A Coordinated Map of the Spatial Arrangement and Cell types in the Mouse Spinal Cord

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Abstract

Data from single-cell RNA sequencing can reveal the molecular variety of different cell types. Recent publications of cell type atlases for the mouse spinal cord have not yet been combined. Here, using single-cell transcriptome data, we create an atlas of spinal cell types, combining the various datasets into a single frame of reference. We present a hierarchical framework of postnatal cell type interactions, with location serving as the highest level of organisation, followed by neurotransmitter status, family, and dozens of refined populations. We map the geographical distributions of each type of neuronal cell in the adult spinal cord and validate a combinatorial marker code for each. We also demonstrate intricate lineage links between several postnatal cell types. To aid in the standardisation of cell type identification, we also create the open-source cell type classifier SeqSeek. An integrated understanding of the various types of spinal cells, their molecular arrangement, and their gene expression profiles is provided by this work.

Keywords: Neurotransmitter • RNA sequencing • Postnatal cell

Introduction

Numerous areas of biology are being transformed by a breakthrough in single-cell sequencing technologies. We can simultaneously define cell types, characterise their molecular signatures, and monitor how each cell type in tissue changes in different biological conditions like development and disease by sequencing the RNA/cDNA or open chromatin from many different cells and using computational analysis to identify shared patterns of gene expression or epigenetic structure. This method may potentially shed light on the cellular underpinnings of behaviour, give marker genes for the creation of genetic tools to influence neuronal function, and shed light on the molecular underpinnings of the astonishing levels of neuronal diversity found within the central nervous system. Multiple articles assessing single-cell RNA expression in the postnatal mouse spinal cord have covered a variety of biological characteristics, including age, tissue area, developmental lineage, and circuit properties. These studies offer a compelling and comprehensive viewpoint on the many spinal cord cell types, however despite this tremendous effort and the abundance of literature describing these cell types, there is still no widely accepted spinal cord cell type atlas. The absence of acknowledged ground truth regarding the cell types in this tissue that may serve as the foundation of a reference atlas is a significant barrier.

Unfortunately, even when the same tissue types and methods are employed throughout research, it can still be challenging to compare the data. It may also represent specific analysis parameters and technological artefacts that obscure underlying similarities between different investigations. This is partly due to biological variations and technology limitations. In fact, it is unclear whether the cell types from the original research are equivalent in their current forms, leaving the spinal cord with a fragmented collection of inconsistent atlases. These are some of the major obstacles that scientists confront when we rediscover the cells and tissues we study from the perspective of single-cell profiling, rather than being unique to the study of the spinal cord. We intended to create a standardised, validated atlas of postnatal spinal cord cell types that may disclose the organisational principles of spinal neuronal diversity and act as a baseline for future research in order to start overcoming these difficulties within the mammalian central nervous system. We started by merging and integrating the raw data from the first six postnatal spinal cord single-cell datasets that were made available to the general public. This meta-cells dataset's and nuclei were grouped, revealing 15 non-neural and 69 neural cell types. This cell type resolution and characterisation surpasses all previous research in both the breadth of general trends and the depth of its detail. We developed a combinatorial panel of many marker genes by examining gene expression profiles across families of cell types in order to quantify the geographic distribution and frequency of each cell type in adult tissue. We then verified this panel using high-content in situ hybridization. This research identified substantial disparities in the cell-type connections and molecular trends of dorsal and ventral neuronal cell types. We were able to deduce possible lineage ties for each postnatal cell type by co-integration with embryonic cell types, and we discovered intricate convergent contributions from numerous lineages to many cell types.

Finally, after evaluating numerous automated classification methods, we determined that a two-tiered approach based on label transfer and neural networks was the most effective strategy for categorising the various types of spinal cord cells. Here, we provide SeqSeek, a web-based tool that allows users to search this data by gene or cell type and access an automated categorization system for every spinal cord cell or nucleus using raw sequencing data.

Results

Combined examination of spinal cord nuclei and cells

First, we combined data from the first six published investigations of the postnatal mouse spinal cord, totaling over 100,000 cells and nuclei. Numerous biological and experimental parameters are covered in these investigations. We started with the raw sequencing reads from each study and processed the data independently using standardised techniques and filters in order to compare the data from these studies as accurately as possible. We utilised standard, liberal filtering levels for inclusion and exclusion after aligning all sequencing reads to a common genomic sequence that contained both exons and introns. As a result, this integrated dataset includes a homogeneous set of genes and more cells and nuclei than were examined in the initial investigations. In order to identify a common set of spinal cord cell types that would only require the resolution of nomenclature differences, our first major objective was to create a harmonized atlas of the major spinal cord cell types that are shared across these studies. To do this, we first considered whether it would be possible to register different studies to one another. We used the combined data (with common cutoff criteria and genes evaluated) and concentrated on dorsal neurons to directly compare the clusters between other research. We determined the average gene expression for each cluster in each study, after which we looked at the correlation between the studies' average gene expression levels.

There were just a few matches across clusters from different research when either all genes or the top 500 highly variable genes were evaluated. We came to the conclusion that it is not enough to merely register the previously released atlases to one another in order to create a reliable reference atlas. This is comparable to other publications that attempted to link cell types across research by correlating gene expression amongst clusters, but even when the same sample age and tissue separation method were employed across studies, this strategy produced weak and/or insufficient correlations. Next, we proposed that co-clustering of cells and nuclei across all studies would increase our capacity to connect different cell types across trials. Principal component analysis was used to reduce dimensionality, and Uniform Manifold Approximation and Projection (UMAP) plots were used to depict the cells and nuclei. The cells or nuclei from each study were, regrettably, virtually entirely separated from one another, suggesting that the study of origin is a significant source of heterogeneity in the dataset.

