Single cell RNA-seq analyses to investigate sub-populations of bone-marrow and adipose derived multipotential stromal cells (MSCs) from 3 donors

Vyacheslav Furtak, Viswanadham Sridhara, and Malcolm Moos

Introduction

Multipotential stromal cells (MSCs) are a promising cell source for regenerative medicine therapy. In spite of many clinical trials, convincing evidence of efficacy is limited. An important obstacle has been difficulty of product characterization caused by cellular heterogeneity, variable bioactivity, inadequate cell surface markers, donor to donor variability etc.

Single cell RNAseq allowing massively parallel analysis of gene expression has become a powerful tool for identifying different cellular subpopulations, some if which might be functionally more active and therefore useful for cell therapy.

Investigation of single cell RNA-seq of MSCs isolated from adipose and bone marrow tissue was aimed to characterize tissue specific MSCs properties at the single cell level.

Materials & Methods

Paired samples of bone marrow and adipose tissue were donated from each of three subjects; stromal cells were isolated and expanded in serum-free medium at 37 °C with 5% CO_2 in a humidified atmosphere. After the cell density reached about 70% confluence, cells were dissociated with TrypLE™ Select (Thermo Fisher Scientific) and used immediately for single-cell library construction (Chromium Single Cell Gene Expression Solution, V2 Chemistry, 10x Genomics) according manufacturer's protocol.

Cell Ranger (10X Genomics) and Seurat were used to convert the single cell raw NGS data to count data. Monocle3 (R package) was then used to identify the clusters (sub-populations), find the differentially expressed markers among the clusters, and finally identify the trajectories of the sub-populations within the entire population.

Single cell methods offer discriminating power that allows exploration of cellular heterogeneity not possible with bulk RNA-seq methods.

There are clear differences between bone marrow- and adipose-derived MSCs populations.

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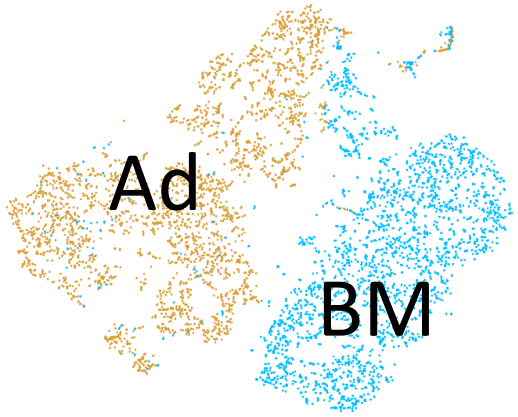
Results

Single cell methods showed promise to help characterize MSCs of bone marrow and adipose origin at high definition



Bone marrow- (BM) and adipose-specific (Ad) clusters of MSCs identified using single cell RNA-seq (10x Genomics).

Conclusions

- **Single cell methods showed promise to help characterize MSCs of bone marrow and adipose origin at high definition**
- **DLX5, Runx3 transcription factors, and CD200 were identified by sc-RNA-seq as specific markers of bone marrow derived MSC cellular subpopulations absent in adipose derived MSCs**
- **Bone marrow derived MSC subpopulations may have a better potential for cell therapy because of specific expression of biologically active molecules**

Acknowledgments

We would like to acknowledge CBER sequencing Core Facility and HIVE Bioinformatics Team for excellent help and support.

Single cell RNA-seq analyses to investigate sub-populations of bone-marrow and adipose derived multipotential stromal cells (MSCs) from 3 donors

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Introduction

Multipotential stem cells (MSCs) also known as mesenchymal/stromal stem cells are considered a promising source of cellular therapy in regenerative medicine. In spite of many clinical trials, convincing evidence of efficacy is limited. An important obstacle has been difficulty of product characterization caused by cellular heterogeneity, variable bioactivity, inadequate cell surface markers, donor to donor variability etc. Single cell RNAseq allows massive parallel analysis of gene expression has become a powerful tool for identifying different cellular subpopulation some if which can be functionally more active and therefore useful for cell therapy.

