Application Note

Improvement of single-cell transcriptomic results of mouse dorsal raphe *Pet1* neurons through gentle cell sorting

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Introduction

Gentle isolation of a target cell population using fluorescent activated cell sorting is an important step for very sensitive downstream applications such as gene expression analysis. The damage induced to cells during cell sorting on electrostatic droplet-based cell sorters can often cause gene expression changes, cell damage and cell death, which can cause gene expression artefacts and extraneous RNA contaminations, and hence false gene expression profiling results. To overcome this problem, our microfluidic chip cell sorter, On-chip Sort, is used for damage-free sorting of fragile and sensitive cell types for effective downstream single-cell genomic analysis. In this study, mouse dorsal raphe (DR) *Pet1* neurons were purified by On-chip Sort and subsequently analyzed by droplet-based single-cell RNA sequencing (scRNA-seq) to characterize their molecular diversity in a high throughput and high resolution manner. The results of this study can contribute to the understanding of the DR functional organization, which can bring more insight into field of neuroscience, and potentially to the development of targeted therapeutics.

Methods

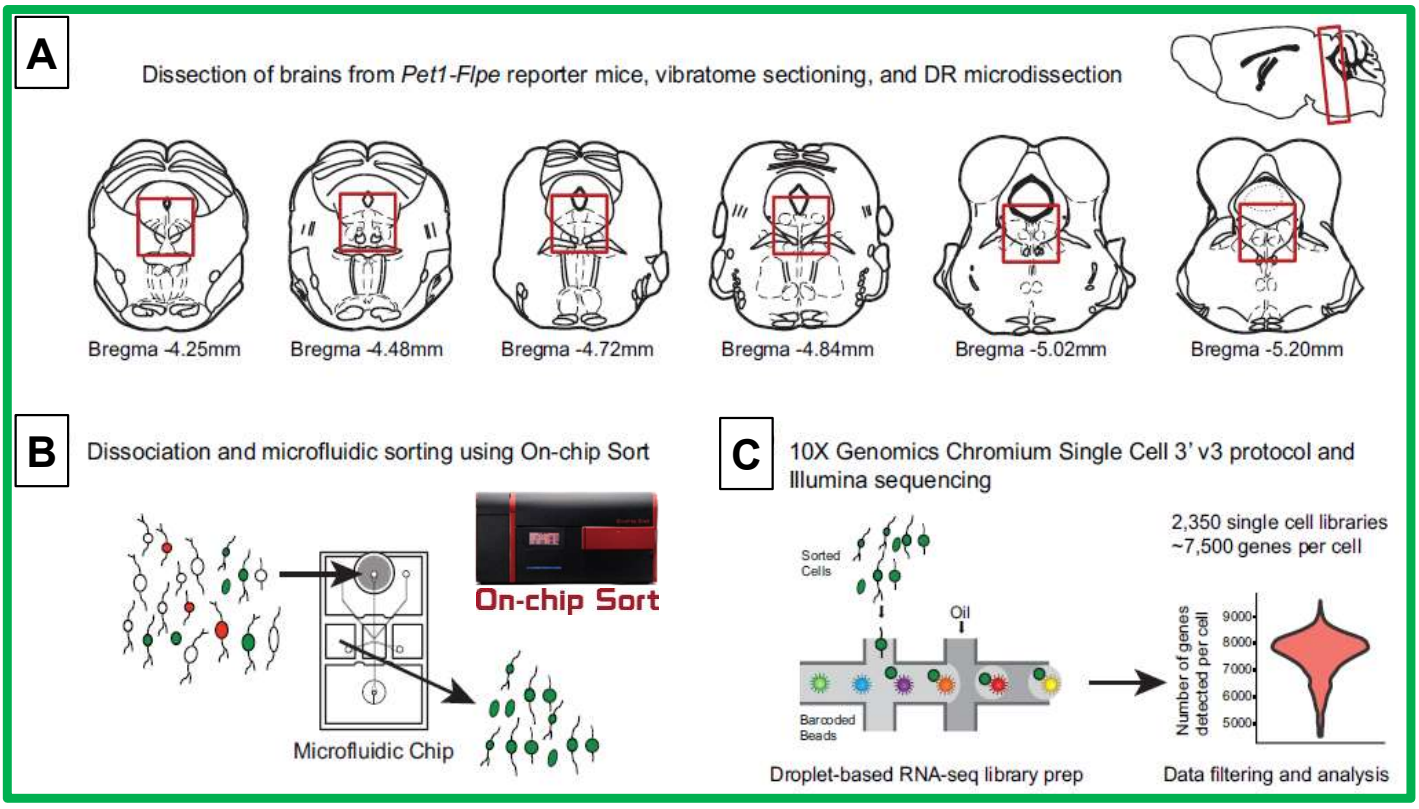

Fig. 1. Schematic diagram of the experimental workflow: (A) dissection of mouse brains, (B) dissociation of DR cells and fluorescence-based sorting using On-chip Sort, and (C) library preparation using 10X genomics, scRNA-seq using Illumina sequencing and data analysis using R package Seurat.

