THE ROLE OF ATOH1 IN REGULATING THE DEVELOPMENT OF PONTINE NUCLEI NEURONS

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Keywords: Single-cell RNA-seq, Dcc, neuronal migration

Background: Pontine nuclei (PN) play an important role in motor function by mediating the communication between cerebral cortex and cerebellum. Previous studies in our lab showed that Atoh1 is required for PN development. Mice carrying an Atoh1 hypomorphic mutation exhibit reduced size and migratory deficits in PN. Although several guidance molecules and their receptors have been shown to govern PN migration, little is known about how these receptors are transcriptionally regulated in PN. Moreover, it remains unclear if the cellular identity of PN neurons is altered when migration is compromised. In this study, we aim to identify the molecular mechanisms by which Atoh1 regulates migration and cellular identity of PN neurons.

Materials/Methods: To test if Atoh1 mediates neuronal migration by upregulating receptors, we studied the genetic interaction between Atoh1 and Dcc, a known receptor mediating PN neuron migration. To unbiasedly identify addtional Atoh1 downstream targets, we perform single-cell RNA-seq (scRNA-seq) on Atoh1-lineage neurons from mouse E14.5 and E18.5 hindbrain. In addition, to determine if the cellular identity of PN neurons is altered by partial loss-of-function of Atoh1 and/or Dcc, we compared scRNA-seq data from wild-type, Dcc heterozygous, Atoh1 hypomorph, and animals carrying both mutant alleles (double mutant).

Results: We have confirmed that Atoh1 regulates Dcc expression level by binding to its gene regulatory elements. Histological analyses reveal that Atoh1/Dcc double mutant mice have profound loss of PN neurons with an uneven distribution at ventral pons. ScRNA-seq analyses elucidate that PN neurons can be molecularly classified into different subtypes at E18.5. Interestingly, the Atoh1 hypomorph and double mutant animals had fewer cells in two of the PN subtypes but had an increased number of cells in the progenitor population. By examining the expression patterns of the marker genes from Allen Brain Atlas, the two affected subtypes seem to be located at the most anterior and dorsal side of PN. In future works, we will use RNA in situ hybridization to validate the spatial information of different PN subtypes.

Conclusions: This study for the first time demonstrates the heterogeneity of PN neurons at single-cell level and suggests that Atoh1 coordinates PN neuron differentiation, specification, and migration by orchestrating downstream target genes.

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