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

Eric Mun, Philippe P. Monnier

1Department of Physiology, University of Toronto, 2Division of Genetics and Development, Krembil Discovery Tower

Introduction

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

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