A harmonized atlas of major cell types

Each study's cell types were determined based on the methods employed to separate the cells or nuclei. In the three experiments, cells in the neuronal sub-clusters and non-neural cells that most likely represented doublets were

primarily generated from the spinal cord neurons by FACS sorting. Additionally, the early postnatal Rosenberg study revealed an enrichment of immature oligodendrocyte lineage cells in comparison to the adult Sathyamurthy study, while the teenage Zeisel study revealed an intermediate distribution among the three studies that looked at all cell types. The only study to examine the spinal cord in detail, including the dorsal and ventral spinal roots, was the only place to find Schwann and peripheral glia cells in these roots.

Overview of harmonized neuronal cell types

We performed a targeted sub-clustering of all mid and ventral cells/nuclei since preliminary analysis showed that putative dorsal horn clusters separated well in principal component space whereas putative mid and ventral horn clusters did not (see Methods). A total of 69 neuronal clusters were found, and by comparing marker gene expression to the results from the initial six experiments, it was possible to identify the neurotransmitter status and likely regional location (dorsal horn, mid-region, ventral horn). These conclusions were supported by later validation investigations. Twenty dorsal excitatory clusters, fourteen dorsal inhibitory clusters, ten deep dorsal/mid excitatory clusters, seven deep dorsal/mid inhibitory clusters, eight ventral excitatory clusters, six ventral inhibitory clusters, three clusters of cholinergic motoneuron, and one cluster of cerebrospinal fluid contacting neurons were all seen (CSF-cN). Due to low counts of genes per cell/nucleus and a lack of marker genes, some ventral neurons from the Sathyamurthy dataset appeared in low-quality clusters that were excluded from the harmonised analysis, whereas some neurons from the Haring dataset were categorised as non-neural cell types or appeared in doublet clusters that were also excluded from the harmonised analysis. However, we discovered that the cells and nuclei from the initial research were dispersed into the harmonised clusters in orderly ways that made it easier to register the original clusters based on their proximity in the neuron principal component space. Last but not least, we compared the patterns of all the marker genes we highlight in this paper to those found in a recent spatial transcriptomics analysis of the spinal cord28 as well as to those found in the Allen and Gensat expression databases, and we discovered a general agreement between these resources. Together, this analysis demonstrates the general reproducibility of spinal cord single-cell sequencing atlases while also highlighting the value of combining data from various sources to identify the most precise and resilient cell types and the necessity of having an annotated reference atlas to aid in cell type analysis in future research.

Discussion

Establishing a consistent collection of cell types is crucial if the study of spinal cord biology is to capitalise on the enormous potential of single-cell technology. In order to define 84 different types of spinal cord cells, we used and built upon previously reported single-cell sequencing investigations of the postnatal mouse spinal cord. We provide a harmonised atlas of these cell types, a validated combinatorial panel of markers to facilitate their study in vivo, in tissue sections, and in vitro cell culture, putative embryonic lineages for each cell type, computational tools for categorising spinal cord cells based on transcriptomics, and a web-based tool, SeqSeek, to enable the community to easily interact with and explore single cell spinal cord data. The first important question to ask is whether or not the biologically accurate cell types in the atlas are confused by technical problems brought on by the initial investigations or analysis decisions that we made here. It's likely, for instance, that combining these research might obscure crucial biological distinctions between them or that combining early postnatal and adult information would muddle accurate descriptions of cell types. It is impossible to provide a comprehensive response to this topic in the absence of a generally acknowledged standard set of spinal cord cell types. But there is evidence to suggest that the classification of spinal cord cell types is valid. Second, these clusters are consistent with previous gene expression analysis of the postnatal spinal cord, including a number of well-known marker gene studies from the past as well as three independent single nucleus sequencing datasets that were left out of the harmonised clustering: a separate dataset that we clustered separately and used to test the SeqSeek Classify algorithm, and two more recent studies that employed different analytic methods but identified similar markers. Thirdly, and most crucially, this atlas does not rely just on a small number of research or on computational methods that could be biassed by the tools and parameter selections used.

To verify the accuracy of anticipated expression patterns in the complete transverse view of adult lumbar spinal cord tissue, we used high content in situ hybridization. This data occasionally varied from the harmonised sequencing data, which would indicate different developmental trends. The resulting data, however, gave the most thorough definition of cell types, their prevalence, and their spatial distribution in the postnatal spinal cord because we validated the great majority of anticipated expression patterns from the harmonised atlas. Dorsal clusters can be roughly categorised into families and are distinct from one another due to their clearly delineated individual cell types. These cell types can be reliably distinguished by machine learning algorithms or in tissue with combinatorial marker genes because they can be found further apart from one another in principal component/UMAP space, have higher measures of robustness (like co-clustering frequency and silhouette score), and are located farther apart from one another. Ventral clusters, on the other hand, have near or overlapped distributions in principal component space as well as similar gene expression patterns. According to a recent study, there may be a second, nested level of spatial trends that organise the different types of ventral neuron cell types. These trends include a Pou6f2-Esrrg trend along the dorsal-ventral axis and Nfib-Zfhx3/4 and birthdate trends along the medial-lateral axis. Although the significance of these distinctions between the dorsal and mid/ventral spinal cords is unknown, it is an exciting potential that discrete versus overlapping sets of cell types could result in different computational features for networks.

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