The Effect of a Histone Deacetylase Inhibitor on PD-L1, HLA-ABC, HLA-E, and HLA-G on Human Breast Cancer Cell Lines

Nikhil Reddy, Alec Toufexis, Dr. Robert O'Donnell
SUNY Geneseo, Geneseo NY 14454

Abstract

Increased expression of human leukocyte antigen (HLA) allows tumor cells to be more easily detected by the immune system. In previous research, epigenetic modifiers including the histone deacetylase inhibitor 3 (HDAC3), RGFP966, has been shown to decrease PD-L1 expression. PD-L1 expression inhibits T cell cytotoxicity, and decreasing it can enhance the immune response. We hope to elucidate whether or not HLA-ABC expression similarly decreases upon exposure to the HDAC3 inhibitor which would be detrimental to immune detection. Two breast cancer cell lines that express HLA-ABC are MCF-7 and MDA-MB-231. Our initial results show that HLA-ABC is expressed on the MDA-MB-231 and MCF-7 cell lines, while PD-L1 is only expressed by MDA-MB-231 cell line as demonstrated by flow cytometry. However, in subsequent experiments testing the effect of RGFP966, there were inconsistent results regarding the change in expression of HLA-ABC which requires further investigation. In addition, we also wish to examine other HLA proteins, such as HLA-E and HLA-G, which have been shown to be detrimental to immune detection. Future experiments are planned to optimize the doses of RGFP966 to maximize HLA expression in these breast cancer cell lines while limiting the expression of PD-L1, HLA-E and HLA-G.

Introduction

Hypothesis:RGFP966 and 5-Azacytidine will independently increase HLA-ABC expression and decrease PD-L1, HLA-G, and HLA-E expression in MDA-MB-231 and MCF-7 cells.

Figure 1: Impact of RGFP966 on Histone deacetylation

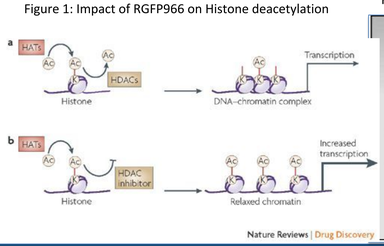
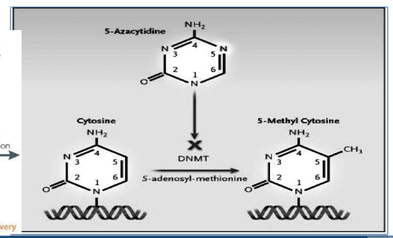
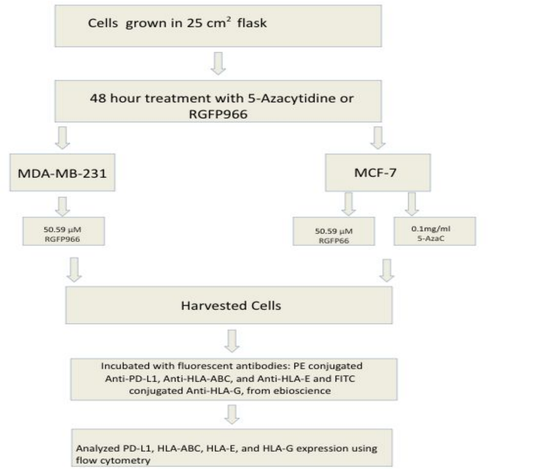


Figure 2: 5-Azacytidine inhibition of DNA methyltransferase



Materials and Methods



1A

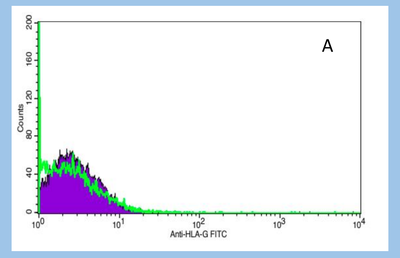
Mean Expression	Control HLA-ABC	HDAC HLA-ABC	Control HLA-ABC	5-Aza C HLA-ABC	Control PD-L1	HDAC PD-L1	Control PD-L1	5-Aza 5C PD-L1	Contro HLA-E	HDAC HLA-E	Control HLA-E	5-Aza C HLA-E	Control HLA-G	HDAC HLA-G
MCF-7 Trial 1	225.75	221.80	32.59	34.72	8.36	6.39	7.20	6.20	5.74	8.03	5.78	5.85	4.56	4.96
MCF-7 Trial 2	47.47	25.41	16.36	29.29	N/A	N/A	5.33	6.97	N/A	N/A	N/A	N/A	11.30	14.71
MCF-7 Trial 3	32.65	28.16	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

