Identification of Alternative Transcription Start Sites that Generate Neuron-Specific nhsl1b Isoform that Regulates Neuron Migration

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Introduction

Neuron migration is a fundamental step in the assembly of neural circuits. The neuron cell body travels significant distances from where they are born to where the nerve cell is needed to function. Mutations in genes that regulate neuronal migration lead to human congenital disorders such as lissencephaly and epilepsy.

It is established that Planar cell polarity (PCP) signaling is required for the alignment of cells within an epithelium, as well as directional migration. Nhsl1b is a novel, neuronal downstream effector of the PCP pathway, involved in the migration of neurons.

As a model system, we study the caudal migration of Facial Branchiomotor (FBM) neurons in the hindbrain of developing zebrafish. As a model system, we study the caudal migration of Facial Branchiomotor (FBM) neurons in the hindbrain of developing zebrafish.

Mutations in a novel gene called Nance-Horan Syndrome-like 1b (Nhsl1b) lead to a dramatic loss of posterior migration of FBM neurons. It remains unclear the function of Nhsl1b in neuron migration.

It remains unclear the function of Nhsl1b in neuron migration. Here we examine which isoform of Nhsl1b is responsible for neuron migration.

In order to determine whether Nhsl1b is required for FBM neuron migration, we generated guide RNAs (gRNA) targeting ex1d for co-injection with Cas9 mRNA in Tg(islet1:GFP) embryos.

When CRISPR guide RNAs are injected in founder (F0) embryos, we can generate mosaic embryos carrying various mutations in different early stem cells. Some cells in the embryos carry predominantly heterozygous mutations, some cells carry mutations in both chromosomes (bi-allelic mutations). We inject high levels of gRNAs in order to promote as many cells carrying bi-allelic mutations as we can.

Conclusion:

Our data suggests:
1) Most Nhsl1b variants are generally expressed throughout the nervous system, particularly in neural progenitor cells.
2) That Nhsl1b is the only Nhsl1b variant that is enriched in FBM neurons and may be a neuron-specific Nhsl1b isoform that is required for FBM neuron migration.

Future Direction:

- Investigate activation of the ex1d promoter.
- Further study neuron expression in the absence of other ex1 isoforms.

Genomic Architecture of nhs1b

Nhsl1b in zebrafish:
- 8 coding exons.
- Exon 5 is variably spliced
- 6 variants of exon 1, each with its own 5’-UTR and transcriptional start site (TSS).

We used a bioinformatics approach to determine the protein domains encoded by Nhsl1b. No known protein domains were found except in isoforms derived from the first alternative transcription start site – encoding Nhsl1b^5

Hypothesis: Nhsl1b^5 variant is the most important isoform to direct neuron migration because it has an N-terminal WAVE homology domain.

What happens to neuron migration in the absence of exon 1d?

In order to determine which Nhsl1b variants is required for FBM neuron migration, we generated guide RNAs (gRNA) targeting ex1d for co-injection with Cas9 mRNA in Tg(islet1:GFP) embryos.

Injections of Nhsl1b-ex1d gRNAs led to variable defects in FBM migration. We scored these defects as normal, partial defects, or severe defects.

We quantified the number of injected embryos that displayed defects.

Conclusion:

Mutations in ex1d/Nhsl1b leads to a severe migration defect of motor neurons consistent with the idea that ex1d/Nhsl1b is a neuron-specific isoform that is essential for neuron migration.

• Investigate activation of the ex1d promoter.
• Further study neuron expression in the absence of other ex1 isoforms.