Translational Research 000 (2022) 1–8

Contents lists available at ScienceDirect

Translational Research

journal homepage: www.elsevier.com/locate/jagp

Review article

Single-cell RNA sequencing in the context of neuropathic pain: progress, challenges, and prospects

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ARTICLE INFO

ABSTRACT

Neuropathic pain, characterized by persistent or intermittent spontaneous pain as well as some unpleasant abnormal sensations, is one of the most prevalent health problems in the world. Ectopic nerve activity, central and peripheral nociceptive sensitization and many other potential mechanisms may participate in neuropathic pain. The complexity and ambiguity of neuropathic pain mechanisms result in difficulties in pain management, and existing treatment plans provide less-than-satisfactory relief. In recent years, single-cell RNA sequencing (scRNA-seq) has been increasingly applied and has become a powerful means for biological researchers to explore the complexity of neurobiology. This technique can be used to perform unbiased, high-throughput and high-resolution transcriptional analyses of neuropathic pain-associated cells, improving the understanding of neuropathic pain mechanisms and enabling individualized pain management. To date, scRNA-seq has been preliminarily used in neuropathic pain research for applications such as compiling a dorsal root ganglion atlas, identifying new cell types and discovering gene regulatory networks associated with neuropathic pain. Although scRNA-seq is a relatively new technique in the neuropathic pain field, there have been several studies based on animal models. However, because of the various differences between animals and humans, more attention should be given to translational medicine research. With the aid of scRNA-seq, researchers can further explore the mechanism of neuropathic pain to improve the clinical understanding of the diagnosis, treatment and management of neuropathic pain.

Introduction

Neuropathic pain, defined as "pain caused by a lesion or disease of the somatosensory nervous system," is one of the most prevalent health problems in the world.[1-3] This type of pain is a major factor contributing to the global burden of disease according to the latest research on global diseases.[4,5] Patients with neuropathic pain tend to report persistent or intermittent spontaneous pain as well as some unpleasant abnormal sensations, revealing the severe somatic and psychological impacts of this condition on patients. In addition to the individual suffering caused by chronic pain, the condition is a considerable health and economic burden on society.[6] Although various therapeutic strategies have been used in attempts to treat neuropathic pain, the efficacy is poor in the majority of patients. This treatment failure is closely related to the fact that the mechanism of neuropathic pain is unexplored.

Single-cell RNA sequencing (scRNA-seq), a next-generation sequencing technique that has emerged in recent years, has been preliminarily used in neuropathic pain research for applications such as the identification of new cell types and the discovery of gene regulatory networks associated with neuropathic pain. Furthermore, scRNA-seq research has been able to generate transcriptomic and epigenetic atlases of the mouse dorsal root ganglion (DRG), revealing the ubiquitous heterogeneity of cell function and providing basic datasets for the study of neuropathic pain. scRNA-seq will clearly demonstrate its power for the exploration of cellular and transcriptomic changes in the course of neuropathic pain, deepen our understanding of neuropathic pain pathogenesis and

Abbreviations: cDNA, complementary DNA; DRG, Dorsal Root Ganglion; FACS, Fluorescence-activated cell sorting; GO, gene ontology analysis; IVT, In Vitro Transcription; LTMR, low-threshold mechanoreceptors; NP, nonpeptidergic; MeCP2, Methyl-CpG binding protein 2; PCA, Principal component analysis; PEP, peptidergic; RNA-seq, RNA-sequencing; rRNA, ribosome RNA; scRNA-seq, single-cell RNA sequencing; snRNA-seq, single-nucleus RNA sequencing; SI, superficial-tissue injury; SNI, spared nerve injury; t-SNE, t-distributed stochastic neighbor embedding; tRNA, transfer RNA; TH, tyrosine hydroxylase; WGCNA, weighted gene co-expression network analysis

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https://doi.org/10.1016/j.trsl.2022.07.004
Received 26 December 2021; Revised 17 July 2022; Accepted 18 July 2022

Please cite this article as: L. Chen et al., Single-cell RNA sequencing in the context of neuropathic pain: progress, challenges, and prospects, Translational Research (2022), https://doi.org/10.1016/j.trsl.2022.07.004

improve the diagnosis and treatment of this condition in the near future. In the current review, we aim to briefly summarize the scRNA-seq technique and then focus on its applications and limitations in the study of neuropathic pain mechanisms. Finally, we will analyze its application prospects and emerging challenges in the future and discuss how it can be used to accelerate the advancement of pain medicine.

Workflow of scRNA-seq

Transcriptome components such as mRNA provide key information directly related to cellular heterogeneity, which leads to the existence of different cell phenotypes.[7] In the past, traditional sequencing techniques such as RNA sequencing (RNA-seq) were performed on cell populations,[8,9] and the results represented only the average gene expression of millions of cells. There was no way to analyze gene expression in individual cells until the development of the scRNA-seq technique, which was first reported by Tang et al.[10] scRNA-seq is a pioneering attempt to perform unbiased, high-throughput and high-resolution transcriptional analysis of single-cell samples. Since the application of scRNA-seq for research on the mechanism of neuropathic pain has been limited, this article will briefly review the process of scRNA-seq.

