RESEARCH HIGHLIGHT

It's me, hi, I solved the problem, it's TF-seqFISH

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In a recent *Cell Research* study, Shi et al. characterize gene expression changes across time and space in the developing human spinal cord and present a new spatial transcriptomics method focused on transcription factors. They identify key features of human spinal cord development, providing an important resource for future investigations.

The spinal cord takes input from the brain and the body's sensory systems and transforms it into motor output. To do this, spinal cord neurons must have specialized functions and integrate into proper circuits. During embryonic development, the spinal cord is made up of a mixture of cells, including neurons, non-neuronal support cells, and various populations of progenitor cells, which give rise to mature cell types at the right time and place. To reveal transcriptional programs involved in human spinal cord development, Shi et al.[1] measured thousands of RNA molecules at a time in individual cells using single-cell RNA-sequencing (scRNA-seq) and in particular locations of the spinal cord using spatial transcriptomics.

Shi et al. started by using scRNA-seq to profile 217,636 cells from developing human spinal cords spanning gestational weeks 7 through 25. To boost their analysis, they combined their data with a dataset from a recent study,[2] resulting in RNA profiles from a total of 912,514 cells. They assigned cells to groups, or 'clusters', corresponding to dividing cells, progenitors, mature neurons and glia, and other cell types. To provide insight into how dividing and progenitor cells give rise to mature, differentiated cell types, the authors used a computational technique called RNA velocity analysis. They found transcriptionally distinct populations of dividing cells that may exclusively give rise to neurons, astrocytes, or oligodendrocytes. This suggests that cell fate could already be specified in these dividing cells. They also identified a population of glial progenitor cells that likely gives rise to both astrocytes and oligodendrocytes. Additionally, the authors found a higher proportion of cells in the neurogenic lineage at earlier time points and a higher proportion of cells in astrocytic and oligodendrocytic lineages later in development, demonstrating that neurogenesis initiates earlier than gliogenesis in the human spinal cord.

Distinct neural progenitor cell populations arise in the developing spinal cord along the dorsal-ventral axis (back-to-front, Fig. 1a) in a region called the ventricular zone. These different populations express unique combinations of transcription factors, proteins that bind to DNA and regulate gene expression, and ultimately give rise to distinct classes of neurons (Fig. 1b).[3,4] Using RNA velocity analysis, the authors identified six neuronal lineages, each consisting of distinct neural progenitor populations and the neuron types they differentiate into. Because transcription factors likely play key roles in specifying each of these neural progenitor populations, the authors developed a new technique called TF-seqFISH, which allowed them to measure the expression of over one thousand transcription factors across space in the developing spinal cord at gestational week 8. Their RNA velocity and TF-seqFISH results were largely consistent with findings from developing mouse spinal cords,[5,6] suggesting conservation of gene expression programs across species. The authors also identified several novel marker genes, which might be primate or human specific.

The authors next extended their TF-seqFISH analyses to other regions of the spinal cord. The cells profiled by TF-seqFISH are grouped into 18 clusters based on gene expression, and interestingly, each cluster belongs to one of five spatially distinct regions of the spinal cord. To infer the expression of non-transcription factors across space, the authors cleverly combined their TF-seqFISH and scRNA-seq data. With the combined dataset in hand, the authors asked how gene expression changes along the medial-lateral axis (middle-to-side, Fig. 1a), mostly focused on the dorsal part of the spinal cord, which is important for processing and transmitting sensory information. They found that cells in the most medial region (ventricular zone) express genes related to cell division and stem cell maintenance. In contrast, cells in the more lateral regions (intermediate zone and mantle zone) express genes involved in neuronal differentiation, migration, and projection development. The authors also classified neurons based on the neurotransmitter they use to send signals to other cells of the body. At gestational week 8, excitatory neurons, which use the neurotransmitter glutamate, are sandwiched between inhibitory neurons, which release GABA or glycine, in the dorsal spinal cord. This sandwich-like structure of neurons is observed transiently in the developing spinal cord, before neurons organize into distinct laminae, or layers, which are evident in the adult.

Shi et al. next switched focus from the dorsal to the ventral spinal cord, where the cell bodies of motor neurons reside. Motor neurons are grouped into distinct 'columns', which have specific locations along the rostral-caudal axis (head-to-tail, Fig. 1a) and make connections with different target muscles. The authors identified populations of motor neuron progenitors, newly born motor neurons, and motor neurons with distinct column identities, and then used RNA velocity analysis to infer gene expression changes that occur over time as progenitors differentiate into motor neurons. They found motor neurons with distinct columnar identities even at very early time points and provided evidence that column-specific transcriptional signatures may already be present in newly born motor neurons.

