The Effects of 5-Azacytidine on HLA-ABC, HLA-E, HLA-G and HLA-A Expression

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INTRODUCTION

Epigenetic modifiers, like the DNA methylation inhibitor 5-Azacytidine (5-AzaC), have been shown to increase human leukocyte antigen (HLA) expression. The expression of HLA is required for a T-cell response to detect tumor cells. A lack of HLA expression allows tumor cells to escape immune detection. It has been previously shown that 5-AzaC is able to upregulate HLA-ABC expression in the cell line MDA-MB-435. However, other HLA proteins like HLA-E and HLA-G may be concurrently upregulated, which would have negative implications on tumor immunity. One flask of MDA-MB-435 cells was treated for 48 hours with 5-AzaC at 0.0025 mg/mL and another was left untreated. The cells were harvested and incubated with control antibodies or the experimental antibodies, Anti-HLA-ABC, Anti-HLA-E, and Anti-HLA-G, and analyzed via flow cytometry. Preliminary experiments testing for the expression of HLA-E and HLA-G have both demonstrated a slight increase in their expression compared to the untreated controls. Although minor, the increase in HLA-E and HLA-G expression may have negative implications on tumor survival and pathogenesis. Understanding individual HLA upregulation may prove to be beneficial and applicable to cancer immunotherapy, an expanding field of improved cancer treatments.

OBJECTIVE

Investigate the impact of 5-azaC on HLA-ABC, -A, -E, and -G expression in MDA-MB-435 Cells

RESULTS

Table 1. Flow Cytometry Fluorescence of HLA-ABC in MDA-MB-435 cells.

<table>
<thead>
<tr>
<th>HLA Type</th>
<th>Control Average (3 trials)</th>
<th>48-hour Incubation Average</th>
<th>Percent Difference Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA A</td>
<td>4.89</td>
<td>6.45</td>
<td>27.51%</td>
</tr>
<tr>
<td>HLA E</td>
<td>18.22</td>
<td>32.30</td>
<td>81.69%</td>
</tr>
<tr>
<td>HLA G</td>
<td>4.35</td>
<td>5.23</td>
<td>18.2%</td>
</tr>
</tbody>
</table>

DISCUSSION

5-AzaC Concentration

- Low doses of 5-AzaC promote cell survivability (Fig 2)
- Previous Results
  - On average, HLA Class I expression of transient absent cells increased by 1.57 fold, compared to the control cells (Fig 4)
  - HLA Class I expression in the continuously treated cell line also increased by a fold of 1.63 compared to the control cells (Fig 4)
- Results
  - HLA Expression of HLA-E displays clear increases, with an increase of 81.69% on average (Table 2)
  - Increases in HLA-E aid cancer cells in evading the human immune system
  - HLA-A and HLA-G both suggest an increase in expression, however, the values are so low no conclusive remarks can be made (Table 2)

CONCLUSION

- HLA expression remains stable following a one-week and two-week absence of 5-AzaC
- 5-AzaC may increase HLA-E, a negative impact on the human immune system response
- More research must be done to review the impact of 5-azaC on HLA-A and HLA-G

FUTURE STUDIES

- Further research into the long-term stability of HLA and the impact 5-azaC has on HLA-A and HLA-E

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REFERENCES


Fig 1. The mechanism of CpG Island Methylation.

Fig 2. 5-AzaC Fluorescence of HLA-ABC in MDA-MB-435 cells.

Fig 3. Week 2 Flow Cytometry Fluorescence of HLA-ABC in MDA-MB-435 cells.

Fig 4. Average increase in HLA-ABC in MDA-MB-435 cells treated with 5-AzaC. Clear increase between the Control and Experimental groups.