Isolation of single cells

The primary challenge of scRNA-seq is to separate individual cells from obtained tissues or cell suspensions. To date, several approaches have been commonly used in neuropathic pain studies, such as micromanipulation, fluorescence-activated cell sorting (FACS), and microfluidics (Fig 1B). Considering the differences among samples, particular methods may be more suitable for single-cell isolation in particular sample types than in others.

Micromanipulation[11-20] can be used to capture a single cell from a small number of cells. Although it is time-consuming and low-throughput, the size of the target neuron can be recorded by means of a scale plate in the microscope. The measurement of neuron size is an important indicator of neuron classification. FACS[11-22] is the most commonly used method for isolating single cells. In this method, researchers use a cell tracker to label

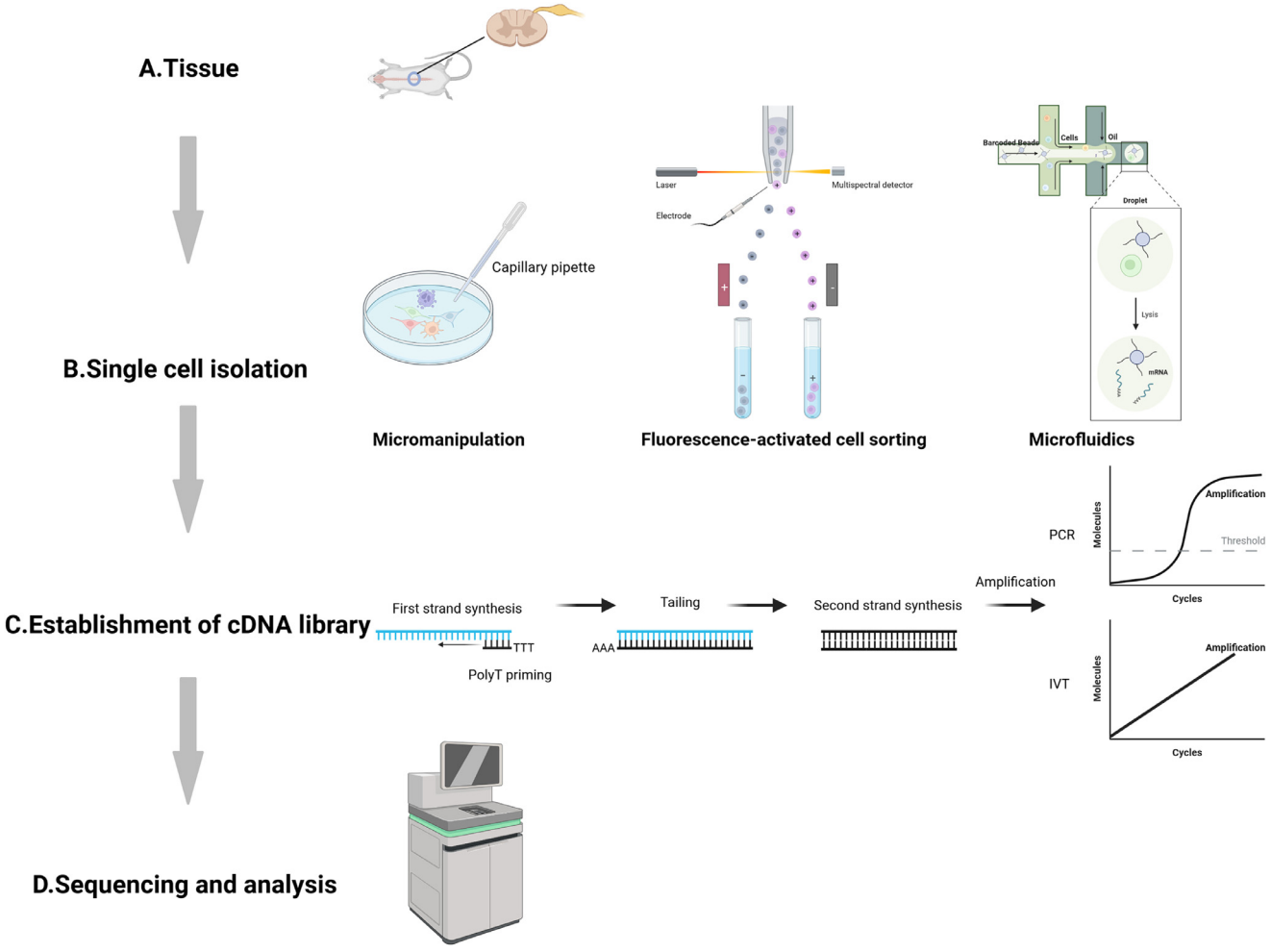

Fig 1. Workflow of scRNA-seq. **A**, In recent neuropathic pain studies, the DRG and spinal cord were the most common sources of samples. **B**, In micromanipulation, individual cells are captured, moved and released with the aid of a micropipette or micromechanical manipulator under a microscope. In FACS, fluorescently labeled antibodies are used to isolate cells of interest based on surface markers. In microfluidics, controlled liquid flow is used to isolate a single cell on a microfluidic chip. **C**, For first-strand synthesis, poly(dT) primers are used to initiate the reverse transcription reaction. For second-strand synthesis, in the poly(A) tailing method, exonuclease I is used for primer digestion, a poly(A) tail is added to the 3′ end of the first strand, and a tagging primer is used for the synthesis of the second strand of cDNA. In the template-switching mechanism, when reverse transcription reaches the 5′ end of the mRNA, the complete cDNA is synthesized by switching the template to the oligonucleotide and continuing the reaction under the guidance of reverse transcriptase. **D**, Analysis of the large volumes of data generated from different experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

DRG neurons and then run them through a FACS instrument.[23] However, this method has some limitations, including the risk of damage to cell activity during sorting and the difficulty in distinguishing subsets of cells expressing similar markers.[24] Microfluidics[11-17,19-22,25] is becoming increasingly popular due to its low sample consumption, precise fluid control and low analytical cost. For neuron isolation, 10X Genomics, In Drop and Drop devices have been used in previous studies.

Establishment of a cDNA library

Constructing a sequencing library for further analysis involves 3 main steps: reverse transcription, complementary DNA (cDNA) amplification, and sequencing library preparation[13-17,26] (Fig 1C). Since the total amount of mRNA in a single cell is very low, the key to establishing a cDNA library suitable for sequencing lies in how the nucleic acid inside the cell is amplified.

