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Introduction

Neuroblastoma is a cancer of primitive sympathetic ganglion cells that typically affects the adrenal glands of children. Significant morbidity and mortality is associated with neuroblastoma as the third most common pediatric cancer and high risk patients having less than 50% 5-year survival rates. Further investigation into the immunosuppressive properties of the neuroblastoma microenvironment may lead to more favorable patient outcomes. Amyloid precursor-like protein 2 (APLP2) has been associated with a more aggressive tumor phenotype, and expression may play a role in altering macrophage sub-populations to the tumor-tolerant M2 phenotype. Furthermore, treatment of neuroblastoma with histone deacetylation inhibitors (HDACi) has led to increased tumor infiltration of macrophages.

Hypothesis

APLP2 expression will alter macrophage polarization and ability to initiate anti-tumor response. Alteration of APLP2 expression and treatment with HDACi will improve macrophage infiltration and response to neuroblastoma.

APLP2 Expression in Polarized Mouse Macrophages

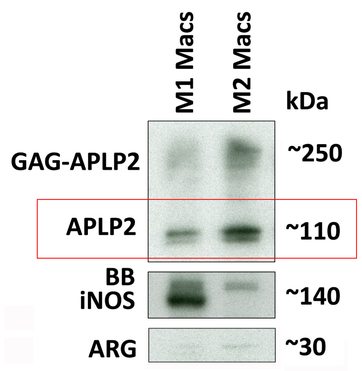

Figure 1: APLP2 is increased in mouse M2 compared to M1 macrophages. Bone marrow from femurs and tibias of euthanized C57BL/6 mice was treated with 50 ng/ml GM-CSF for 7 days, and then polarized for 24 hours. M1 macrophages were generated with 20 ng/mL IFNγ and 20 ng/mL IL-4 to generate M2. BB=background band; iNOS=inducible nitric oxide synthase (indicating M1); ARG=Arginase-1 (indicating M2)

Generating APLP2-Knock Out Mouse Model

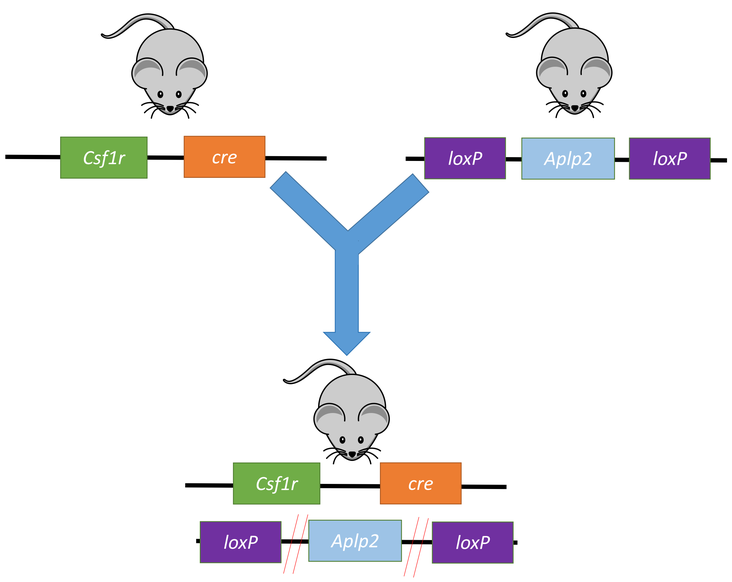

Figure 2: Breeding strategy used to generate macrophage-targeted APLP2-knockout (KO) C57BL/6 mice. Macrophage-targeted APLP2-KO mice were generated by breeding mice expressing cre recombinase housed under the *Csf1r* promoter, expressed by macrophages and dendritic cells, with mice containing floxed *Aplp2*. Genotypes of offspring were verified by PCR for *Csf1r-cre* and *Aplp2*.

