Difluoromethylornithine Promotes Expression of Genes Correlated with T-Cell Activation and Cytotoxicity in Neuroblastoma: A Multiplex mRNA Profile

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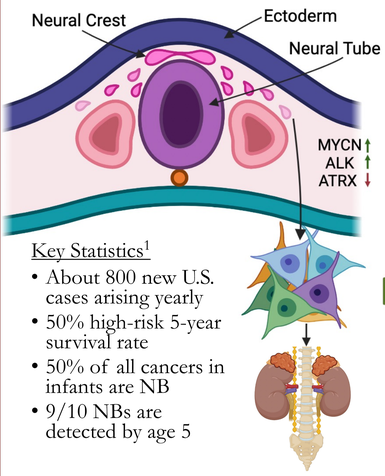
Introduction: Neuroblastoma and DFMO

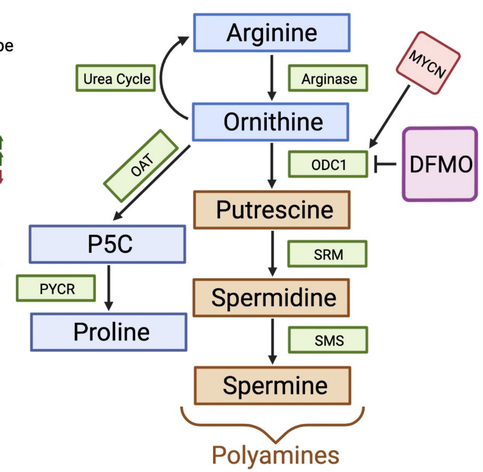
I. NB is neural crest derived



Key Statistics[1]

- About 800 new U.S. cases arising yearly
- 50% high-risk 5-year survival rate
- 50% of all cancers in infants are NB
- 9/10 NBs are detected by age 5

II. DFMO hinders polyamine synthesis



Figures 1 & 2. Neuroblastoma (NB) arises from neural crest-derived cells, where driver genes like MYCN are overexpressed and irregular peripheral nervous system cell growth occurs, usually at the adrenal glands or other sympathetic ganglia. In high-risk NB, tumor cell proliferation is driven by MYCN upregulating polyamine synthesis via ODC1. DFMO irreversibly inhibits ODC1 to hinder polyamine synthesis. [2,3]

