Axon pruning is the process of selectively degenerating axons in neurons. Axon pruning is important for peripheral nervous system development. Aberrant axon pruning is implicated in neurodegenerative diseases such as Alzheimer's Disease.

NLRP1 (NOD-like receptor pyrin domain containing 1) is required for axon pruning to occur.

NLRP1 is a non-canonical inflammasome with a unique mechanism of activation.

NLRP1 plays a novel role in axon pruning that is independent of the immune system.

Figure 1. Proposed axon pruning pathway. Pro-apoptotic proteins c-Jun, Bax, Caspase-9, and Caspase-3 are required for axon pruning. Apaf-1 is not required for pruning.

Figure 2. Schematic of canonical and non-canonical inflammasome activation. NLRP1 is non-canonically activated by N' cleavage and proteasomal degradation.

Results

Determinates of Best Lentiviruses for Experiment:

Figure 6. Plan to determine best lentivirus plasmid for experiments. Neurons are plated in dishes rather than microfluidic devices to assess transduction. Virus efficacy is measured by strength of HA and 3X FLAG tags/mNeonGreen expression, as seen by fluorescent microscopy.

Figure 7. Phase/Fluorescent images of lentivirus expression. Highest expression was seen with mNG and 3X FLAG. None of the constructs showed good HA expression. Mutant NLRP1 exhibits higher expression of mNG and 3X FLAG.

Conclusion & Forward Directions

Through this experiment, we identified which plasmid constructs were best to use in the main experiment.

HA signal is absent in both of the best constructs, though there is green fluorescent signal (mNG or 3X FLAG), indicating that HA tag may not be viable.

Currently testing plasmid HA expression in different cells than neurons, as neurons may be the issue.

After verifying HA tag expression, the main experiment can proceed with the selected lentiviruses.

If the NLRP1 FiIND mutant cannot restore pruning, then the FiIND domain is required.

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