The first step is reverse transcription. Most studies have used poly primers to prime reverse transcription to eliminate unwanted ribosomal RNA and transfer RNA.[27,28] The synthesis of the second strand can be accomplished through 2 approaches: the poly(A) tailing method and the template-switching method.[29,30] After reverse transcription, the obtained trace cDNA must be amplified to construct the sequencing library for scRNA-seq analysis, which can be completed by PCR or in vitro transcription.

In subsequent sections, we will discuss the current status of scRNA-seq research in the field of neuropathic pain as well as the advantages, disadvantages and future challenges associated with this technique.

ScRNA-seq for DRG atlas preparation

DRG neurons, which are primary afferent neurons of pain that play important roles in the process of pain production, transduce and modulate sensory information and transmit it to the spinal cord. DRG neurons not only contain neurotransmitters and modulators that transmit nociception but also express some ion channels associated with chronic pain.[31] Therefore, further research on the DRG is extremely important to elucidate the mechanism of chronic pain, especially chronic neuropathic pain, and may contribute to the identification of new therapeutic targets. According to traditional neuron classification methods, primary sensory neurons of the DRG are divided into small- and large-diameter neurons. These neurons can be further classified according to the expression of some specific substances, such as neuropeptides, calcitonin gene-related peptide and neurofilament-200.[32,33] However, this type of classification is not accurate for DRG neurons because the coding genes that seem to be markers in fact label multiple neuron types. scRNA-seq has unique single-cell resolution and recognition capabilities and is therefore promising for use in the discovery of previously rare or even undetected cell subpopulations. Thus, the primary use of scRNA-seq is to define cell subsets based on the single-cell transcriptome, and its application in the typing of DRG neurons has been demonstrated.

Usoskin et al[34] first applied scRNA-seq to classify the DRG neurons of mice in an unbiased manner. For researchers, the transcriptome data of new DRG neuron types can be used as a resource for the investigation of sensory physiology, especially with regard to pain mechanisms. In that study, DRG neurons were divided into 4 clusters with the aid of comprehensive transcriptomic analysis and the expression of known markers: the neurofilament cluster, the peptidergic nociceptor cluster, the non-peptidergic (NP) nociceptor cluster and the tyrosine hydroxylase (TH) cluster. The 4 main classes were further identified by principal component analysis (PCA) to contain further subtypes of neurons and then validated by immunohistochemical staining. In addition to discovering and classifying new cell types, scRNA-seq was used to link the identified sensory neuronal types with their functions. With the single-cell transcriptome data of the DRG, researchers could even predict some functions of neuron types. For example, lysophosphatidic acid receptors 3 and 5, which are expressed in NP1 neurons, were predicted to be involved in neuropathic pain caused by nerve injury. However, marked variation in gene expression was detected among cells due to the limited number of genes tested (3574 ± 2010 genes per neuron).