Polyamines Contribute to Immunosuppressive TME

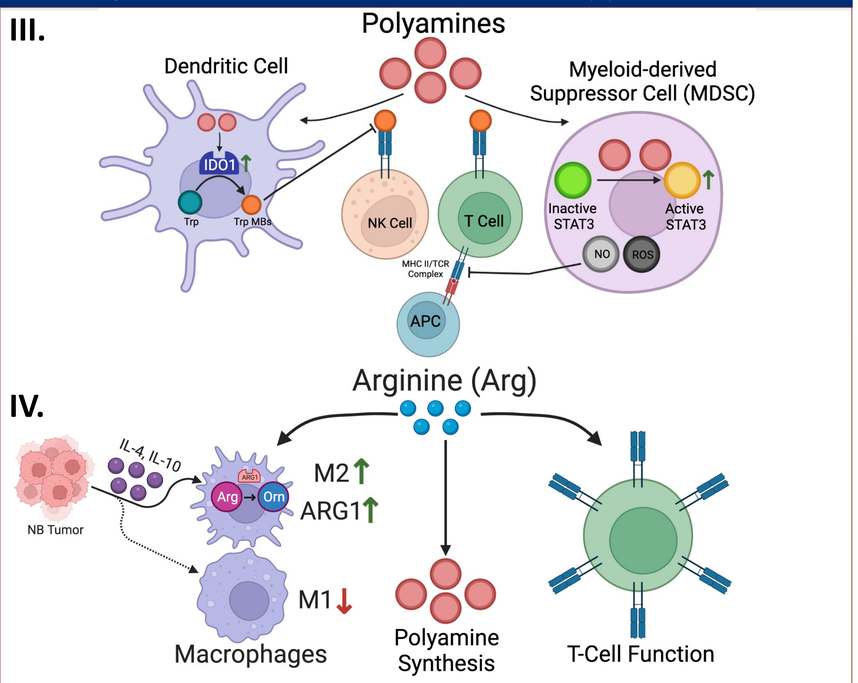
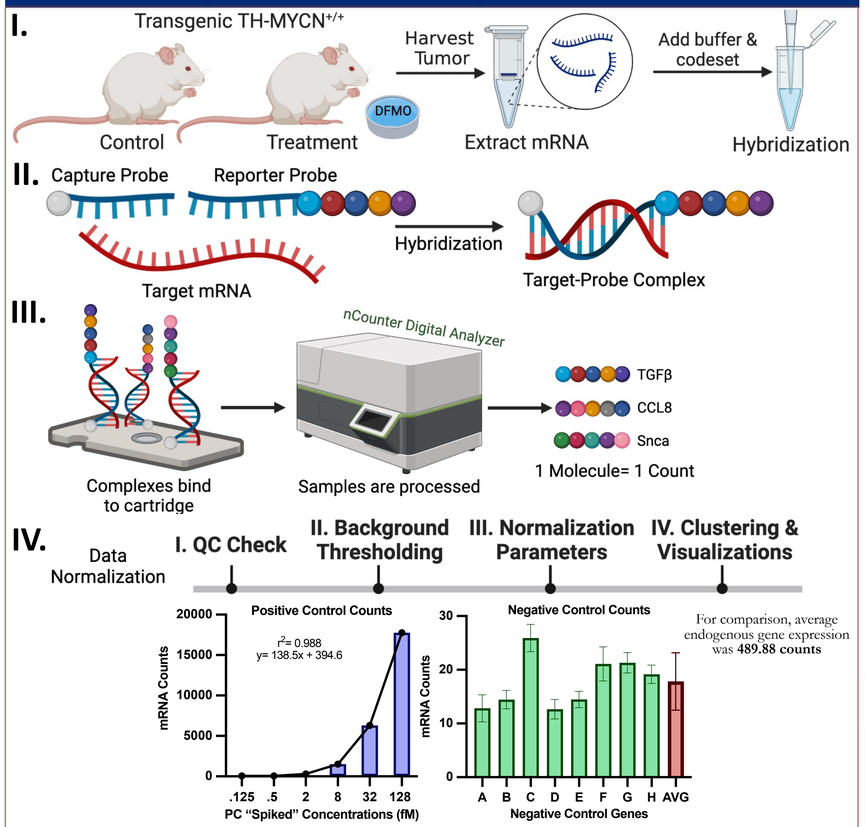


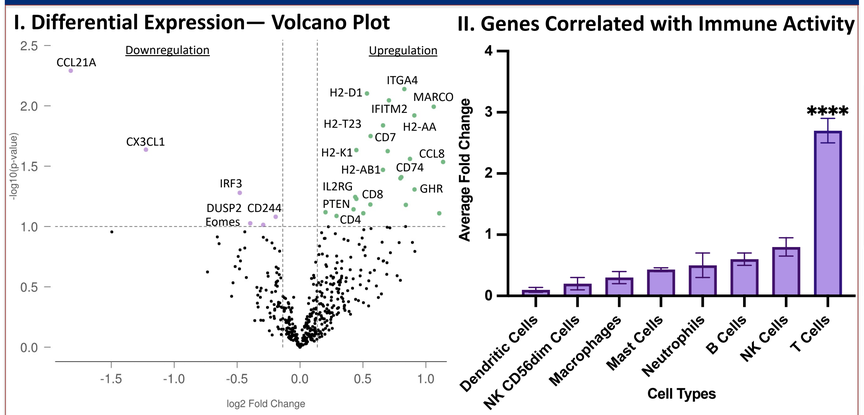
Figure 3. Polyamines induce the expression of IDO1, which metabolizes tryptophan (Trp). The Trp metabolites inhibit receptor activation of T & NK cells. Polyamines also promote MDSC survival via STAT3 activation. MSDCs produce nitric oxide (NO) and reactive oxygen species (ROS) that disrupt MHCII-TCR complexes. **Figure 4.** Cancer cells release IL-4/IL-10 to promote M2 differentiation, which expresses high levels of ARG1 that converts Arg to Orn. Since Arg is used by macrophages, polyamines, and T-cells, there is heavy competition for limited Arg supply. This results in weakened T-Cell activity. [4]

Methods: Digital mRNA Profiling and Data Normalization



Steps I-IV. Transgenic TH-MYCN$^{+/+}$ mice received 1% DFMO *ad libitum* in drinking water. Resultant tumors were harvested per laboratory standards. mRNA was extracted and hybridized with Nanostring nCounter probes, which allowed for complexes to bind to the cartridges. Samples were processed in the nCounter Digital Analyzer. Data was normalized using positive/negative controls and housekeeping genes.[5]

