The Role of *Ndufs4* and *Slc2a1* on D4M-UV2 Melanoma Tumor Metabolism and Growth

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Introduction

•Melanoma is one of the most aggressive and metastasis-prone forms of cancer.

•Melanoma brain metastases (MBMs) are a common and devastating complication of advanced melanoma.

•Oxidative Phosphorylation (OXPHOS) has been observed to be particularly elevated in MBMs.

•Previous studies have found that tumor metabolism may be involved in suppressing immune responses to cancer.

•The role of tumor metabolism in immune responses remains unclear.

Hypothesis

We hypothesize that elevated tumor metabolism suppresses the immune response in MBMs.

Aim

- To develop models of High- and Low-OXPHOS or glycolysis using CRISPR technology.
- To better understand the role of tumor metabolism in MBM pathogenesis.

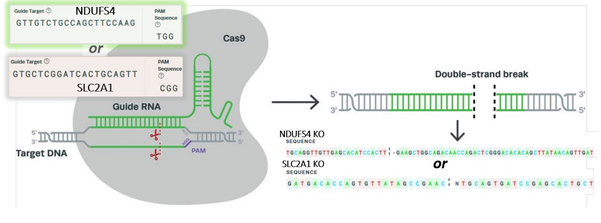
Methods and Materials

Cell Culture: D4M-UV2 cells were cultured in DMEM media containing 10% FBS and 1% NEAA. Cells were incubated at 37^{0}C and 5% CO_2 and were grown *in vitro.*

Cellular Metabolism Analysis: The MitoStress Test was run on Seahorse 96-well XF Analyzer to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of the D4M-UV2 cell line.

Cell Proliferation Assay: D4M-UV2 cells were incubated with Cell Titer Blue reagent until color change was observed. Then, fluorescence of cell media was analyzed on a Tecan plate reader at 560 nm excitation / 590 nm emission.

CRISPR Knockouts



Results

Metabolic Effects on NDUFS4 KO

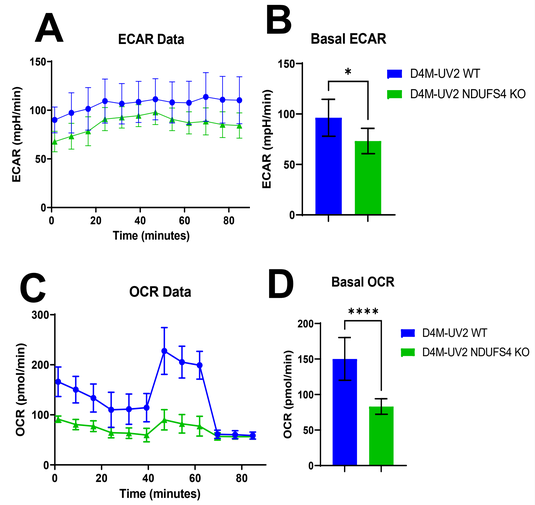


Figure 1: Observation of Metabolic Effects on NDUFS4 KO conducted by MitoStress Test. **(A)** Tumor glycolysis is represented by the Extracellular Acidification Rate (ECAR) of treated cells (n = 5). **(B)** KO of NDUFS4 resulted in a small 20.6% decrease in basal ECAR (p = 0.0439). **(C)** Tumor mitochondrial OXHPOS is represented by the Oxygen Consumption Rate (OCR) of treated cells (n = 5), **(D)** KO of NDUFS4 decreased basal OCR by 44.6% (p<0.0001). Dots in MitoStress Test curves represent individual replicates (n = 3) and lines represent SD. Bars in ECAR and OCR histograms represent mean values and lines represent SD.