Based on Usoskin et al research, Li et al[33] found a viable approach to reclassify sensory neuron types by combining high-coverage scRNA-seq and hierarchical clustering based on neuron size. That study provided new insights into somatosensory neurons, particularly nociceptors. According to the average size of neurons combined with PCA and unsupervised hierarchical clustering analysis, a new classification method for DRG neurons was proposed based on single-cell transcriptomes. Specifically, small neurons were divided into 6 clusters (C1-C6), and large neurons were divided into 4 clusters (C7-C10). It is worth mentioning that the C7 cluster could not be detected in low coverage sequencing. The neurons were further subdivided on the basis of the obtained cluster tree. For example, the differentially expressed gene of the C4 cluster was Mrgpra3, and C4 could be further divided into 2 subtypes, C4-1 and C4-2, based on the expression of Megpra4. Except for the use of an additional gene network for further classification, neuron subclusters can also be formed by the expression of representative genes of other clusters, which is called the “hybrid state”. After the DRG neuron types were redefined, immunohistochemistry and in situ hybridization were used to validate the new cell classifications. Moreover, the research showed that single-cell transcriptome data could be used to partially predict the functions of different neuron types, which needs more experimental verification.

Following these 2 seminal papers, DRG somatosensory neurons have been profiled many times with the aid of single-cell transcriptome sequencing. For example, Renthal et al[35] performed snRNA-seq (single-nucleus RNA sequencing) on lumbar DRGs from naïve mice and processed their transcriptional profiles. Nine DRG neuron subtypes were observed and followed Usoskin et al in principle. Compared with scRNA-seq, snRNA-seq yields similar results and can be successfully used to generate a cell atlas. Recently, Wang et al[36] performed 10 × Genomics scRNA-seq on spared nerve injury (SNI) mouse DRG neurons and proposed slightly different classification methods due to the sequencing depth and technical limitations. In addition, Sharma et al[37] obtained similar somatosensory neuron classifications, in which DRG neurons were distributed into subclusters based on achievements of low-threshold mechanoreceptors (LTMRs) and previous studies on trigeminal neurons.[38,39] With the aid of gene function analysis, some studies have raised a few hypotheses regarding the somatosensory functions of each neuron type, which need further verification. For example, the functions predicted by analyzing the gene expression of TH clusters, such as Piezo2 and Slc17a8, seem to be consistent with those previously reported to be involved in mechanical pain and pleasant touch.[40-42] After comparing the traditional somatosensory neuron classification with the classification methods proposed in these papers, the major types and subtypes of DRG neurons that scRNA-seq can offer seem to have been identified. Here, we summarize several representative studies, compare different nomenclatures and match their corresponding functions, which are shown in Table I. However, the existence of some rare types cannot be completely ruled out due to the relatively small numbers and similarities in their gene expression patterns.

Since the current scRNA-seq applications in the field of neuropathic pain mainly apply mouse models and choose the DRG as the object of study, we focus on the research progress of the mouse DRG atlas. However, single-cell datasets of the human DRG have already been generated.[43] Comparing the scRNA-seq dataset between human and mouse DRG neurons, consistency was found in gene expression across many neuron types. For example, neurotrophic tyrosine receptor kinase (NTRK)-positive cells identified in human DRG neurons showed a similar transcription pattern to proprioceptors in mice. In addition, the analysis revealed several human DRG neuron clusters without clear transcriptomic equivalents in mice. This aspect of the difference, to some extent, clarifies why some neuropathic pain-related studies on mice or other rodents do not completely apply to humans. Moreover, spatial transcriptomics helped further verify the consistency between mouse and human DRG neurons.[44] The comparison even reveals the

Table I
Comparison of different nomenclatures and matching of their corresponding functions

