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

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Background: A handful of neural progenitors give rise to thousands of diverse neuronal cell types that perform complex functions. In the developing hindbrain, the proneural transcription factor, Atonal homolog 1 (Atoh1), is expressed in the rhombic lip (RL) and gives rise to all excitatory neurons in the cerebellum and dozens of brainstem nuclei responsible for critical functions including balance, hearing, and breathing. However, the transcriptional changes that instruct Atoh1 progenitors to leave the RL and migrate to spatially diverse locations throughout the hindbrain are still unknown. The hypothesis of this work is that Atoh1 progenitors undergo temporal and spatial fate decisions driven by a defined molecular network.

Materials/Methods: To determine the molecular network that drives Atoh1 lineage development, I have utilized the combination of two genetic mouse models that express independent fluorescent reporters during distinct stages of Atoh1 development. We have performed immunostaining and 3D light sheet imaging to determine the spatial distribution of Atoh1 progenitors compared to Atoh1 migrating cells. Further, to determine the transcriptional changes that drive Atoh1 lineage development, we have performed single cell RNA sequencing on the fluorescently sorted Atoh1 cells at multiple embryonic time points.

Results: We have demonstrated with immunostaining and 3D lightsheet imaging that fluorescent reporter expression can distinguish Atoh1 expressing neural progenitors at the RL from migrating Atoh1 lineage traced cells. To perform transcriptome analysis on just the Atoh1 lineage, we have successfully sorted live Atoh1 progenitors and lineage traced cells from whole hindbrain tissue at several embryonic stages. Initial bioinformatic analysis from the fluorescently sorted populations reveals that the Atoh1 population clusters by transcripts that define maturation state and regional identity. In future work, we will perform trajectory analysis to identify key nodes driving neural fate specification in the Atoh1 lineage.

Conclusions: Together, this study aims to elucidate the transcriptional programs of Atoh1 progenitors to understand the complexity of neuronal fate decisions in the developing hindbrain.