Molecular mechanisms of phrenic motor neuron development and connectivity

Alicia N. Vagnozzi and Polyxeni Philippidou

Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH

The acquisition of neuronal identity through distinct transcriptional programs is critical for establishing synaptic specificity during development. Despite evidence that transcriptional programs dictate subtype-specific features, it is not clear how they impact circuit connectivity and behavior. The neural circuits that control respiration provide an excellent model to probe this question, as phrenic motor neurons (MNs) in the spinal cord provide the only motor input to the diaphragm, the major inspiratory muscle. Therefore, changes in the identity and connectivity of phrenic MNs have a robust impact on breathing. We have established that the transcription factors Hoxa5 and Hoxc5 are required for the development of the Phrenic Motor Column (PMC). Mice with a MN-specific deletion of Hox5 genes (Hox5MNΔ) exhibit defects in PMC cell body clustering and a dramatic reduction in axonal branching. Here, we show that Hoxa5MNΔ mice display changes in respiratory behaviors, such as elevated breathing frequency. Phrenic nerve recordings showed that PMC neurons in Hoxa5MNΔ mice displayed irregular and more frequent bursting, suggesting a reconfiguration of respiratory circuits. Consistent with this hypothesis, the dendritic orientation of PMC neurons was altered in Hoxa5MNΔ mice, indicating potential changes in PMC synaptic inputs. To identify the downstream pathways responsible for these effects, we performed RNA-seq in Hox5MNΔ embryos and identified 213 differentially expressed genes. Gene ontology analysis indicated a predominance of targets involved in cell adhesion, and several of these genes were validated as Hox5 targets by in situ hybridization. We will further analyze promising candidates by examining knockout mice for defects in phrenic MN development and respiratory behavior. Our analysis will provide insight to a fundamental principle of nervous system development: how transcriptional networks control the assembly of neural circuits and, ultimately, behavior.