Traditional classification	Usoskin. et al, 2015	Wang. et al, 2021	Functions	Li. et al, 2016
PEP (Calca/Tac1)	PEP1 (Ntrk1/Plxnc/Tac1/Calca)	C1-1 (Cldn9)	Undetermined	C1-1 (Gal/Cldn9)
		C1-2-1 (Zcchc12/Sstr2)	Thermal Sensation (Trpm8 for cold, others for heat)	C1-2 (Gal/Zcchc12/Sstr2)
		C1-2-2 (Zcchc12/Dcn)		
		C1-2-3 (Zcchc12/Trpm8)		
		C1-2-4 (Zcchc12/Rxfp1)		
	NP3 (Plxnc1/Sst/Il31ra/P2 × 3low)	C2 (Nppb/Sst/Il31ra)	Itch sensation	C2-1 (Nppb/Sst/Il31ra)
				C2-2 (Nppb/Sst/Il31ra/S100b)
	TH (Th)	C3 (Th/Fam19a4)	c-LTMR	C3 (Th/Fam19a4)
NP (IB4)	NP2 (Plxnc1/Ntrk/Calca/P2 × 3?/MrgprA3)	C4-1 (Mrgpra3)	Itch sensation	C4-1 (Mrgpra3)
		C4-2 (Mrgpra3/Mrgprb4)	Massage-like stroking	C4-2 (Mrgpra3/Mrgprb4)
	NP1 (Plxnc1/P2 × 3high/Mrgprd)	C5-1 (Mrgprd/Lpar3)	Itch sensation (β-alanine) and mechanical sensation	C5 (Mrgprd)
		C5-2 (Mrgprd/Gm7271)		C5 (Mrgprd)
			——	C6 (Mrgprd/S100b)
NF(S100b/Nefh/Pvalb)	NF4 (LDHB/CNTNAP2/SPP1/PV/Runx3)	C7 (Wnt7a)	Proprioceptive receptor	C7 (Wnt7a)
	NF5 (LDHB/CNTNAP2/SPP1/PV/Runx3)			
	NF2 (LDHB/CACNA1H/CALB1)	C8-1 (Trappc3l/Ntrk3/Gfra1)	Aβ field-LTMR	C8-1 (Trappc3l/Ntrk3)
	NF3 (LDHB/FAM19A1)	C8-2 (Trappc3l/Prokr2)	Aβ rapidly adapting (RA)-LTMR	C8-2 (Trappc3l/Ntrk1)
	PEP2 (Ntrk1/Fam19a1/Nefh/Calca) (?)	C8-3 (Trappc3l/Smr2)	Undetermined	C8-2 (Trappc3l/Ntrk1)
	NF1 (LDHB/CACNA1H/TrkBhigh/NECAB2)	C9 (Baiap2l1/Ntrk2)	Aδ-LTMR	C9 (Baiap2l1)
			——	C10 (Gal/S100b)

Abbreviations: LTMR, low-threshold mechanoreceptors; *NF*, neurofilament containing; *NP*, nonpeptidergic; *PEP*, peptidergic; *TH*, tyrosine hydroxylase.

differences in nociceptors, which is of great significance and can help identify potential drug targets. By studying the gene expression profiles of pharmacologically relevant targets such as potassium channels, scRNA-seq can advance our understanding of pain mechanisms and open up a new pathway for the development of pain treatments.

Applications in neuropathic pain

Several mechanisms are involved in neuropathic pain, the development of which involves dysfunction in afferent pathways.[45] In addition, ectopic nerve activity,[46] central and peripheral nociceptive sensitization[47-49] and many other potential mechanisms may participate in neuropathic pain. In the past, because of the unclear mechanism of neuropathic pain, treatment methods were unsatisfactory. The rapid development of scRNA-seq has given researchers an opportunity to elucidate the mechanism and may provide new strategies for individualized treatment.

To date, the applications of scRNA-seq in the study of chronic pain have mostly focused on neuropathic pain (Table II), mainly because of the existence of sufficient studies on DRG neurons and the ease of modeling chronic neuropathic pain. However, due to the various differences between animals and humans,[50] greater attention must be given to translational medicine research. Although different scRNA-seq methods differ in throughput, sensitivity, and precision, they provide new perspectives for researchers to identify cellular heterogeneity in neuropathic pain-associated tissues and to study the pathogenesis and treatment at the gene expression level.

Table II
Research status of single-cell RNA sequencing in neuropathic pain models

Year	First author	Species	Modeling	Tissue	Database	Ref.
2014	Dmitry Usoskin	Mouse	None	DRG	GSE59739	34
2016	Chang-Lin Li	Mouse	None	DRG	GSE63576	33
2016	Ganlu Hu	Mouse	SNT	DRG	GSE71453	57
2018	Ming-Dong Zhang	/	/	/	GSE59739	63
2020	Wenjuan Tao	Mouse	CCI	Brain	GSE128916	66
2020	Zhun Wang	/	/	/	GSE71453	68
2021	Kaikai Wang	Mouse	SNI	DRG	GSE155622	36
2021	Jesse K. Niehaus	Mouse	SNI and SI	Spinal cord	GSE134003	62

Abbreviations: CCI, chronic constraint injury; *SNT*, sciatic nerve transection; *SNI*, spared nerve injury; *SI*, superficial-tissue injury; "/", previous databases were analyzed to obtain results; thus, no laboratory animals and models were used.

ScRNA-seq for DRG neurons after nerve injury

In a previous study, the scRNA-seq technique was mainly applied to generate a cell landscape of mouse DRG neurons under physiological conditions; however, the gene expression patterns of DRG neurons in neuropathic pain models caused by peripheral nerve injury or inflammation are significantly different.[35,51] DRG microarray data have revealed the regulation in gene expression of DRG neurons after nerve injury.[52,53] Changes in the expression of genes encoding some proteins, such as brain-derived neurotrophic factor and neuropeptide Y, are associated with neuropathic pain,[54] consistent with previous studies.[55,56] To precisely understand the transcriptional responses in neuropathic pain, scRNA-seq can certainly demonstrate its power to explore the mechanisms associated with altered patterns of gene expression in neuropathic pain. Nevertheless, bulk DRG neuron analysis does not explain the individual transcriptome changes in each neuron type and their role in neuropathy. In this section, we discuss how scRNA-seq has been combined with other methods to reveal changes in the composition and gene expression of DRG neurons after nerve injury to explore the potential mechanism of neuropathic pain.

