Interferon-Gamma Stimulation Upregulates MHC-I, MHC-II, and PD-L1 in Yumm1.7-3.D8.B7 Melanoma Cell Line

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Background

- T cell recognition of tumor-derived antigens presented on major histocompatibility complex (MHC) is critical for effective anti-tumor immune responses
- MHC class I (MHC-I) presents antigens to CD8+ T cells, whereas MHC-II presents antigens to CD4+ T cells
- T cells become activated upon recognition of peptide-MHC along with co-stimulation
- Activation and T cell stimulation can also upregulate the immune checkpoints CTLA-4 and PD-1 and inhibit T cells
- Interferon-gamma (IFN-γ) can induce upregulation of MHC-I and PD-L1 (a ligand for PD-1) on most tumors and MHC-II on a minority of tumors including some melanomas (1-3)
- MHC-II is usually expressed on antigen presenting cells (APCs) like macrophages and dendritic cells
- The role of MHC-II expression in melanoma and how this affects that anti-tumor immune responses is unclear
- We evaluated expression of MHC-I, MHC-II, PD-L1, and the co-stimulatory molecule CD80 on the Yumm1.7-3.D8.B7 melanoma cell line engineered to express model tumor antigens in the presence of absence of IFN-γ stimulatory conditions

Hypothesis

IFN-γ will induce upregulation of MHC-I, MHC-II, and PD-L1 in a time-dependent manner in the Yumm1.7-3.D8.B7 mouse melanoma model.

Methods

Thawing and Growing cells

- Frozen cells were thawed in a 37°C water bath.
- Thawed cells were washed and transferred to a T75 flask
- Cells were grown in R10+BME media at 37°C/5% CO_2
- After cell expansion, 500,000 cells were seeded in 6 T-75 flasks
- Cells were treated with 0, 100, or 300 u/mL of recombinant mouse IFN-γ for 24 or 72 hours

Harvesting and Staining cells

- After the incubation period, the cells were harvested following standard cell splitting protocol and transferred to a 96 well plate to their assigned well
- The plate was centrifuged for 2 minutes at 2000 rpm and resuspended in 50 uL of 1x Fc block master mix (0.5 Fc block, 49.5 FACS buffer per well) after decanting the supernatant post-centrifugation
- The antibodies (Figure 1) and NIR dye were centrifuged for 5 minutes and used to make master mixed based on the FACS panel designed prior the harvesting

Methods (Continued)

List of Fluorescently Conjugated Antibodies	
Fluorescent Antibody	Expression Implication
PerCP-Cy5.5-CD274 PD-L1	PD-L1(+)
I-A/I-E MHC class II-BV650	MHC-II(+)
Isotype control BV650	MHC-II(-)
H-2D^b MHC class 1-APC	MHC-I(+)
H-2K^b MHC class 1-PE	MHC-I(+)
CD80 (B7.1)-PE/Cy7	CD80(+)
Isotype Control. Hamster IgG-PE/Cy7	CD80(-)

Figure 1. List of fluorescently conjugated antibodies used for the experiment. Isotype-matched control conjugated antibodies were added in separate staining panels to act as controls.

- The cells were stained in dark at 4°C for 30 minutes. Afterwards, they were washed with 200 ul of FACS buffer
- Cells were and resuspended in 400ul of buffer and transferred FACS tubes
- Compensation single color controls were used to correct fluorescence spillover and prevent spectral overlap

Fluorescence-activated single cell sorting (FACS) analysis

- The compensations were ran first on the BD LSR FACS machine.
- Data were downloaded on our working computers and analyzed using FlowJo to generate plots and gates to determine surface molecule expressions.

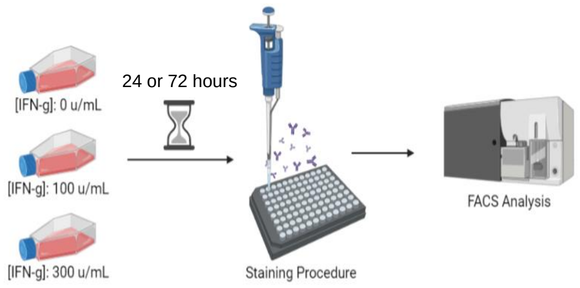

Figure 2. Experimental layout

Results

- Yumm1.7-3.D8.B7 cells without IFN-γ stimulation expressed little to no MHC-I, MHC-II, and PD-L1. (Figure 4 and 5)
- IFN-γ induced upregulation of MHC-I and PD-L1. (Figure 3, 4 and 5)
- MHC-I and PD-L1 expression dropped for cells stimulated with 300 u/mL IFN-γ for 72 hours compared to 100 u/mL treatment group. (Figure 3)
- MHC-I expression was identified on more than 90% of cells treated with IFN-γ, peaking near 99% at 300 u/mL of IFN-γ. (Figure 5)
- IFN-γ induced upregulation of MHC-II, but only on a subset of melanoma cells.
- The percent of MHC-II expressing tumor cells increased from about 12.7% to 18.2% when increasing dosage of IFN-γ from 100 u/mL to 300 u/mL. (Figure 4)
- CD80 was constitutively expressed on Yumm1.7-3.D8.B7. (Figure 6)

Results (Continued)

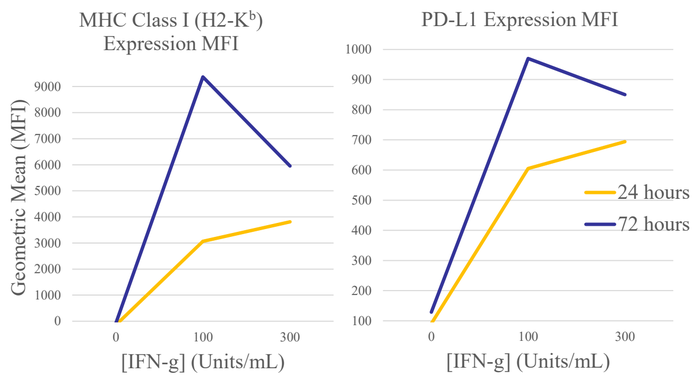

Figure 3. MHC-I and PD-L1 expression charts based on mean fluorescent intensity (MFI).

