Introduction

Melanoma is the rarest yet most aggressive of the common forms of skin cancer. Melanoma brain metastases (MBMs) are a leading cause of morbidity and mortality for patients with advanced melanoma. Previous studies have implicated oxidative phosphorylation (OXPHOS) in the pathogenesis of MBM and in suppression of the anti-tumor immune response. Clinically, MBMs with high OXPHOS had decreased sensitivity to immunotherapy and targeted gene therapy. However, the role of OXPHOS in modulating tumor immunity in MBM remains unclear.

Hypothesis

We hypothesize that OXPHOS suppresses the immune response in MBMs.

Aim

To better understand the role of OXPHOS in MBM pathogenesis and immunosuppression, we investigated the metabolic and immunologic effects of pharmacological OXPHOS inhibitors using IPN-60090 and IACS-010759 on D4M melanoma cells.

Methods and Materials

Fluorescence Tagging: D4M murine melanoma cells were cultured and stably transfected with lentiviral particles co-expressing firefly luciferase and RFP, and blasticidin antibiotic resistance (GenTarget) Selection: D4M sensitivity to blasticidin (Bsd) treatment (0.1 µg/mL – 1000 µg/mL) was evaluated by Cell Titer Blue (CTB) assay. Transfected D4M cells underwent dual-selection with blasticidin antibiotic treatment (100 µg/mL) and FACS

Drug Treatments: Cells were treated with complete media, DMSO, and 0.1 - 5 µM of IPN-60090 (glutaminase inhibitor) and IACS-010759 (mitochondria complex I inhibitor).

Cellular Metabolism Analysis: The MitoStress Test was run on Seahorse 96-well XF Analyzer to measure oxygen consumption rate (OCR) of D4M cells 24h post-drug treatment

Cytokine Analysis: Cell media supernatant was collected at 24h post-treatment with 1 µM IACS-010759. Cytokine array analysis was performed using the Mesoscale U-PLEX platform to assess the effect of OXPHOS inhibition on D4M melanoma secretion of cytokines and chemokines involved in immune system regulation

Results

D4M Blasticidin Sensitivity

Figure 2: D4M cell vitality after blasticidin antibiotic treatment analyzed by CTB Assay. Dots represent mean of replicates (n=4) and lines represent SEM.

Immune Effect of IACS-010759 on D4M Cells

(A) Anti-tumorigenic Cyto-/Chemokines

(B) Pro-Tumorigenic Cyto-/Chemokines

(C) Other Cyto-/Chemokines

Figure 5: OXPHOS inhibition: (A) Increased expression of anti-tumorigenic GM-CSF (p<0.0001) and CXCL10 (p=0.0144), (B) Decreased secretion of pro-tumorigenic CCL2 (p=0.0005), CXCL1 (p<0.0001), and CXCL2 (p=0.034), (C) Highly increased expression of VEGF (p<0.0001) and CCL4 (p<0.0001).

Oxidative Phosphorylation (OXPHOS) Modulation of Immune Response in Melanoma

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References