Previous studies on bulk DRG neurons are not capable of distinguishing gene expression changes in different subtypes of neurons after nerve injury. Thus, to reveal heterotypic injury responses in different subtypes of DRG sensory neurons, Hu et al[57] studied DRG neurons in a mouse sciatic nerve transection model with the aid of scRNA-seq. Their study identified not only the transcriptomic responses of different subtypes of DRG neurons on the nerve-injured side but also damage-responding genes associated with abnormal pain and cell death. By performing DRG

neuron sequencing in combination with weighted gene coexpression network analysis (WGCNA) and gene ontology (GO) analysis, the researchers found a total of 7 coregulated injury-responsive gene clusters that were functionally related to different physiological or pathological processes. Some of the neuropathic pain-related genes proposed in the study, such as those related to ion channels, are consistent with previous studies. In addition, new potential targets, such as Kcng3 and Kcnn1, have also been proposed to contribute to neuropathic pain.

Similarly, Renthal et al[35] studied transcriptional reprogramming in different subtypes of neurons after axonal injury. Some newly generated neuronal clusters of the "injured state" were identified because of the high-level expression of injury-induced genes, such as Atf3 and Sprr1a. Further analysis revealed that different DRG neuron types have unique responses to nerve injury, which may lead to their different roles in some physiological processes and even neuropathic pain. Enrichment analysis showed significant overlap of common injury genes compared with those in the previous study. Notably, they found that the cell-specific marker gene that defines the identity and functional specialization of each subtype was reduced after nerve injury. This cell-type-specific gene reduction seems to be due to the loss of transcriptomic identity, while many are ion channels that affect neuronal excitability and may participate in neuropathic pain. In addition, nonneuronal cells, such as satellite glia or Schwann cells, are also sequenced and show significant injury-induced gene expression.

Recently, Wang et al[36] studied the gene expression profiles of DRG neurons in SNI mice. With the aid of PCA and t-Distributed Stochastic Neighbor Embedding (t-SNE), 3 clusters of SNI-induced neurons were revealed by 10 × Genomics. Consistent with previous studies, known injury-induced genes such as Atf3 and Sprr1a,[51,58,59] were highly expressed in these types of neurons. By comparing marker gene expression and calculating the transcriptomic correlations among different neuron types after SNI, researchers found that injury-induced neuron types originate from naïve neuron types such as Cldn9-positive neurons (C1-1). For example, the gene expression patterns of C1-1 significantly changed from the expression of Cldn9 to high expression of Atf3 with the help of some regulons. Among the 3 types of SNI-induced neurons, SNIIC2, which highly expresses Atf3 and Mrgprd, seems to be a transition state between C5 and SNIIC1. Notably, during the SNI-induced neuronal switching process, multiple genes associated with neuropathic pain or inflammatory responses were identified. The study revealed striking transcriptomic changes during the development of neuropathic pain-associated neurons to identify potential analgesic targets.

Current studies on DRG neurons subjected to scRNA-seq after nerve injury are limited, but they share consistent results, while injury-induced genes are the common focus of their studies. It is believed that nerve injury induces changes in gene expression patterns, including but not limited to genes related to nerve regeneration, immune inflammation, and cell death. Hu et al and Renthal et al treated damaged DRG neurons as a state of injury, investigating genes that differ from naïve samples. However, Renthal et al proposed a bioinformatics analysis method to distinguish the types of neurons after injury and trace their development, and nonneuronal cells, such as satellite glial cells, have also been partially explored. Furthermore, Wang et al defined several SNI-induced neurons and discovered the origin and transformation process of each type. The present studies seem to gradually delve deeper into the gene expression patterns of DRG after nerve injury, which can promote research exploring the mechanisms of neuropathic pain and developing therapies for pain.

ScRNA-seq for identification of the immune inflammatory mechanism of neuropathic pain

Diverse types of immune cells and inflammatory factors have been found to provide seminal contributions to the formation and maintenance of neuropathic pain. Immune cells such as macrophages, T lymphocytes and microglia affect the occurrence and development of neuropathic pain mainly by participating in peripheral and central sensitization.[60] Due to the potential immunological mechanism of neuropathic pain,[61] studies on immune cells are also helpful for exploring the mechanism of neuropathic pain.

To reveal novel modulators during the development of neuropathic pain, Jesse K. N et al,[62] performed scRNA-seq on lumbar spinal cord segments of mice after SNI and superficial tissue injury (SI), concentrating on transcriptional responses in spinal macrophages. The changes in cell proportions between SNI and SI were mainly due to the difference in macrophages and microglia. Notably, the expansion of macrophages was blunted in SNI animals compared with SI animals. In the SI model, MRC1 + spinal macrophages upregulated the anti-inflammatory mediator CD163, which may facilitate the resolution of persistent pain and microgliosis, but this response was not obvious in the SNI model. The research suggested, for the first time, that the spinal neuroimmune response after nerve injury is bidirectional instead of unidirectional. However, the experimental method used in that study ablated macrophages nonselectively, which increased the cell type specificity.