APLP2-Knock Out Alters Macrophage Polarization

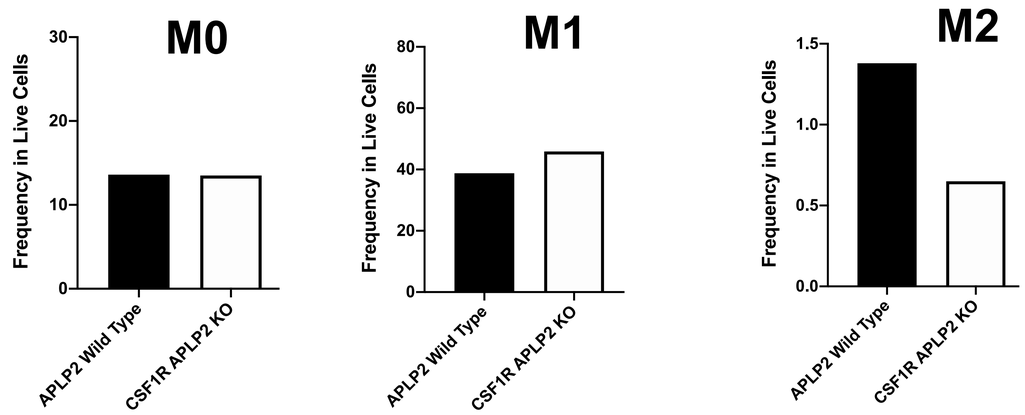

Figure 3: CD11b⁺ CD11c⁻ F4/80⁺ cells in APLP2-KO mice have increased ability to polarize to M1 phenotype and decreased M2 polarization. Bone marrow was extracted from C57BL/6 mice with wild type APLP2 (APLP2 Wild Type) and mice with APLP2-KO in macrophages (CSF1R APLP2-KO). Macrophages were cultured in L929 conditioned-media for 7 days then polarized to M1 (LPS + IFN-γ) or M2 (IL-4) for 3 days prior to flow cytometry analysis.

HDACi Treatment Decreases Neuroblastoma Viability

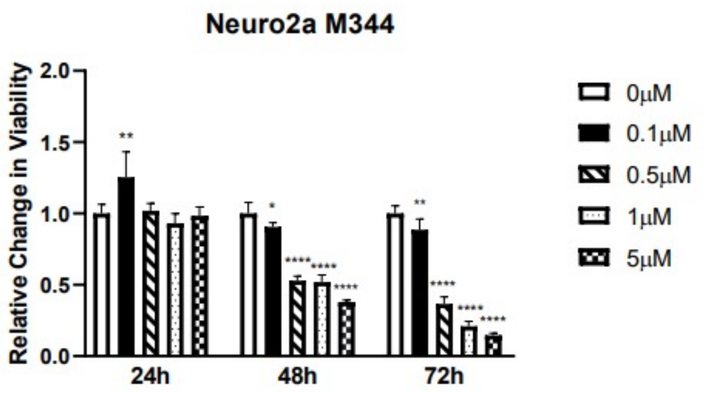

Figure 4: M344, an HDACi, causes a dose-dependent decrease in Neuro2a viability. Neuro2a cells were treated with increasing concentrations of M344 at 24, 48, and 72 hour time points, and viability was evaluated through MTT assay. *p<0.05, **p<0.01, p****<0.0001

Conclusions and Future Directions

In conclusion, APLP2 expression is associated with the tumor-tolerant M2 macrophage phenotype. Decreasing APLP2 expression may also encourage anti-tumor M1 polarization, and generating APLP2-KO mouse lines allows for further characterization of APLP2's role in macrophage physiology. M344 causes a potent negative effect on Neuro2a viability, and will be further explored in the context of neuroblastoma's metastatic potential.

Future directions include investigating the tumor-infiltrating and phagocytic ability of macrophages in APLP2-KO mice with orthotopically implanted neuroblastoma. Additionally, HDACi, such as M344, will be investigated for ability to traffic macrophages to tumors and inhibit tumor migration.

Acknowledgements

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