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

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ABSTRACT

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-G, and Anti-HLA-E 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 cells. 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.

INTRODUCTION

- In 2020, 100,350 melanomas are expected to be diagnosed in the United States
  - Estimated death from melanoma in 2019: 6,850 (CDC)
- 5-AzaC: nucleoside-based DNA methyltransferase inhibitor that induces demethylation and gene reactivation

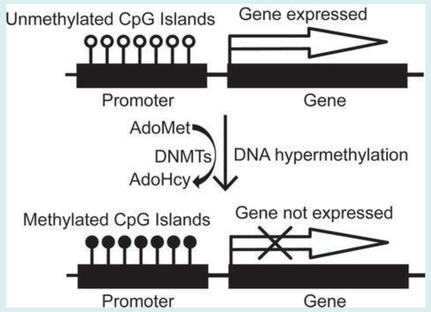

Fig 1. The mechanism of CpG Island Methylation.

OBJECTIVE

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

METHODOLOGY OVERVIEW

MDA-MB-435 Cell Culture

IMEM medium was used and cells were kept at 5.0% CO_2

Cells grown in 25cm^2 flask at 1 x 10^6 cells/mL

Treatment Selection

Cells were split into a control and experimental flask

- Control Flask
- Experimental Flask: 5-AzaC (0.00025 mg/mL) (48 hours)

Flow Cytometry

FITC conjugated Anti-HLA Class I antibodies added (ebiosciences)

- HLA - ABC, -A, -E, -G
- HLA-ABC, -A , -E, -G

PREVIOUS RESULTS

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

	Control	Transient Absence (One-week)	Transient Absence (Two-week)	Long-term
Mean	239.20	421.49	368.00	375.37
Median	181.06	305.05	266.55	254.83
Peak Channel	212	330	268	259

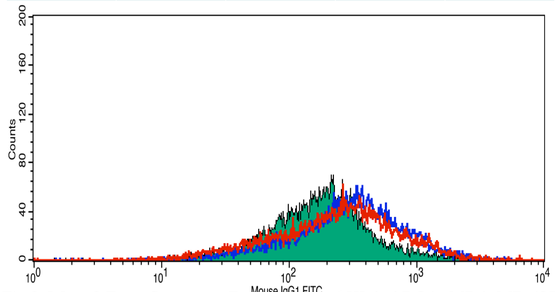

Fig 3. Week 2 Flow Cytometry Fluorescence of HLA-ABC in MDA-MB-435 cells.

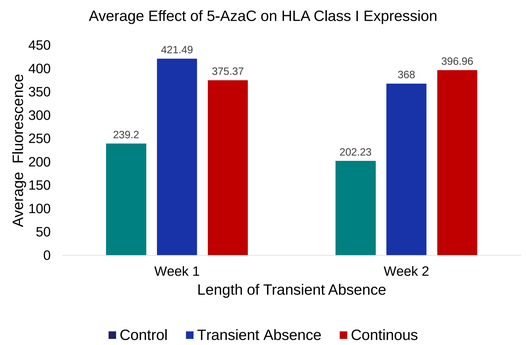

Fig 4. Average increase in HLA-ABC in MDA-MB-435 cells treated with 5-AzaC. Clear increase between the Control and Experimental groups.

5-AzaC CONCENTRATION

- 96 well plate MTT cytotoxicity analysis
  - Varying amounts of 5-azaC
  - 0.10 to 0.000198 (mg/mL)
  - Cell survivability

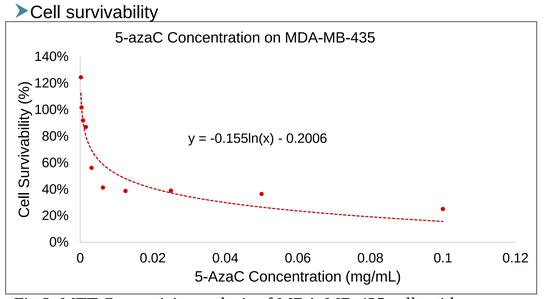

Fig 2. MTT Cytotoxicity analysis of MDA-MB-435 cells with a decreasing concentration of 5-AzaC (mg/mL).

