Identifying an Inhibitor for Vertebrate Lonesome and its Effect on Retinal Ganglion Cell Regeneration

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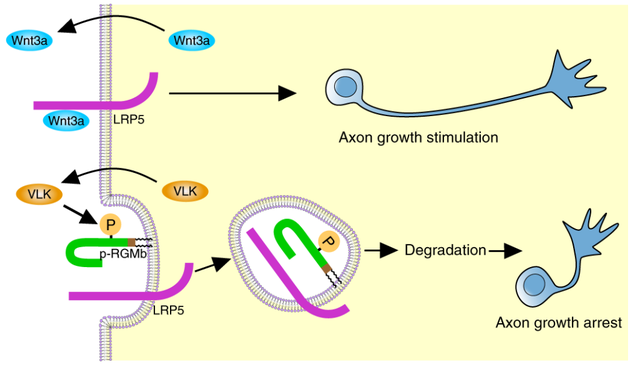
Introduction

- Vertebrate Lonesome Kinase (VLK) is a secreted tyrosine kinase which phosphorylates a variety of extracellular matrix and membrane associated targets[1]
  - Involved in a wide array of processes including guidance of retinal ganglion cells (RGCs) to the optic tectum[2] and phosphorylation of secreted trabecular meshwork proteins involved in glaucoma[3]
- During RGCs guidance VLK phosphorylates Repulsive Guidance Molecule B (RGMb) allowing for proper growth and guidance[2]
- However, phosphorylated RGMb leads to the internalization of the Wnt3a receptor LRP5[2]
  - Internalization of LRP5 can promote axon growth arrest[4]
- During neurodegeneration there is an increase in extracellular ATP as a result of cell death[5]
  - Thus, providing an ATP source for VLK function



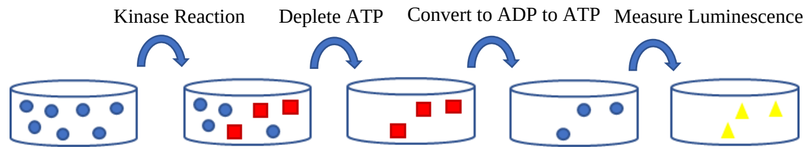
Hypothesis + Aims

- We hypothesize that inhibition of VLK may lead to retinal ganglion cell regeneration/protection during degeneration. We aim to:
  1. Identify VLK inhibitor using high throughput screening
  2. In vitro experiments applying VLK inhibitors to retinal ganglion cells

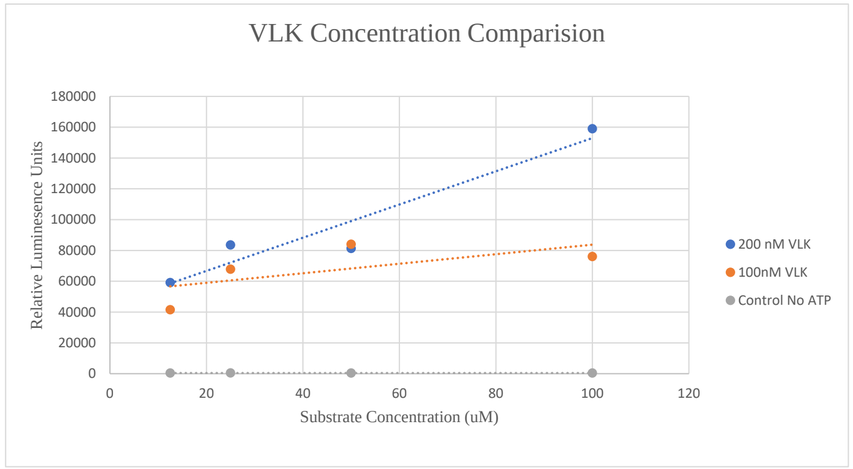
Methods

Optimizing VLK kinase reaction for screening

- To find optimal substrate concentrations for optimal VLK activity, 2-fold dilutions of substrate were made, and kinase reaction was performed at a constant ATP and kinase concentrations
- Assay was performed using ADP-Glo assay
- After kinase reaction was completed, remaining ATP in well was depleted
  - ADP formed from reaction was reconverted to ATP
- ATP then underwent a luciferase light reaction and relative luminescence was measured



Preliminary Results



- Observed difference in relative luminescence at 100µm of peptide between 200nM of VLK and 100nM
- No increase in luminescence past 80µm at 100nM VLK

Future Directions

- Using optimized kinase reaction conditions, screen kinase inhibitor library to identify specific VLK inhibitor
- Identify Km for VLK and IC 50 of inhibitors
- Apply inhibitor to retinal ganglion cells to evaluate effects of inhibitor on cell growth
- Assess if in vivo injections of inhibitor can protect/rescue neurodegeneration

Significance

- Our experiments will identify a specific inhibitor for VLK
- Apart from the importance of finding an inhibitor for an extracellular kinase involved in a wide array of targets, it can potentially be used to as a therapy for retinal degeneration and other visual disorders

References

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