ELUCIDATING THE MOLECULAR BASIS OF ATOH1 LINEAGE DIVERSITY IN THE DEVELOPING HINDBRAIN

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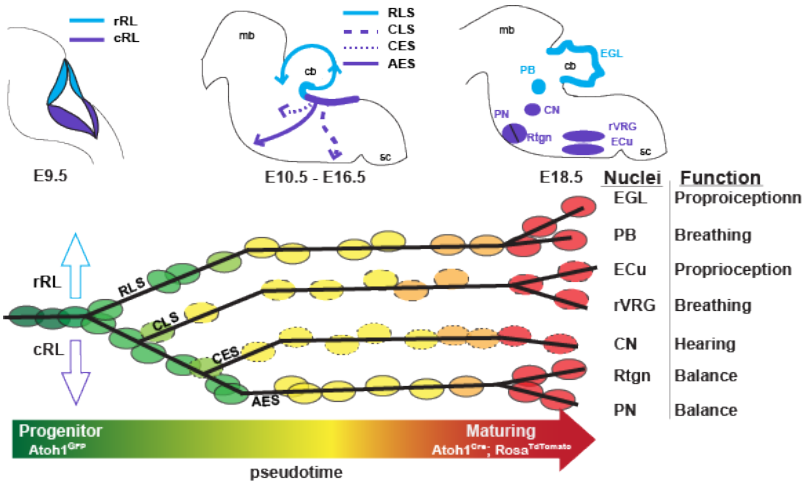
Background: The proneural transcription factor, Atoh1, gives rise to a progenitor population in the rhombic lip of the developing hindbrain and is responsible for differentiation of over 40 different nuclei in the cerebellum and brainstem involved in breathing, hearing, and proprioception. Studies have qualitatively described how the Atoh1-lineage leaves the rhombic lip in time-specific migration streams. However, the transcriptional cascade that drives a seemingly homogenous population to differentiate through space and time while acquiring diverse function is unknown. We hypothesize that there are different transcriptional cues to drive neural fate decision of diverse Atoh1-lineage nuclei from one common progenitor.

Materials/Methods: To profile Atoh1-lineage cells, two florescent genetic mouse models were used to detect Atoh1-expressing cells in real-time and to trace cells derived from the Atoh1-lineage. To reveal the transcriptomic cascade through developmental time, single cell RNA-sequencing (scRNA-seq) was performed in Atoh1-lineage cells from genetically modified E9.5 to E16.5 embryos. In addition, we profiled DNA binding of Atoh1 at E12.5 and E14.5 to understand the role of Atoh1 binding in regulating the transcriptomic changes.

Results: A transcriptomic map of Atoh1-lineage development was built from the scRNA-seq including identification of the progenitor population, migration streams, and mature Atoh1-derived nuclei. The Atoh1 progenitor population was found to be transcriptionally heterogenous at distinct stages, suggesting the rhombic lip progenitor pool changes throughout development. As the progenitors begin to differentiate, Notch receptors were down-regulated and Notch ligands were increased, implicating Notch signaling as a potential cue to begin migration and differentiation. To determine if Atoh1 binding reflects gene expression changes, differentially expressed genes (DEGs) were compared to genes that contained an Atoh1 binding peak. We found that 89.9% and 65.9% of DEGs contained an Atoh1 peak at E12.5 and E14.5, respectively. Future work will corroborate transcriptional decision points with Atoh1 binding data to determine when Atoh1 is directly involved in neural fate decisions.

Conclusions: In conclusion, we are elucidating the transcriptional mechanism behind Atoh1-lineage fate decisions in the developing hindbrain.

Images / Graph / Table


rRL
cRL
E9.5
RLS
CLS
CES
AES
mb
cb
sc
E10.5 - E16.5
EGL
PB
CN
PN
Rtgn
rVRG
ECu
E18.5
Nuclei
Function
EGL
Proproiceptionn
PB
Breathing
ECu
Proprioception
rVRG
Breathing
CN
Hearing
Rtgn
Balance
PN
Balance
rRL
cRL
RLS
CLS
CES
AES
Progenitor
Atoh1GFP
Maturing
Atoh1Cre; RosaTdTomato
pseudotime