Functional Activity of NLRP1 in Axon Pruning

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INTRODUCTION

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

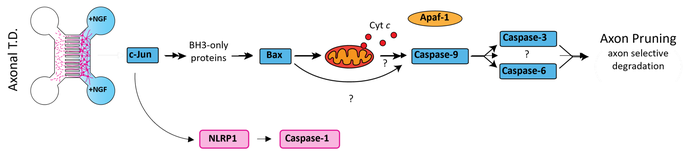

Figure 1. Proposed axon pruning pathway. Pro-apoptotic proteins cJun, Bax, Caspase-9, and Caspase-3 are required for axon pruning. Apaf-1 is not required for pruning.

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

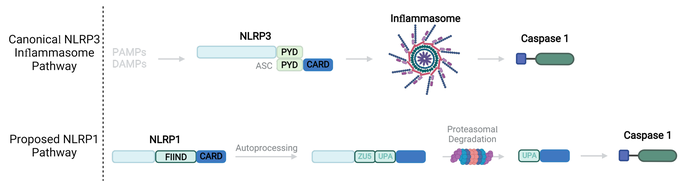

Figure 2. Schematic of canonical and non-canonical inflammasome activation. NLRP1 is non-canonically activated by N' cleavage and proteasomal degradation

METHODS

Experimental Approach:

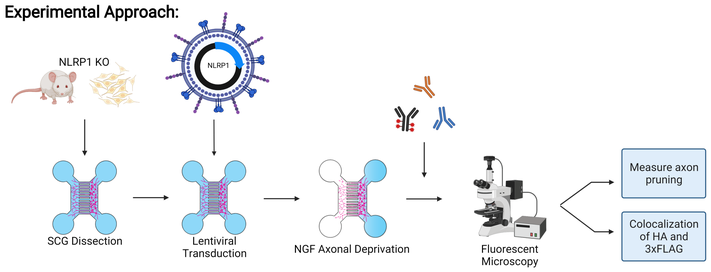

Figure 3. Experimental approach: Superior cervical ganglia (SCG) neurons are isolated from mice and plated into microfluidic devices. After 3 days in vitro (3 DIV), the neurons are transduced with lentivirus. After another 2 days in vitro the neurons are deprived of axonal NGF to induce pruning for 98 hours. Chambers are then processed with fluorescent antibodies to measure axon pruning and co-localization of HA and 3X FLAG.

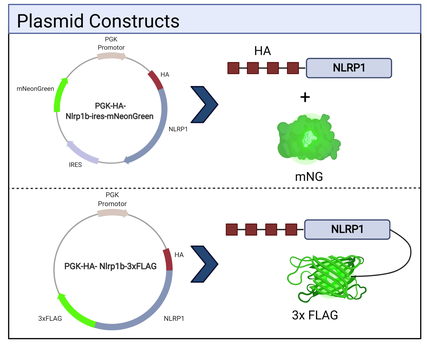

Figure 4. NLRP1 lentivirus constructs. 1) NLRP1 with N' HA tag and mNeonGreen (mNG) expressed after an IRES element. 2) NLRP1 with N' HA tag and C' 3X FLAG tag.

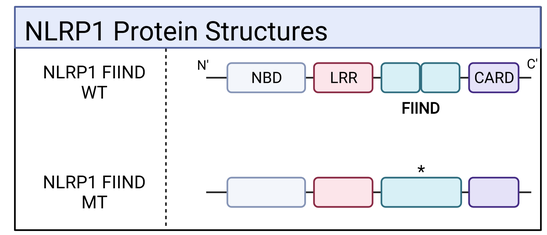

Figure 5. Schematic of mouse NLRP1 structure. NBD= nucleotide binding domain; LRR = Leucine Rich Repeats; FIIND = function-to-find (also known as the ZU5-UPA domain); CARD = Caspase activation and recruitment domain. Mutant (MT) NLRP1 has a single nucleotide mutation that prevents FIIND domain autocleavage, resulting in NLRP1 being constitutively inactive.

RESULTS

Determination of Best Lentiviruses for Experiment:

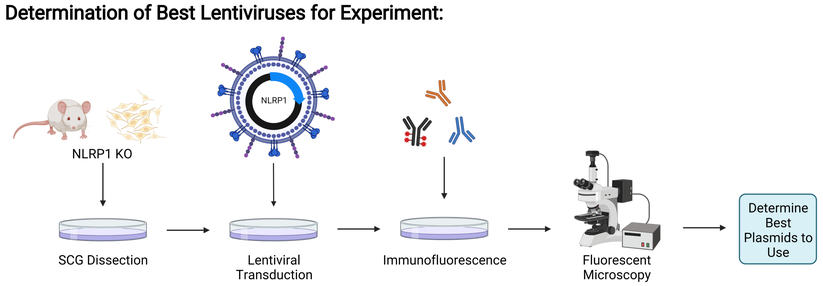

Figure 6. Plan to determine best lentivirus plasmid for experiments. Neurons are plated in dishes rather than microfluidic devices to assess transduction. Virus efficacy is measured by strength of HA and 3X FLAG tags/mNeonGreen expression, as seen by fluorescent microscopy.

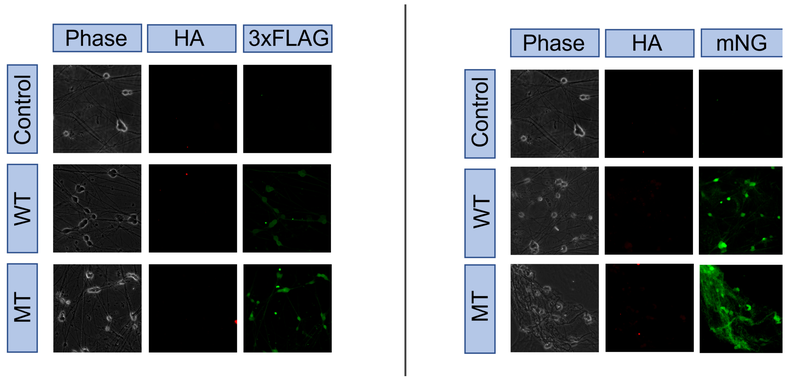

Figure 7. Phase/Fluorescent images of lentivirus expression. Highest expression was seen with mNG and 3X FLAG. None of the constructs showed good HA expression. Mutant NLRP1 exhibits higher expression of mNG and 3X FLAG.

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

ACKNOWLEDGEMENTS

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