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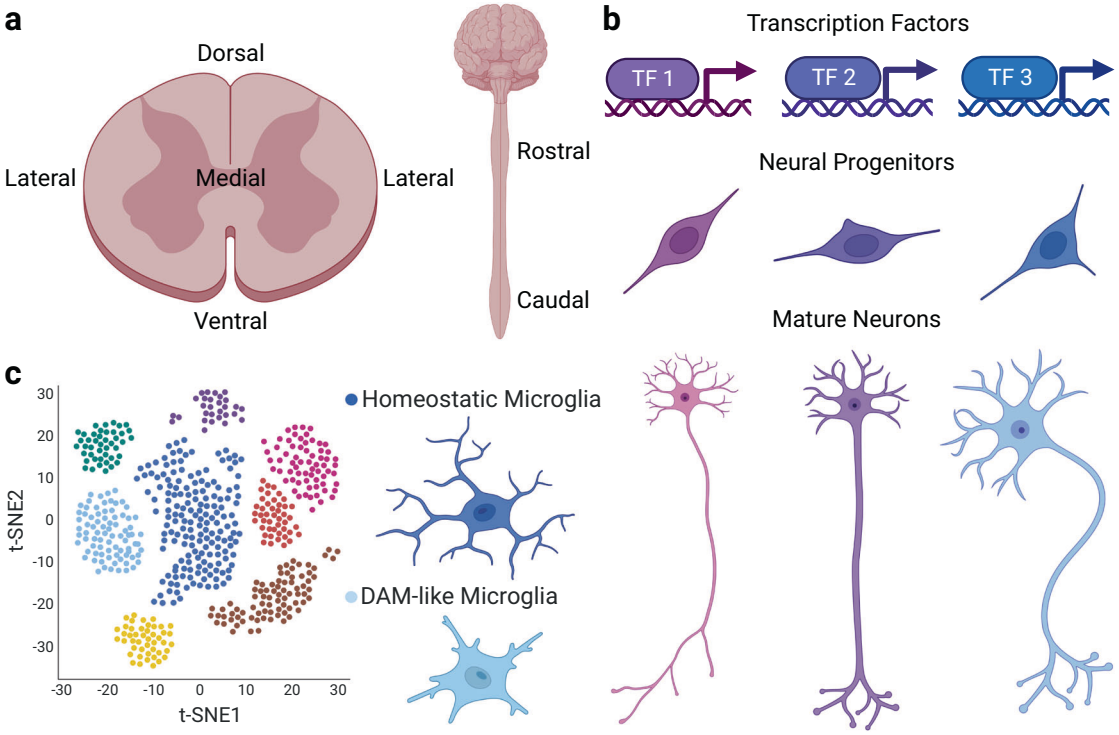

Fig. 1 Anatomical orientation and cell type diversity in the human spinal cord. a Anatomical axes of a cross-section of the adult spinal cord (left) and the brain and spinal cord (right). **b** Different populations of neural progenitor cells in the developing spinal cord express unique combinations of transcription factors and give rise to distinct neuron types. **c** Representative scRNA-seq results from microglia of the developing human spinal cord. Dots represent single cells, and the colors mark different clusters. Clusters correspond to different types of microglia, such as homeostatic and DAM-like microglia.

Motor neurons selectively degenerate in the neurodegenerative disease amyotrophic lateral sclerosis (ALS).[7] Shi et al. identified ALS risk genes with enriched expression in particular spinal cord cell types, including motor neurons, microglia, endothelial cells, and pericytes. The microglial subpopulations with the strongest connection to ALS genetics also exhibit transcriptional characteristics of microglia associated with neurodegenerative diseases (disease-associated microglia (DAM), Fig. 1c). ALS is typically an adult-onset disorder; therefore, it will be interesting to compare transcriptional signatures from the developing spinal cord to their counterparts in adult.

In summary, Shi et al. performed scRNA-seq and spatial transcriptomics throughout human spinal cord development, and invented a new technology, called TF-seqFISH. Their work and other recent studies[1,2,8–10] have elucidated important aspects of human spinal cord development and offer valuable resources for future research. These kinds of techniques can be used to compare gene expression profiles from developing and adult spinal cords in health and in disease, potentially paving the way for new treatment approaches.

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ADDITIONAL INFORMATION

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