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.

Methods and Materials

Cell Culture: D4M-UV2 cells were cultured in DMEM containing 10% FBS and 1% NEAA. Cells were incubated at 37°C and 5% CO2 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

• Future studies will evaluate the rationale for OXPHOS or glycolysis and how it can apply proliferation of tumor cells in vitro.
• The 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.

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References


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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 correlates 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 correlates with cell proliferation. SLC2A1 KO did not result in a significant change in cell proliferation. (p = 0.1768)

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