Investigating Early Transcriptional Targets of Neuroprotective LXB4 in Neuronal Injury Models

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Introduction: Glaucoma is an incurable group of optic neuropathies that lead patients down a road of worsening tunnel vision to eventual total blindness. The degeneration of retinal ganglion cells (RGCs) and their axons in the optic nerve is an ever present characteristic noted as a major factor to the pathology. We have identified neuroprotective activity of the endogenous lipid mediator Lipoxin B4 (LXB4) in acute and chronic models of glaucomatous injury. LXB4 was found to be significantly more potent than similar lipid mediators, with its activity mediated through a separate unknown mechanism than its well-studied isomer, LXA4. LXB4's signaling and associated targets have yet to be identified, yet in injury models is most potent as a 1 hour pretreatment to injury. This requirement points to transcriptional regulation as the driving factor in neuroprotective activity. We have previously performed bulk RNA sequencing and now aim to refine an approach with single cell RNA sequencing, coupled with our existing injury model to recapitulate and possibly extend these findings in primary neurons.

Methods: "Bulk RNA Sequencing: HT22 neuronal cells were seeded at 1x106 cells/well in DMEM media overnight. 4 groups; LXA4, LXB4,15-HETE (inactive LXB4 precursor) and Vehicle were treated at 1µM for 1 hour. Total RNA was isolated, purified and sequenced on an Illumina platform. Data was then bioinformatically analyzed with differential gene expression to identify lipoxin-specific transcripts. Top Hit Validation: TERT and telomerase activity was studied through TERT agonists in neuronal injury models. This was coupled with further in-vivo validation of previously identified functions of TERT in neurodegenerative disease models. Single cell RNA sequencing: C57BL/6 mice were intravitreally injected with 10µM LXB4 and Vehicle 1-hour before 10mM kainic acid (KA) insult. Retinas were dissected after defined timepoints and RGCs positively selected using CD90.2 microbeads following neural dissociation.

Results: Compared to Vehicle, 242 LXB4-specific genes, 539-LXA4, and 440 15-HETE-specific genes were identified ($p<0.01$). 10 LXB4-specific genes passed the statistical test of p.adj<0.05 and log2FC -3</>3 to be used in subsequent analyses. Of those TERT was the most high differentiated gene unique to the LXB4 group.

Discussion/Conclusion: LXB4 is a potent neuroprotective lipid with ambiguous activity. The current work seeks to capture early transcriptional responses in relation to glaucoma models. Next steps include validation of signature genes to further define their relation to LXB4 neuroprotective activity.