A0407

GENERATION OF A TRANSCRIPTIONAL SIGNATURE DESCRIBING NEUROPROTECTIVE LIPOXIN ACTIVITY

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Background

- Lipoxin A_4 (LXA_4) and Lipoxin B_4 (LXB_4) are lipid isomers that produce direct neuroprotection of retinal ganglion cells in acute and chronic rodent models of glaucomatous injury.
- The general bioactivity of LXA_4 has been well characterized through its primary receptor; Lipoxin A_4 receptor/Formyl peptide receptor 2 (ALX/FPR2). Yet, LXB_4 activity is not mediated through the ALX receptor, and its specific bioactivity remains unidentified.
- The signaling cascades induced by lipoxins in a neuronal context are unknown.

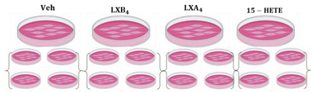
Purpose and Hypothesis

- To identify a molecular signature describing the early transcriptional changes of neuronal LXA_4 and LXB_4 treatments to identify key genes involved in their activities.
- Hypothesis: Characterizing transcriptional changes specific to LXA_4 and LXB_4 signaling will identify a molecular signature that includes key genes necessary for their neuroprotective effects.

Methods

RNA Sequencing collection:

- HT22 neuronal cells were seeded into 6 well plates and grown overnight at 37°C. Cells were treated with Veh, LXB_4, LXA_4 & 15-HETE for 1 hour at 1µM. RNA was then collected, purified and sequenced.



Bioinformatic Analysis:

- Hisat2 was used to map transcripts to a reference mouse genome, HTSeq to count reads, and DeSEQ2 for differential gene expression analysis. Subsequent comparisons were performed in R to determine transcripts specific to the treatment groups.
- 15-HETE is an LXB_4 precursor with similar structure to LXB_4 and no neuroprotective activity. Shared 15-HETE and lipoxin treatment transcripts were subtracted to give LXA_4 and LXB_4 specific gene lists.

Results

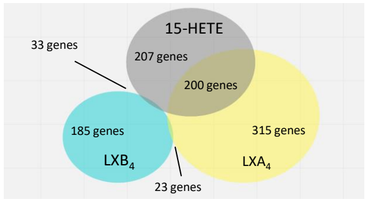


Figure 1: Transcript counts of lipoxin treatment groups and control. Groups of interest are marked with a star.

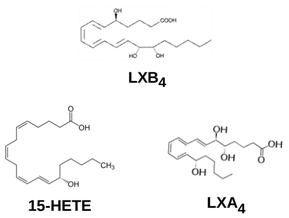


Figure 2: Molecular structure of LXB_4, LXA_4 and 15-HETE.

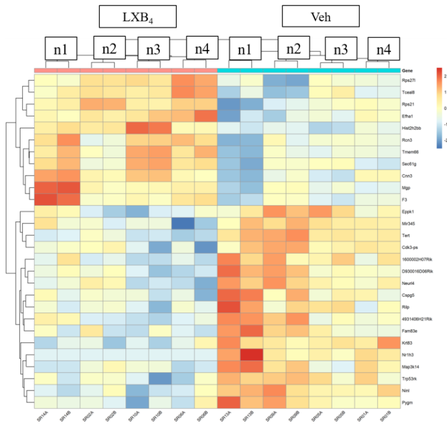


Figure 3: Gene expression with LXB_4 treatment (*left*) shows a negative pattern to control (*right*). N=4. p<0.01.

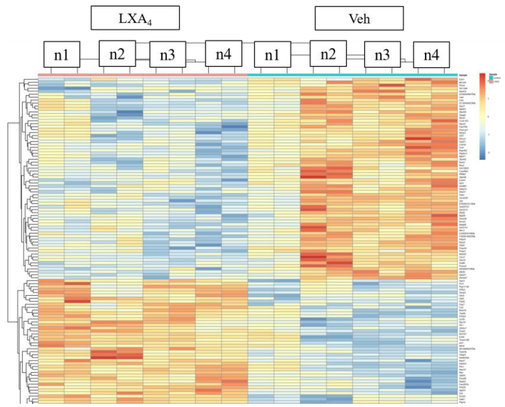


Figure 4: Gene expression with LXA_4 treatment (*left*) shows a negative pattern to control (*right*). N=4. p<0.01.

Figure 3 & 4 Legend

- Each replicate has 2 reads for a total of 8 replicates per group.
- Relative to control, lower normalized read counts are denoted in blue, and higher normalized read counts in red.

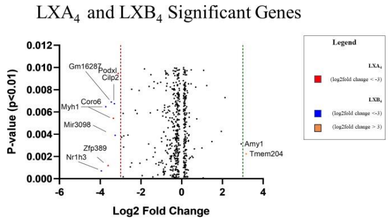


Figure 6: Comparison of differential gene expression with LXB_4 (*blue, orange*) and LXA_4 (*red*) treatment.

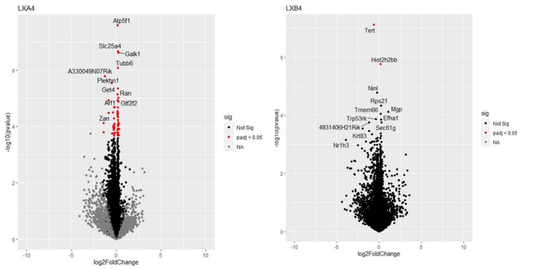


Figure 5: Bioinformatic analysis of LXA_4 (*left*) and LXB_4 (*right*) specific transcripts at p.adj <0.05. Only 2 genes pass threshold in LXB_4 group, and 10> genes in LXA_4 group.

Conclusion

- Despite their activities as neuroprotective agents, LXA_4 and LXB_4 bioactivities have not yet been fully characterized.
- The current project identified a key set of genes that may play a role in lipoxin activities by way of next generation sequencing.
- A list of LXA_4 and LXB_4 specific genes were identified and next steps include validation and characterization of top hits to determine their roles in a neuroprotective context.

References

1. Livne-Bar et al. Astrocyte derived lipoxins A4 and B4 promote neuroprotection from acute and chronic injury. Journal of Clinical Investigation. 2017; 127(12):4403-4414. Published 2017 Dec 1. doi: 10.1172/JCI77398.

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