1B

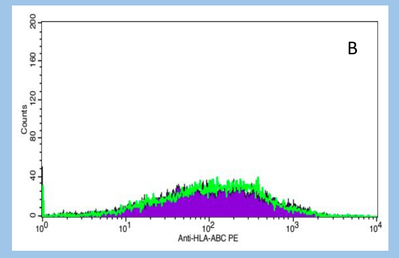
Mean Expression	Control HLA	HDAC HLA	Control HLA-G	HDAC HLA-G	Control HLA-E	HDAC HLA-E	Control PD-L1	HDAC PD-L1
231 Trial 1	100.49	262.18	6.11	7.83	N/A	N/A	N/A	N/A
231 Trial 2	204.26	203.22	N/A	N/A	N/A	N/A	36.61	33.44
231 Trial 3	57.91	55.23	8.63	5.18	N/A	N/A	N/A	N/A
231 Trial 4	116.24	131.56	N/A	N/A	17.11	16.59	45.84	62.44

Figure 1A (MCF-7) and 1B (MDA-MB-231) show the results for different control and drug conditions for HLA-ABC,HLA-E,HLA-G, and PD-L1

MCF-7 Cell Line

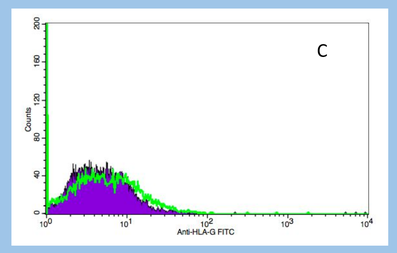


Anti-HLA-G

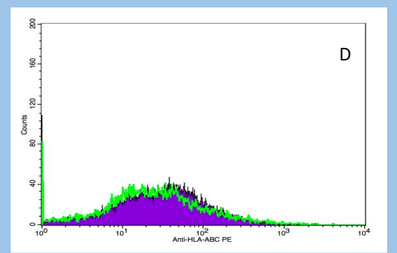


Anti-HLA-ABC

MDA-MB 231 Cell Line



Anti-HLA-G



Anti-HLA-ABC

Figure 4 A and C show levels of expression of HLA-G in the controls(purple) and the drug RGFP966(green), and B and D show HLA-ABC expression. In 231 cells treated with RGFP966 , HLA-G(C) and HLA-ABC (D) expression remained the same. In MCF-7 cells,HLA-G (A) and HLA-ABC(B) expression also remained the same after treatment with RGFP966.

Conclusions

- The RGFP966 treatment showed mixed results for the MDA-MB-231 cell line when it came to HLA-ABC expression. One trial showed an increase while the others showed no change at all.
- MDA-MB-231 cells showed little HLA-E or HLA-G expression, and PD-L1 expression remain unchanged after RGFP966 treatment
- The RGFP966 treatment had no significant effect on the HLA-ABC, HLA-E, HLA-G or PD-L1 expression in the MCF7 cell line.
- 5-Azacytidine had no significant effect on the expression of HLA-ABC or PD-L1 in the MCF-7 cell line

Future Studies

- Test different concentrations of 5-AzaC or RGFP966 to determine an optimal concentration for increasing HLA-ABC, while not increasing HLA-E or HLA-G
- Study other HDAC inhibitors, like the novel OK-179, which as shown inhibitory effects on growth of MDA-MB-231 cells

Works Cited (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.
- Finelli, Carlo, Martinelli Giovanni, Constanza Bosi, and Michelle Bacarani. "Azacytidine: First Therapeutic Opportunity for Myelodysplastic Syndromes." *Nephrology Reviews* 2.7 (2009): 57-59. doi: 10.4081/hmr.v2i7.415
- Kumar, S., & Sharawat, S. K. (2018). Epigenetic regulators of programmed death-ligand 1 expression in human cancers. *The Journal of Laboratory and Clinical Medicine*, *202*, 129–145. Retrieved from https://www.translationalres.com/article/S1931-5244(18)30094-X/fulltext
- Kim,KiBem et al. "Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells."*Proceedings of the National Academy of Sciences of the United States of America* vol. 111,32 (2014): 11774-9. doi:10.1073/pnas.141
- Ritter, Cathrin et. al. "Epigenetic priming restores the HLA class I antigen processing machinery expression in Merkel cell carcimona."*Scientific Reports* vol. 7,1 2290 (2017). doi:10.1038/s41598-017-02608-0
- OKI-179 is a novel, oral, class I specific histone deacetylase inhibitor in phase 1 clinical trials
- Jennifer R Diamond, Jodi A Kagihara, Xuedong Liu, Gilad Gordon, Amy M Heim, James Winkler, John A DeMattei, Anthony D Piscopio and S. Gail Eckhardt
- Mol Cancer Ther December 1 2019 (18) (12 Supplement) B007; **DOI:** 10.1158/1535-7163.TARG-19-B007