In some studies, scRNA-seq is used in combination with histochemical analyses to help researchers locate target genes of interest. Zhang et al[63] found that the Ca^{2+}-binding protein NECAB2 is located in some neurons of the DRG and spinal cord based on the expression data for DRG neurons published by Usoskin et al[34] By incorporating other methods, such as quantitative polymerase chain reaction (qPCR) and Western blotting, researchers have revealed that NECAB2 regulates inflammatory pain hypersensitivity by limiting brain-derived neurotrophic factor release and inhibiting the excitatory transmission of spinal interneurons. Through analysis of the published scRNA-seq database, researchers may be able to discover some other pathways related to chronic neuropathic pain, but further experiments are needed to verify the existence of these pathways.

ScRNA-seq in transgenerational transmission of chronic pain after nerve injury

Several types of chronic pain, such as migraine,[64] frozen shoulder and tennis elbow,[65] have a strong genetic component, but the mechanism of transgenerational transmission of pain is still unclear. Tao et al[66] studied mice that underwent chronic constraint injury and identified a potential iterative genetic pathway for chronic pain. Research has shown the vital function of methyl-CpG binding protein 2 (MeCP2) in the transgenerational transmission of pain through actions on Glu^{S1} neurons. In that study, the gene expression profiles of mice and their progeny were investigated through scRNA-seq and chromatin immunoprecipitation analysis (ChIP), which revealed differentially expressed genes with the same direction of change. Some of these differentially expressed genes were regulated by MeCP2 and may be involved in the transgenerational transmission of chronic pain. Therefore, future studies can also incorporate scRNA-seq to explore the transmission of chronic pain and to investigate some types of chronic pain that are highly related to genetics.

ScRNA-seq in neuropathic pain associated with cancers

Neuropathic pain associated with cancer can be caused by the disease itself or by the cancer treatment, and it is exacerbated by sensitization of sensory neurons in nociceptors.[67] There is currently no effective way to relieve this pain. However, a research team has applied several scRNA-seq and RNA-seq databases to help identify the intercellular interactions of neuropathic pain associated with cancer so that a viable treatment for this severe pain can be found in the future.

Wang et al[68] selected dendritic cells from lung adenocarcinoma cases as research samples and revealed the effect of tumor microenvironment-induced dendritic cells on neuropathic pain. The contributions of infiltrated dendritic cells induced by the tumor microenvironment to neuropathic pain were unveiled by using RNA-seq, scRNA-seq, and ChIP-seq data as well as bioinformatics assays. Compared with that of peritumoural dendritic cells, the transcriptome of infiltrated dendritic cells is significantly different. The upregulated genes, such as histamine synthesis

genes, were enriched in pain-related pathways, suggesting that dendritic cells induced by intercellular communication or paracrine factors in the tumor microenvironment can regulate neuropathic pain through the synthesis and release of pain mediators such as histamine. Infiltrated dendritic cells also exhibited upregulation of the expression of dozens of paracrine inflammatory factors, which promoted cancer-related neuropathic pain by sensitizing neurons. TNF, WNT10A, PDGFA and NRG1 may be involved in the pathogenesis of cancer-related neuropathic pain. The interactions of these paracrine factors with their receptors can cause activation of downstream transcription factors, such as BCL3, E2F1, SMAD5 and REST, and upregulation of the expression of CDH5, ARRB2 and other pain-related genes. Wang et al also found that paracrine factor-sensitized neuropathic pain is widely involved in a variety of cancers, suggesting that this pathway may be a common mechanism of cancer-related neuropathic pain; thus, blocking the paracrine factor-sensitized signaling pathway may be a novel therapeutic target for the relief or treatment of cancer-related neuropathic pain.

Limitations

Since the advent of scRNA-seq, this technique has become a powerful means for biological researchers to address the complexity of neuropathic pain. However, the application of this new technology has limitations. The first is the high cost of sequencing. Although technological innovations have provided a substantial reduction in the sequencing cost per cell, the price for reagents remains a formidable issue. Another limitation is the limited number of neuropathic pain studies available for reference. In recent years, researchers have begun to apply scRNA-seq to explore the mechanism of neuropathic pain, but the research has not been intensive, and the number of studies is limited. This means that it will be difficult for future researchers to obtain sufficient information. In addition, analysis of the data from scRNA-seq research results in countless assumptions, which are difficult to verify. In addition, the ethical issues of clinical research are complex. Neuropathic pain research is mostly conducted using the DRGs and spinal cords of mice, as it is rather complicated to obtain materials from clinical patients. Most of the time, due to ethical concerns, researchers can take only peripheral blood or even skin from patients, even though nerve tissues contain more information.

