Single-cell transcriptomic analysis reveals genetic drivers of slow/fast motor neuron identity

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Abstract

Skeletal motor neurons are the essential cells connecting the spinal cord and muscles, translating our thoughts and needs into actions and response. To match the specific muscles they control, motor neurons must tune their functional properties to the unique demands of their environment. "Slow-firing" motor neurons innervate "slow-twitch" muscle fibers, and "fast-firing" motor neurons innervate "fast-twitch" fibers. Interestingly, among other electrophysiological differences, slow-firing motor neurons are strikingly more resistant to the neurodegenerative disease amyotrophic lateral sclerosis (ALS), while fast-firing motor neurons are vulnerable. Here, we analyze a previously published single-cell dataset to explore the gene expression differences that distinguish slow and fast-firing motor neuron subtypes. Surprisingly, we find that the two subtypes do not transcriptionally cluster into separate, discrete populations. Rather, we find that all motor neurons exist on a continuous spectrum that corresponds to electrophysiological identity. We establish an individual per-neuron 'Fast Score,' and we show that canonical markers of slow and fast motor neurons exist on opposite ends of a continuous spectrum. This finding challenges the conventional wisdom that slow and fast motor neurons are discrete cell types – instead arguing for a new model of motor neuron identity. Using regression analysis, we explore the hypothesis that transcription factors are responsible for polarizing motor neurons along this continuous spectrum. We discover modules of genes controlling canonical properties of slow and fast motor neurons, affirming our hypothesis that gene expression differences encode functional motor neuron properties. Finally, we granularize our data into transcription factor networks, allowing us to resolve the master regulators of cell identity in slow and fast motor neurons. Defining the regulatory logic that polarizes fast and slow-firing motor neurons holds the potential to unlock interconversion between these important and disease-relevant cell types. In the future, manipulation of these networks may present viable therapeutic strategies in neuromuscular diseases like ALS and inform our ability to generate motor neuron subtypes in induced pluripotent stem cell (iPSC)-derived models of disease.