Metabolic Effects on SLC2A1 KO

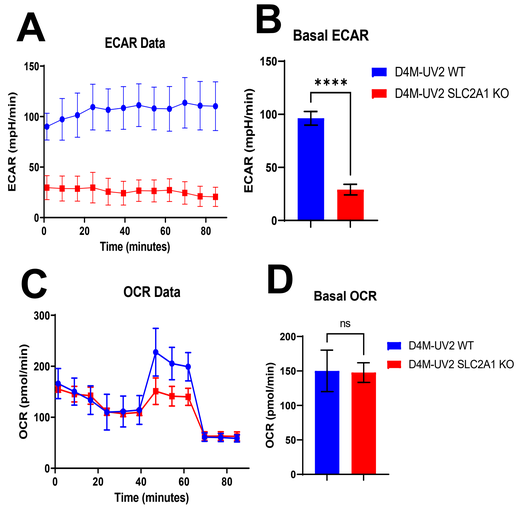


Figure 2: Observation of Metabolic Effects on NDUFS4 KO conducted by MitoStress Test. **(A)** Tumor glycolysis is represented by the Extracellular Acidification Rate (ECAR) of treated cells (n = 5). **(B)** KO of SLC2A1 resulted in a 69.8% decrease in basal ECAR (p < 0.0001). **(C)** Tumor mitochondrial OXHPOS is represented by the Oxygen Consumption Rate (OCR) of treated cells (n = 5), **(D)** KO of SLC2A1 did not significantly change Basal OCR (p = 0.8891). Dots in MitoStress Test curves represent individual replicates (n = 3) and lines represent SD. Bars in ECAR and OCR histograms represent mean values and lines represent SD.

Effects on Cell Growth

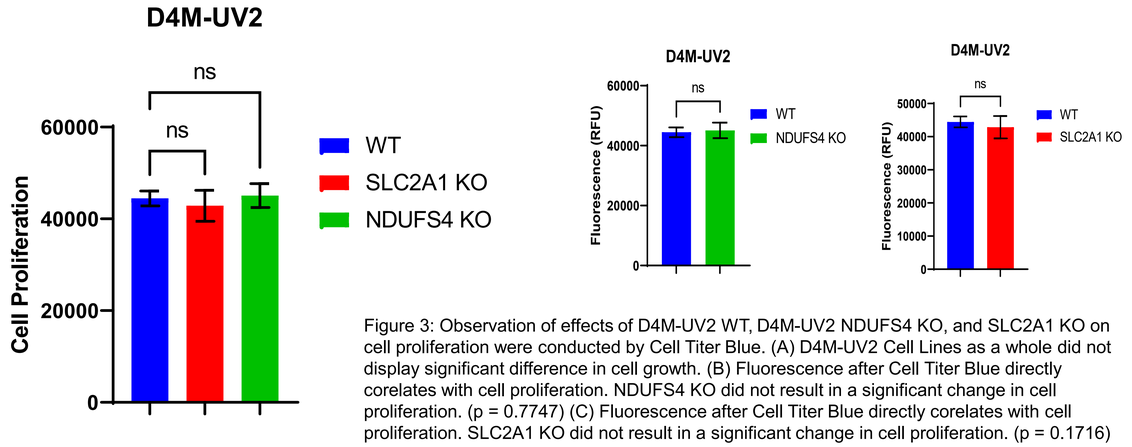


Figure 3: Observation of effects of D4M-UV2 WT, D4M-UV2 NDUFS4 KO, and SLC2A1 KO on cell proliferation were conducted by Cell Titer Blue. (A) D4M-UV2 Cell Lines as a whole did not display significant difference in cell growth. (B) Fluorescence after Cell Titer Blue directly corelates with cell proliferation. NDUFS4 KO did not result in a significant change in cell proliferation. (p = 0.7747) (C) Fluorescence after Cell Titer Blue directly corelates with cell proliferation. SLC2A1 KO did not result in a significant change in cell proliferation. (p = 0.1716)

Conclusions

- Gene KO of NDUFS4 strongly inhibited OXPHOS and slightly inhibited glycolysis *in vitro.*
- Gene KO of SLC2A1 resulted in specific inhibition of glycolysis *in vitro.*
- KO of NDUFS4 OR SLC2A1 does not impact the proliferation of tumor cells *in vitro.*
- We plan to study these models of high and low OXPHOS or glycolysis and how it can apply to tumor metabolism and anti-tumor immune response.
- D4M-UV2 Cell Lines will be evaluated for *in vivo* growth in mice.
- Future studies will evaluate the rationale for development of combinatorial treatment strategies targeting tumor metabolism and immunotherapy.

Future Directions

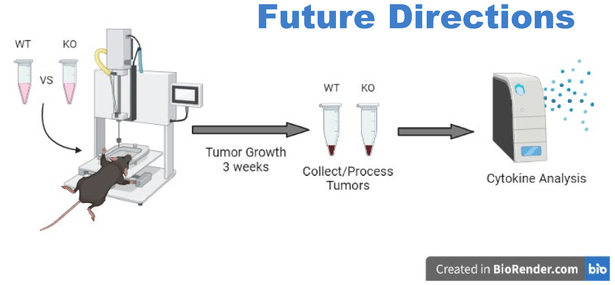


Figure 4: Evaluation of D4M-UV2 WT and KO Cell Lines can be conducted in mice models via intercranial injections.

Acknowledgements

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