Found in neuropathic pain studies, the scRNA-seq technique sometimes has its own limitations. The first is the chosen sequencing platform; for example, Wang et al was unable to distinguish neuron cluster 10 from cluster 1 because the neuronal size could not be recognized by 10 × Genomics technology. Second, sequencing depth affects how many genes are tested. Insufficient sequencing depth may result in loss of changes in gene expression after injury. Moreover, bioinformatics analysis techniques are also one of the conditions that limits the acquisition of conclusions. Renthal et al observed a reduction in cell-type-specific genes, which makes it difficult to distinguish clusters of neurons. They proposed a new bioinformatics analysis technique that could make it feasible, which needs further verification and research.

Perspectives

Although some limitations remain, scRNA-seq is a very promising technique for exploring the mechanism of neuropathic pain. First, somatosensory neurons participate in sensory transduction under both physiological and pathological conditions. With the development of single-cell transcriptome sequencing, the understanding of somatosensory neurons is also gradually advancing, which provides a better understanding of pain mechanisms. This review chose the mouse DRG as the focus of attention; however, some parts of the nervous system, such as the trigeminal nerve[38] and brain,[69,70] have also been studied at the single-cell level. In contrast, the classification of trigeminal neurons is highly consistent with that of DRG somatosensory neurons. Due to the complexity of the nervous system, high-precision technologies such as scRNA-seq can be powerful tools allowing for the study of more sophisticated neuronal classification, and neuron-type transcriptome data can be used as a resource for studying sensory mechanisms and pain treatment.

Second, most current studies of chronic pain are based on neuropathic pain models after nerve injury. Some other types of chronic pain, such as migraine,[71,72] have also been studied at the single-cell level and the tissue localization of migraine-related genes have been determined. Therefore, scRNA-seq can be used not only to study the transcriptional changes of neuropathic pain after nerve injury but also to explore the mechanism of gene-related chronic pain. In addition, inflammatory and chemotherapy-induced pain models can also be used as research objects. The etiologies that result in chronic pain are complex and may be related to diseases of nerves, bones, muscles, viscera and other tissues.[73-75] Thus, in the selection of study samples, blood,[76] skin,[77] muscle[78] and nerves can be chosen for sequencing and further analysis.

Moreover, neuropathic pain is defined as pain caused by a lesion or disease of the somatosensory system. Current treatments do not provide particularly satisfactory results due to the unexplored mechanisms of neuropathic pain. Adverse reactions are sometimes unacceptable due to the nonspecificity of drug targets in the nervous system. Here, scRNA-seq can be used to explore the changes in the gene expression profile of somatosensory neurons after peripheral injury, thus identifying a series of potential drug targets, which can serve as the theoretical basis for drug development and aid the development of new drugs. For example, cardiotrophin-like cytokine factor 1, which is upregulated in genes after nerve injury, may be a potential analgesic target in the future.[36]

Finally, different types of pain have similar clinical manifestations, so different combinations of symptoms and signs may reflect different pathophysiological mechanisms and responses to treatment. This leads us to the need for stratified treatment of patients with chronic pain, especially neuropathic pain. Currently, the stratified treatment of neuropathic pain mainly adopts some nonobjective indicators, such as symptoms or clinical questionnaires, as well as some objective indicators, such as quantitative sensory testing or molecular characteristics analysis. ScRNA-seq provides insights into the changes that occur during neuropathic pain at the cellular and gene expression levels and can be a powerful tool for stratified treatment.

Conclusions

Single-cell sequencing has provided insights for the study of neuropathic pain and has helped pain researchers have a more comprehensive understanding of this incurable condition. This technique has enabled compilation of a DRG atlas, analyses of gene expression changes after nerve injury and even prediction of the potential functions of some types of neurons. Briefly, further application of scRNA-seq in neuropathic pain research can further expand our understanding of the individual heterogeneity of patients with chronic pain and enable deeper understanding of the molecular mechanisms. Such research will aid in the search for new treatment methods to achieve more appropriate individualized chronic pain management.

Acknowledgments

Conflicts of Interest: All authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare. All authors have read the journal's authorship statement, and the manuscript has been reviewed and approved by all named authors.

This work was supported by the Key R&D Program of Zhejiang (No. 2022C03081) and the Medical and Health Technology Program of Zhejiang (No. WJKJ-ZJ-2025).

Author contributions are as follow: Lei Chen Writing - review & editing; Yunze Li Writing - review & editing; Lina Zhu Writing - review & editing; Haifei Jin Writing - review & editing; Xianhui Kang Writing - review & editing; Zhiying Feng Writing - review & editing.

The authors express their thanks to Professor Changlin Li and Xiangyao Li for their helpful discussions.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.trsl.2022.07.004.

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