RESULTS

Table 2. Flow Cytometry Fluorescence of HLA –A, -E, -G in MDA-MB-435 cells.

HLA Type	Control Average (3 trials)	48 – hour Incubation Average	Percent Difference Average
HLA-A	4.89	6.45	27.51%
HLA-E	18.22	32.30	81.69%
HLA-G	4.35	5.23	18.2%

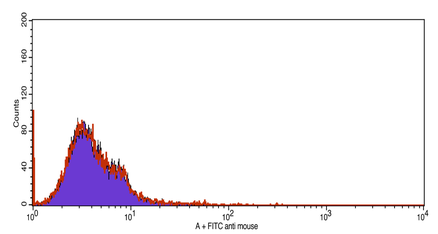

Fig 5. Flow Cytometry Fluorescence of HLA-A in MDA-MB-435 cells. No conclusive change seen.

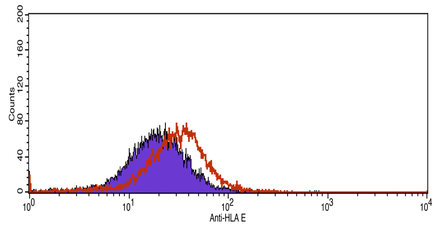

Fig 6. Flow Cytometry Fluorescence of HLA-E in MDA-MB-435 cells. Clear increase in HLA- E expression seen.

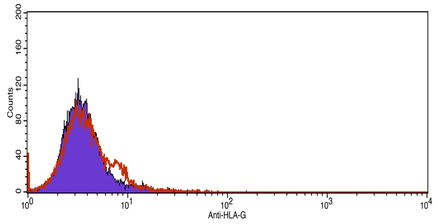

Fig 7. Flow Cytometry Fluorescence of HLA-G in MDA-MB-435 cells. No conclusive change seen.

DISCUSSION

5-AzaC Concentration

- Low doses of 5-AzaC promote cell survivability (Fig 2.)

Previous Results

- On average, HLA Class I expression of transient absence 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

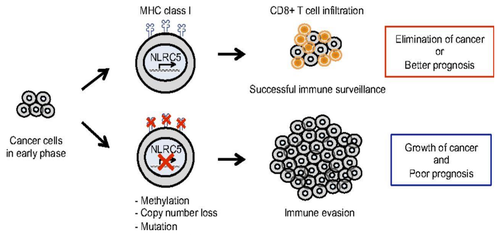

Fig 8. Role of MHC Class I HLA expression .

FUTURE STUDIES

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

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REFERENCES

1. Mendonça-Torres, M. C., & Roberts, S. S. (2013). The translocator protein (TSPO) ligand PK11195 induces apoptosis and cell cycle arrest and sensitizes to chemotherapy treatment in pre- and post-relapse neuroblastoma cell lines. Cancer Biology & Therapy, 14(4), 319–326.
2. Chen, Y., Sajjad, M., Wang, Y., Batt, C., Nabi, H. A., & Pandey, R. K. (2011). TSPO 18 kDa (PBR) Targeted Photosensitizers for Cancer Imaging (PET) and PDT. ACS Medicinal Chemistry Letters, 2(2), 136–141
3. Ostuni, M. A., Ducroc, R. , Péranzi, G. , Tonon, M. , Papadopoulos, V. and Lacapere, J. (2007), Translocator protein (18 kDa) ligand PK 11195 induces transient mitochondrial Ca2+ release leading to transepithelial Cl− secretion in HT-29 human colon cancer cells. Biology of the Cell, 99: 639-647.
4. Cancer Statistics, 2019 Rebecca L. Siegel, MPH ; Kimberly D. Miller, MPH ; Ahmedin Jemal, DVM, PhD, CA CANCER J CLIN 2019;0:1–28