligands have been found on TNBC cells and its expression is associated with more pathological manifestations of the disease. Specifically, a blockade of both PD-L1 and PD-1 lead to promising results in TNBC.

Another checkpoint receptor, which has shown to provide synergistic activity when targeted with PD1, is Lymphocyte Aktivation-activation gene 3 (LAG-3). LAG-3 is present on immune cells, including natural killer cells, B cells, TILs, and T-cells. In healthy cells, the binding of ligands with LAG-3 receptors will downregulate the immune response. Studies have shown elevated levels of LAG-3 antigens or ligands in cancer cells, which result in chronic suppression of the T-cell response. Currently, the guidelines do not address checkpoint inhibitors. This proposed research will further determine the ability of checkpoint inhibitors to enhance TNBC cell killing in vitro, and provide a foundation for further research on these drug molecules in vivo.

Approach

This experiment involved observing the interactions of CD8+ T-cells and MD-MB-231 breast cancer cells within a microfluidic device after incubation with the checkpoint inhibitors anti-PD1 and anti-LAG3 or none as a control.

- The microfluidic device allowed us to observe single-cell interactions between the cancer and T-cells (see figure 1).
- The cells were imaged every 15 minutes for a total of 24 hours.
- Number of T-cells, number of cancer cells, time points of contact between cancer and T-cells, and time of cancer and T-cell death were recorded for analysis.

Data/Results

<table>
<thead>
<tr>
<th>% of Cells with Contact</th>
<th>anti-LAG3/PD1</th>
<th>Control</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95.83</td>
<td>78.18</td>
<td>p&lt;0.002*</td>
</tr>
<tr>
<td>Average % of Time in Contact</td>
<td>48.62</td>
<td>52.83</td>
<td>p=0.629**</td>
</tr>
</tbody>
</table>

Table 1: Differences in contact between anti-LAG3/PD1 cells and control. Where more anti-LAG3/PD1 incubated T-cells made significantly more contact, but did not have significantly longer average contact time. *Using chi square test **Using unpaired two tailed t-test

Impact/Conclusion

The use of anti-PD1 and anti-LAG3 checkpoint inhibitors were not associated with increased MD-MB-231 breast cancer cell killing as hypothesized. A higher percentage of cancer cell death was found in the control group. However, incubation with anti-PD1 and anti-LAG3 was found to significantly increase the number of cells in contact. Additionally, MD-MB-231 breast cancer cells showed significantly greater percent of time spent in contact among dead cells compared to alive cells. We would assume that checkpoint inhibitors would enhance killing since it engages more MD-MB-231 breast cancer cells in contact. However, the study found no significant difference in the average proportion of time in contact among anti-PD1 and anti-LAG3 compared to control. Therefore, although checkpoint inhibitors engage more cells in contact, it does not necessarily increase cancer cell killing. It is possible that small differences in experimental set-up lead to increased cell killing in general in the control, but T-cell death rates were similar between the checkpoint inhibitor and cancer experiments (59.7% and 80.6%).

Areas for Future Research:

- Further studies are necessary to evaluate the effect of checkpoint inhibitors on T cell activity. This single study may not be representative of its effects due to limitations, such as study design.
- If MD-MB-231 breast cancer cell death is associated with contact time and checkpoint inhibitors can enhance the number of breast cancer cells involved with contact, then this may be a potential area for its use.