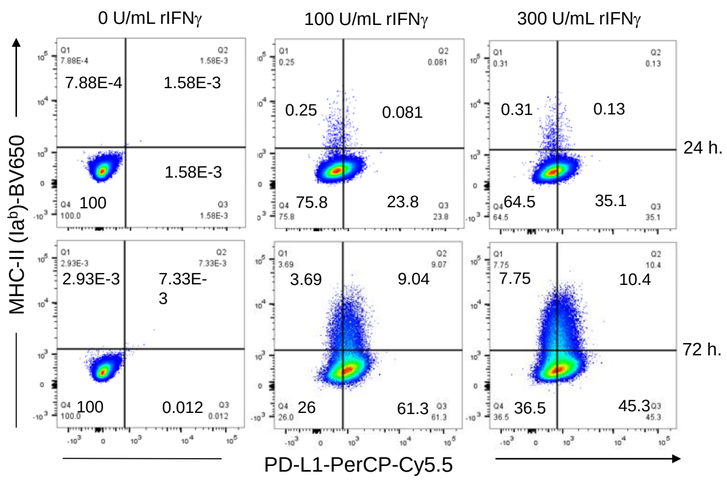

Figure 4. MHC-II (vertical) and PD-L1 (horizontal) expression dot chart.

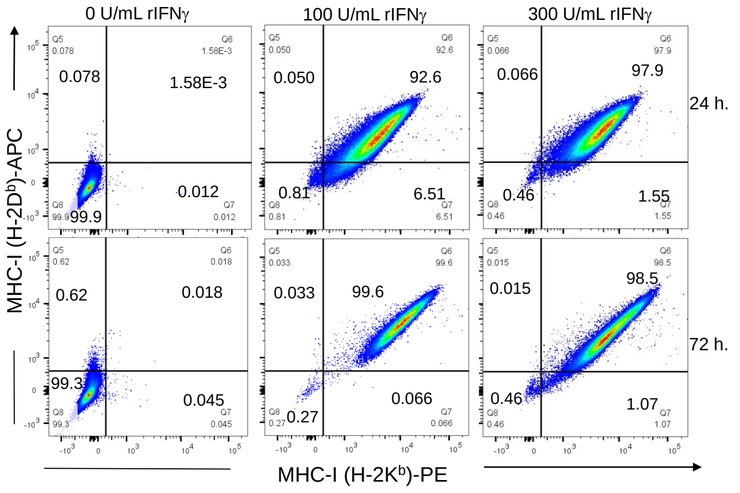

Figure 5. MHC-I expression dot chart. Double positive would imply MHC-I expression

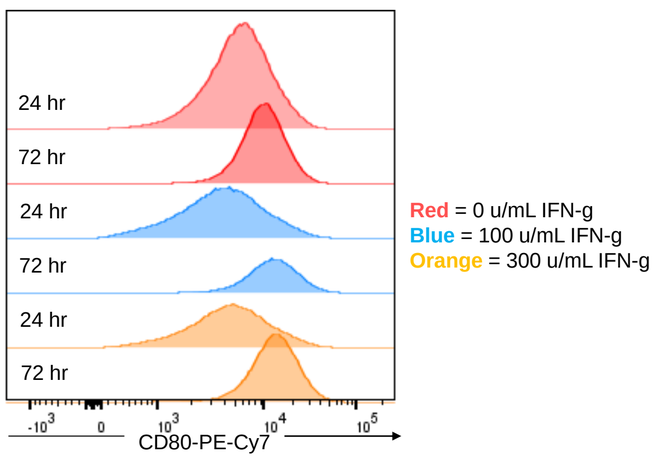

Figure 6. CD80 expression histograms between 24 and 72 hour incubation group

Discussion (or) Conclusions

•Consistent with our hypothesis, IFN-γ upregulated MHC-I and PD-L1

•IFN-γ stimulation leads to MHC-II expression by some tumor cells, specifically less than 20% of all live tumor cells

•Further research may be necessary to identify whether the relationship is significant enough to be taken into consideration as a potential target to tackle for cancer immunotherapy.

•It appears that the amount of time the cells are exposed to IFN-g seems to also play a role in the level of MHC-II expression, where longer exposure time would lead to more MHC-II expression.

•Perhaps, by upregulating IFN-γ, we could force solid tumors to express more MHC-II than they usually do, making them more susceptible to CD4+ T-cells.

•When comparing the 100 U/mL and 300 U/mL groups under the same incubation times, there does not seem to be any significant difference in MHC-II expression, possibly due to saturation of IFN-γ

•Oversaturation of IFN-γ stimulation over a long period of time may down regulate MHC-I and PD-L1 expression.

•Future research could be done to check if there is an optimal concentration for IFN-γ for upregulating surface molecule expression.

•While IFN-γ is a dominant cytokine for upregulating MHC and PD-L1 expression, other inflammatory signals (such as IFN-a) can be explored upon to examine whether they also regulate surface molecule expression.

References

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