Abstract

Vorinostat is a histone deacetylase inhibitor with epigenetic effects. 5-Azacitidine is a methylation inhibitor. In previous research, it has been shown that both drugs can result in an increase in gene expression. The goal of our experiments was to see if Vorinostat and/or 5-Azacitidine could upregulate HLA expression without upregulating PD-L1 expression. While an increase in HLA expression would be beneficial for immune cells to kill cancer cells, an increase in PD-L1 expression would be detrimental because it prevents the activation of T cells, thereby allowing cancer cells to evade the immune response. To demonstrate the effects of Vorinostat and 5-Azacitidine on cancer cells, we conducted flow cytometry experiments on the MCF-7 and MDA-MB-231 cancer cell lines, two breast cancer cell lines. For Vorinostat, the initial results indicated that HLA expression for both cancer cell lines unexpectedly decreased while the PD-L1 expression remained unchanged. For 5-Azacitidine, MCF-7 cells showed an increase in HLA expression with no change in PD-L1 while MDA-MB-231 cells showed no change in HLA or PD-L1 expression. Experiments are planned to modify the concentration of the drug used and to test other epigenetic modifiers of gene expression.

Introduction

Hypothesis: 5-Azacitidine and Vorinostat will independently increase HLA-ABC expression and decrease PD-L1 expression in MDA-MB-231 and MCF-7 cells.

Materials and Methods

Cells grown in 25 cm² or 75 cm² flasks

48 hour treatment with 5-Azacitidine or Vorinostat

MDA-MB-231

MCF-7

Harrasted Cells

5.51 µM Vorinostat

5.51 µM Vorinostat

Harrested Cells

Results

Figure 3A and 3B show the cell survival curve for varying concentrations of Vorinostat in the MTT Assay. Higher drug concentrations resulted in a decrease in cell number.

MCF-7 Cell Line

MDA-MB-231 Cell Line

Figure 4 A, B, C, and D show levels of expression of HLA Class I antigens in the controls (purple) and the drugs (green) Vorinostat (A), and Aza (B, D). In MCF-7 cells treated with Vorinostat (A) and Aza (B), HLA Class I expression increased. In MDA-MB-231 cells treated with Vorinostat (C), HLA Class I expression decreased. In MDA-MB-231 cells treated with Aza (D), HLA Class I expression was unaffected.

Conclusions

- The 5-AzaC treatment for the MCF-7 cell line showed an increase in HLA Class I antigen expression while the PD-L1 expression remained the same. Treatment with Vorinostat showed a slight increase in HLA Class I expression and constant PD-L1 expression.
- The 5-Azac treatment for the MDA-MB-231 cell line showed no change for both HLA Class I and PD-L1 expression while treatment with Vorinostat showed a decrease in HLA Class I expression while PD-L1 expression remained the same.

Future Studies

- Test different concentrations of 5-AzaC and Vorinostat to determine the optimal dose for increasing HLA Class I antigen expression while decreasing or not increasing PD-L1 expression.
- Study if other histone deacetylase inhibitors, such as HDAC3, could decrease PD-L1 expression.

Acknowledgements

Thank you Dr. O’Donnell for guiding us and being an excellent mentor. Also, thank you to the others in Dr. O’Donnell’s lab for assisting us throughout the experiments and to the Biology Department for supporting our efforts.

References


Ritter, Cathrin et al. “Epigenetic priming restores the HLA class I antigen processing machinery expression in Merkel cell carcinoma.” Scientific Reports vol. 7,1 1230 (2017). doi:10.1038/s41598-017-02688-0