Expanding the Tumor Mutation Burden in Mouse Melanoma Cells

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Introduction

- Melanoma is one of three types of skin cancer, predominately caused by exposure to UV radiation.[1] Previous studies indicate that melanomas have a high tumor mutation burden (TMB), promoting immunogenicity and significant anti-tumor immune responses due to the translation of mutations into neoantigens.[1,2]
- Neoantigen-containing tumors are known to be responsive to immunotherapies[3], however, many do not respond to such treatments. It is unclear what differentiates responding tumors from non-responders.
- New high TMB melanoma cell models that could be studied in immunocompetent mice would be beneficial to help understand anti-melanoma immune responses.
- ***We hypothesize that expanding the TMB of melanoma cells by irradiation with UV-A and UV-B will increase immunogenicity and inhibit tumor growth in immunocompetent mice.***
- We tested our hypothesis using the following specific aims:

➢ Irradiate cell lines YUMM 5.2 and B16-F0 with solar-simulating UVA/B radiation.

➢ Perform DNA sequencing to determine the effect of the UV treatments on the cells' TMB.

➢ Evaluate *in vitro* cell growth and *in vivo* tumor growth of the high TMB cells in immunocompetent mice.

➢ Evaluate cellular energetics and antigenic responses of the high TMB cells.

References

[1]RP;, Sahu. "Deciphering Mechanisms of UVR-INDUCED TUMORAL Immune CHECKPOINT Regulation against Melanoma." *Cancer Research*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/31160307/.

[2]Wang, Jake, et al. "Uv-Induced Somatic Mutations Elicit a Functional t Cell Response in the yummer1.7 Mouse Melanoma Model." *Pigment Cell & Melanoma Research*, U.S. National Library of Medicine, July 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5820096/.

[3]Snyder A;Makarov V;Merghoub T;Yuan J;Zaretsky JM;Desrichard A;Walsh LA;Postow MA;Wong P;Ho TS;Hollmann TJ;Bruggeman C;Kannan K;Li Y;Elipenahli C;Liu C;Harbison CT;Wang L;Ribas A;Wolchok JD;Chan TA; "Genetic Basis for Clinical Response to Ctla-4 Blockade in Melanoma." *The New England Journal of Medicine*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/25409260/.

Results

UVA/B-induced TMB in B16 and YUMM5.2 cells

B16-F0 UVR Clones & UVR Exposure Times	# of New Mutations
B16 Clone 1.1 : 52.5 sec	1758
B16 Clone 1.2: 52.5 sec	1420
B16 Clone 2 : 1:45 min	1471
B16 Clone 3 : 3:30 min	1485

YUMM5.2 UVR Clones & UVR Exposure Times	# of New Mutations
YUMM5.2 Clone 1.1 : 52.5sec	2218
YUMM5.2 Clone 1.2 : 52.5 sec	2681
YUMM5.2 Clone 1.3 : 52.5sec	2549
YUMM5.2 Clone 2 : 1:45 min	2955

Table 1 & 2. Effect of the UV treatments on the treated B16 and YUMM cell lines' DNA.

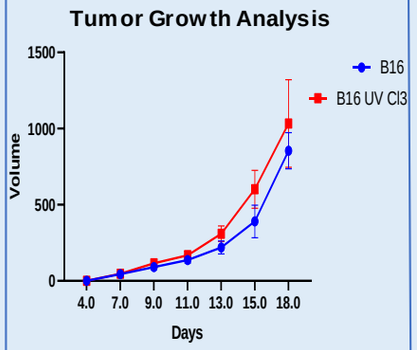

Figure 2. Sub-cutaneous tumor growth of B16 parental and B16.UV.Cl3 cells in B6 mice.

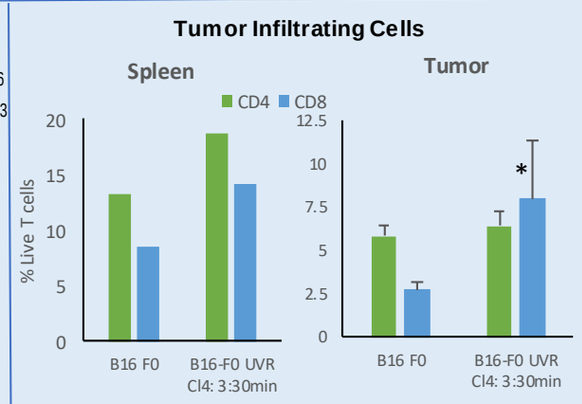

Figure 3: Percentage of different types of tumor-infiltrating cells.

