New emerging single-cell profiling techniques provide unprecedented opportunities for researchers to explore the heterogeneity among individual cells. Great efforts have been made by scientists around the globe trying to build the references of all cell types in the human body, such as the human cell atlas [1] and more focused atlas of blood cells by using state-of-the-art single-cell omics techniques. However, the throughput is limited and the cost is relatively high. Building comprehensive references of the whole body still highly depends on extensive and efficient multi-lab collaborations.

Han et al. developed a new single-cell RNA sequencing method (Microwell-seq) with much higher throughput and lower cost [2]. In Microwell-seq, customized agarose microarrays and magnetic beads are used to harvest single cells, followed by Smart-seq2 [3] pipeline to construct barcoded libraries to estimate gene expression simultaneously in ten thousand individual cells. This high throughput is comparable to Drop-seq, a high-throughput RNA-seq method which can analyze thousands of single cells each time [4]. Moreover, the optimized experimental pipeline and reusable silicon and PDMS chips reduce the cost of library construction of single cells to less than 0.02 USD, which is much lower than the most commonly used platform for single-cell RNA-seq, such as Fluidigm C1 system and 10X Genomics Chromium system [5].

Remarkably, this group applied the Microwell-seq technique to profile more than 400,000 single cells from 51 mouse organs, tissues and cell lines. The large numbers of samples and cell types make this study the most comprehensive and complete mammalian single cell data resource to date.

By integrative analysis of single-cell gene expression profiles, more than 800 cell types were classified. Several types of cells were revealed within multiple tissues including hematopoietic cells, stromal cells, endothelial cells, neurons and myocytes, suggesting multi-source hematopoiesis and the shared microenvironment supporting different tissues. Differential gene expression analysis further showed heterogeneity existed in cells of the same cell type that obtained from different tissues. In addition, Han et al. also constructed correlation networks among tissues based on gene expression dynamics. They found extensive connections between mesenchymal and epithelial cells, endothelial and hematopoietic cells, supporting the M-E transitions and endothelia-derived hematopoiesis.

Importantly, a single-cell mouse cell atlas (scMCA) database archiving single-cell gene expression profiles was established in this study for easily public browsing and accessing. Furthermore, a cell type classification tool based on a gene expression correlation ranking approach was also developed. This effort will promote further investigation of expression networks of individual cells at the level of a whole organism.

The Microwell-seq technique reaches on average 10% single cell capture efficiency, which is enough to harvest majority of cell types in a bulk sample. Further improvements are needed to enhance the capture efficiency for analyzing rare cell types. Though massively parallel transcriptome profiling of single cells has been achieved, exploring single-cell genome and epigenome remains at low throughput. Prospectively, cellular identity will be defined more precisely by the combination of regulatory networks composed of multiple layers of information, including but not limited to...
genome, epigenome, transcriptome and proteome, with the improvement and development of new single-cell multi-omics techniques [6-11].

In summary, Han et al. proposed a high-throughput and low-cost single-cell RNA sequencing technique and profiled the gene expression of single cells covering mouse major cell types. This work built the first comprehensive reference map of transcription diversity in mouse at single-cell resolution and provides valuable resources for the research community performing in-depth biological data mining. The successful application of Microwell-seq in mouse will largely accelerate the single cell transcriptome study on human and other species in a living system. In the meantime, clinical values of applying this Microwell-seq technology will be revealed, for which perhaps different nucleated blood cells in the hematopoietic and immune system would be the most feasible targets to begin with in a variety of diseases.

References