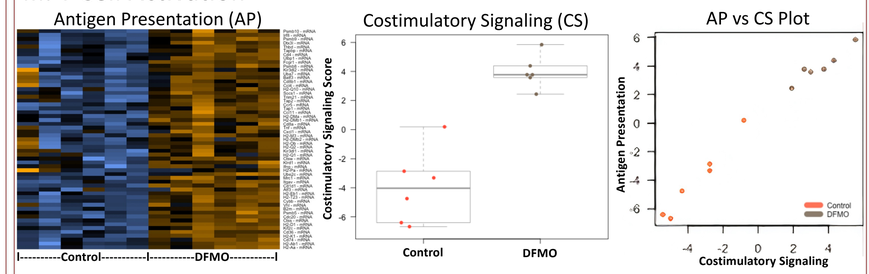
Summary of Differential Immune Gene Expressions



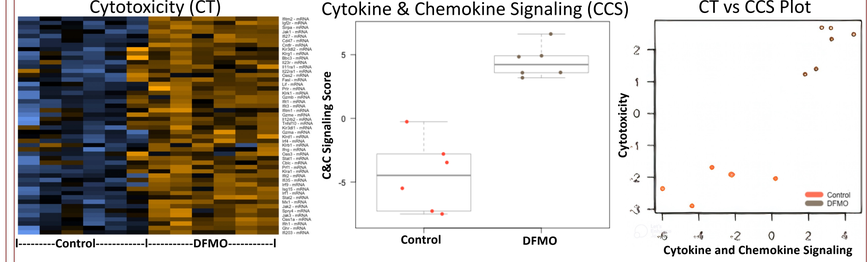
Graph 1. Gene expressions of 750 tested genes were mapped based on fold change between Control and DFMO samples. **Graph 2.** Genes were grouped based on correlated activity in various immune cells. A positive fold change indicates that DFMO-treated tumors showed an increase in correlated gene expression.

Elevated Expression of T Cell Activity-related Genes

III. T-Cell Activation

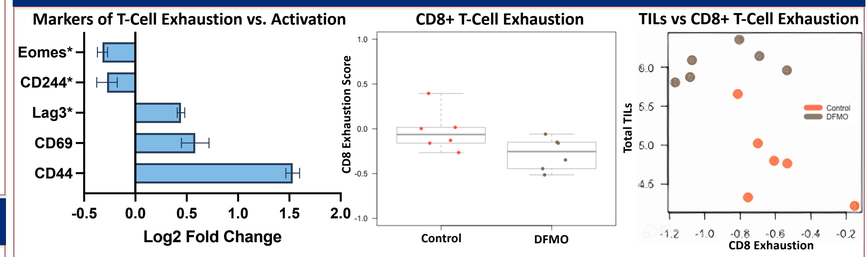


IV. T-Cell Cytotoxicity



Graphs III & IV. Genes correlated with T-Cell activation and cytotoxicity were plotted in heat maps, box plots, and PCA plots. These genes were significantly upregulated in DFMO-treated tumors compared to the control tumors. In the heat maps, gold shows relative upregulation while blue shows relative downregulation.

Reduced Expression of T-Cell Exhaustion Marker Genes



Genes that are markers of T-Cell exhaustion (Lag3, Eomes, CD244) were plotted alongside T-Cell activation markers (CD69, CD44). Markers of CD8+ T-Cell exhaustion were plotted, and they were compared against markers of TIL activity. Overall, DFMO-treated tumors showed lower expression of exhaustion genes.

Conclusion & Future Directions

- Conclusion: DFMO inhibiting polyamine synthesis in NB lead to differential expression of genes correlated with T-cell activation, cytotoxicity, and exhaustion
- Future Direction: Explore combining DFMO with arginine/proline depletion to potentially create a more clinically-viable treatment for neuroblastoma

Acknowledgements & References

Thank you to everyone in the Hogarty Lab for their mentorship, guidance, and support. Figures were created using BioRender, Prism9, nSolver, and Rosalind. This research was supported by: NCATS of the NIH under award number TL1TR001880, Institute for Translational Medicine and Therapeutics of the UPenn Perelman School of Medicine, Department of Defense Translational Team Science CA 170257.

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