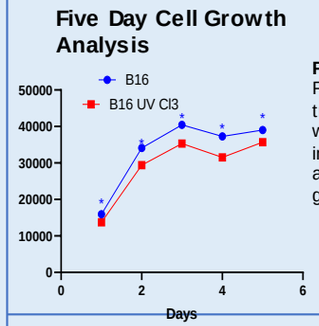

Figure 1. Parental and UV treated cell lines were assessed in triplicates for a five-day cell growth assay.

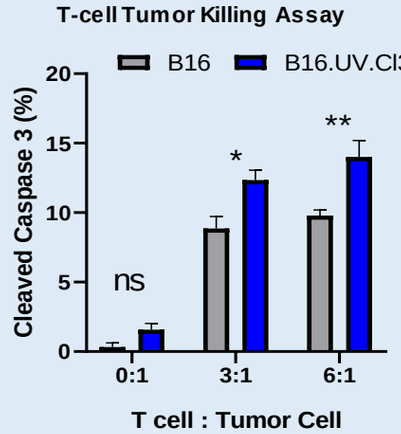

Figure 4. B16 and B16 UV Cl3 apoptotic rates were evaluated by caspase 3 cleavage.

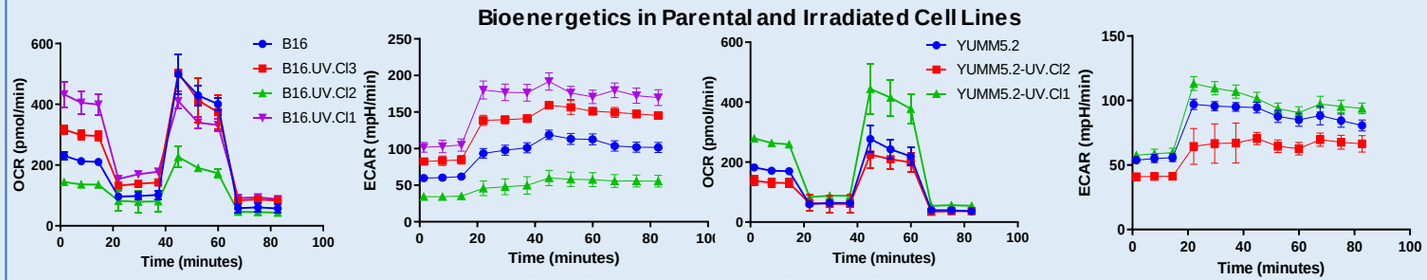

Figure 5. Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) were measured in the indicated cell lines.

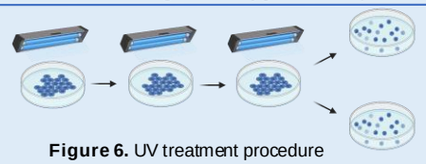

Figure 6. UV treatment procedure

UVA/B Radiation Treatments

Methods

- **UV Treatments:** Cell lines B16 and genetically engineered YUMM 5.2 were irradiated three times at intensities that generate severe skin burns at time intervals of 52.5 seconds, 1:45 minutes, 3:30 minutes and 7:00 minutes. After the three rounds of treatments, surviving clones were selected and seeded.
- **Whole Exome Sequencing (WES):** DNA was extracted from UV treated and parental cell lines using QIAamp DNA Mini Kit (250), quantified and WES was performed.
- **Seahorse Assay**: Parental and UV treated cells were plated in Seahorse assay plates and mito-stress test was performed following the manufacturer's instructions.
- **Cytotoxic T-cell tumor killing assay:** DDAO-labeled tumor cells were seeded in 96 well plates and co-cultured with IL2-activated T Cells from Black 6 mice for 3 hours, and apoptosis induction in tumor cells was detected using FACS analysis for cleaved Caspase 3.
- **Cell growth and tumor growth studies:** Parental B16 and UV-treated cells were seeded in 96 well plates and cell growth over a period of 5 days was evaluated using Cell Titer Blue. Parental B16 and UV treated cell line, B16 3:30 Clone 3, were implanted into mice at a concentration of $3x10^5$ cells per injection. After the implantations, tumor progression/regression after one week was evaluated.

Conclusions

- We successfully generated high TMB clones of B16 and YUMM5.2
- *In vitro* cell growth assay shows a small decrease of cell growth in the high TMB B16 cells compared to parental cells.
- *In vivo* tumor growth data shows that the UV treated B16 cell line has faster growth.
- T cell tumor killing assay shows activated T cells induce significantly higher rate of apoptosis in the high TMB B16 clone versus parental.
- Seahorse mito stress test shows that high TMB clones have differentially altered cellular bioenergetics compared to parental cells.
- FACS analysis of the tumor and spleen indicate that a high TMB recruits a greater number of immune cells.

Acknowledgements

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