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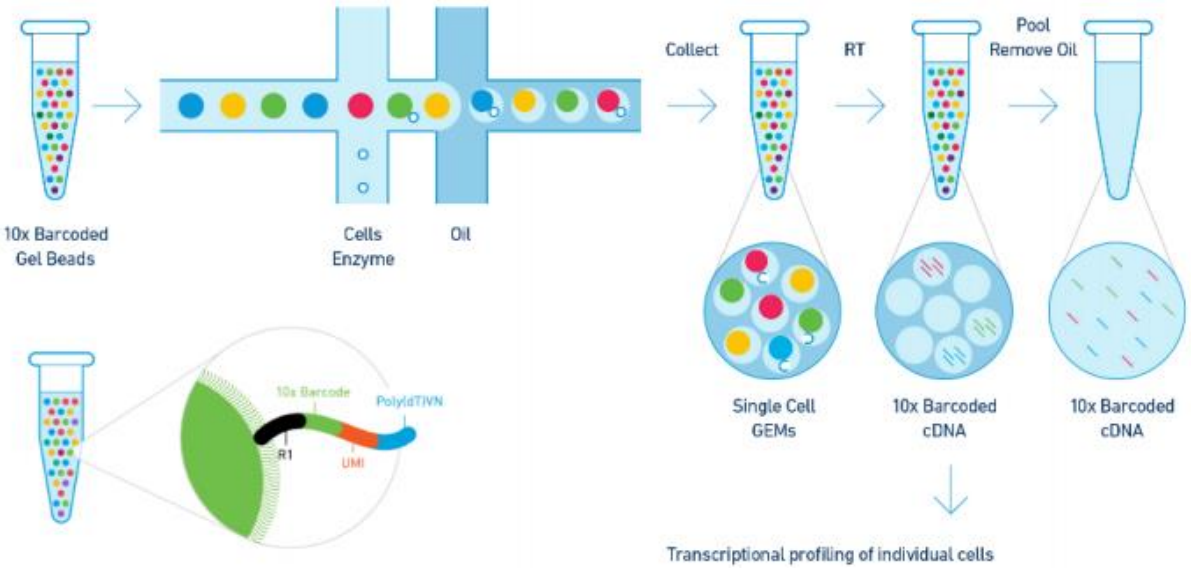

10X Genomics sample processing pipeline for preparation of single cell RNA-seq libraries.

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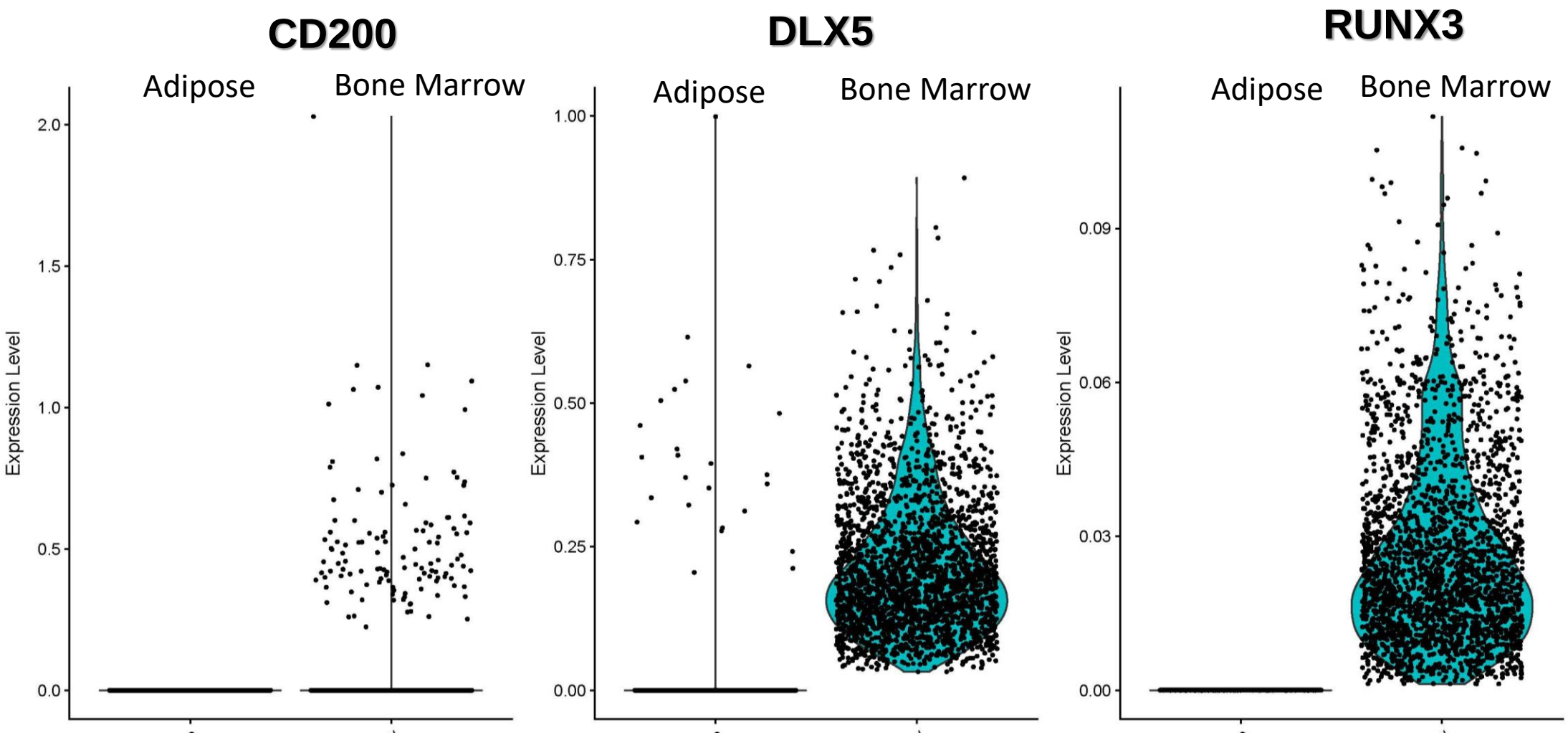
CD200, DLX5, and RUNX3 Distinguish Bone Marrow from Adipose-derived MSCs