Fig. 1 shows the experimental workflow. Brains of mice aged 6 to 10 weeks from two genotypes were dissected (Fig. 1A) and DR cells were dissociated (Fig. 1B). DR-*Pet1* cells marked by EGFP expression were sorted by On-chip Sort (Fig. 1B). Sorted neurons were run through the 10X Genomics Chromium Single Cell 3' v3 protocol, then by Illumina NextSeq 500 sequencing (Fig. 1C). Single-cell RNA-seq data analysis was performed with R Package Seurat. After stringent data filtering and analysis, 2,350 single cell libraries were further analyzed (Fig. 1C).

Results

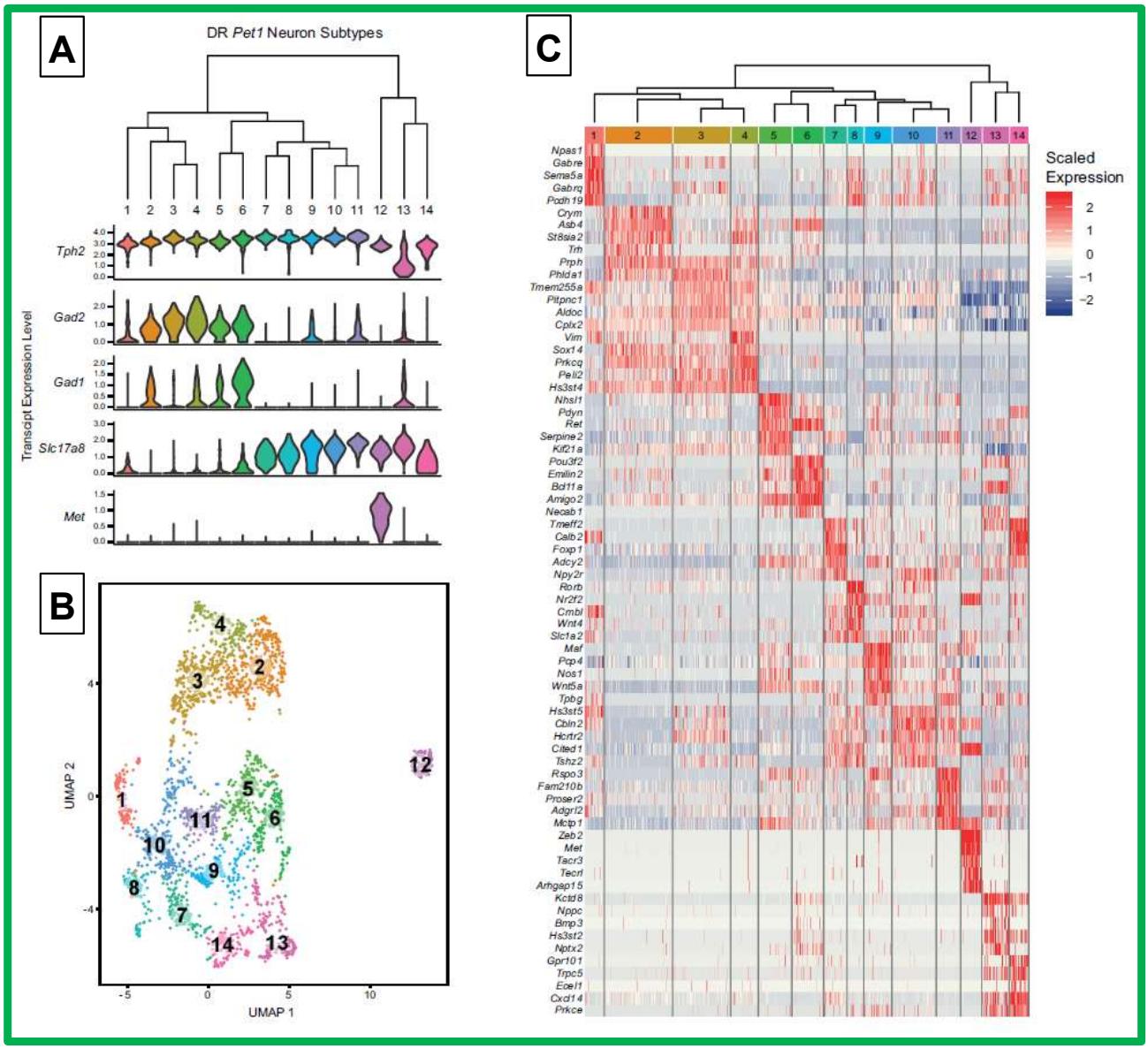

Fig. 2. Characterization of molecular diversity of single DR neurons by clustering analyses. (A) Hierarchical clustering of DR-*Pet1* neuron subtypes (clusters) identified by Louvain clustering, violin plot depicting the log-normalized expression of a common set of genes. (B) UMAP visualization of single-neuron transcriptome community/similarity structure. Each color represents a discrete cluster, with the same clustering parameters used in (A). (C) Heatmap depicting the scaled expression of the top five marker genes for each cluster.

Many of the DR neurons sorted by On-chip Sort were observed to be viable and had intact processes emanating from their somas, indicating that On-chip Sort was capable of gentle sorting. In order to characterize the molecular diversity of DR-*Pet1* neurons, genes with significantly variable transcript expression across single neurons were identified and based on these gene expression differences, 14 distinct molecularly-defined subtypes of *Pet1* neurons were classified in mouse DR using clustering analyses shown in Fig. 2A-C. Overall, gentle sorting of DR neurons using On-chip Sort is an important preliminary step upstream of scRNA-seq to ensure the delivery of improved single cell transcriptomic results.

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