Violin plots generated with Seurat showed significantly higher expression of CD200, DLX5, and RUNX3 in bone marrow-specific MSCs.

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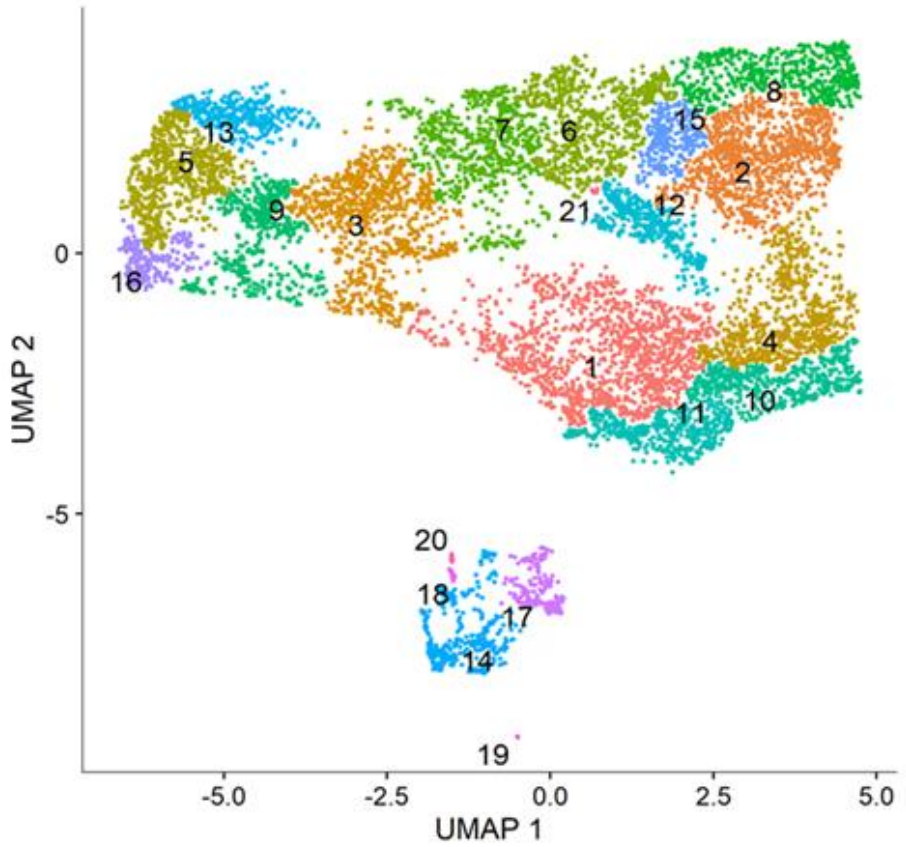
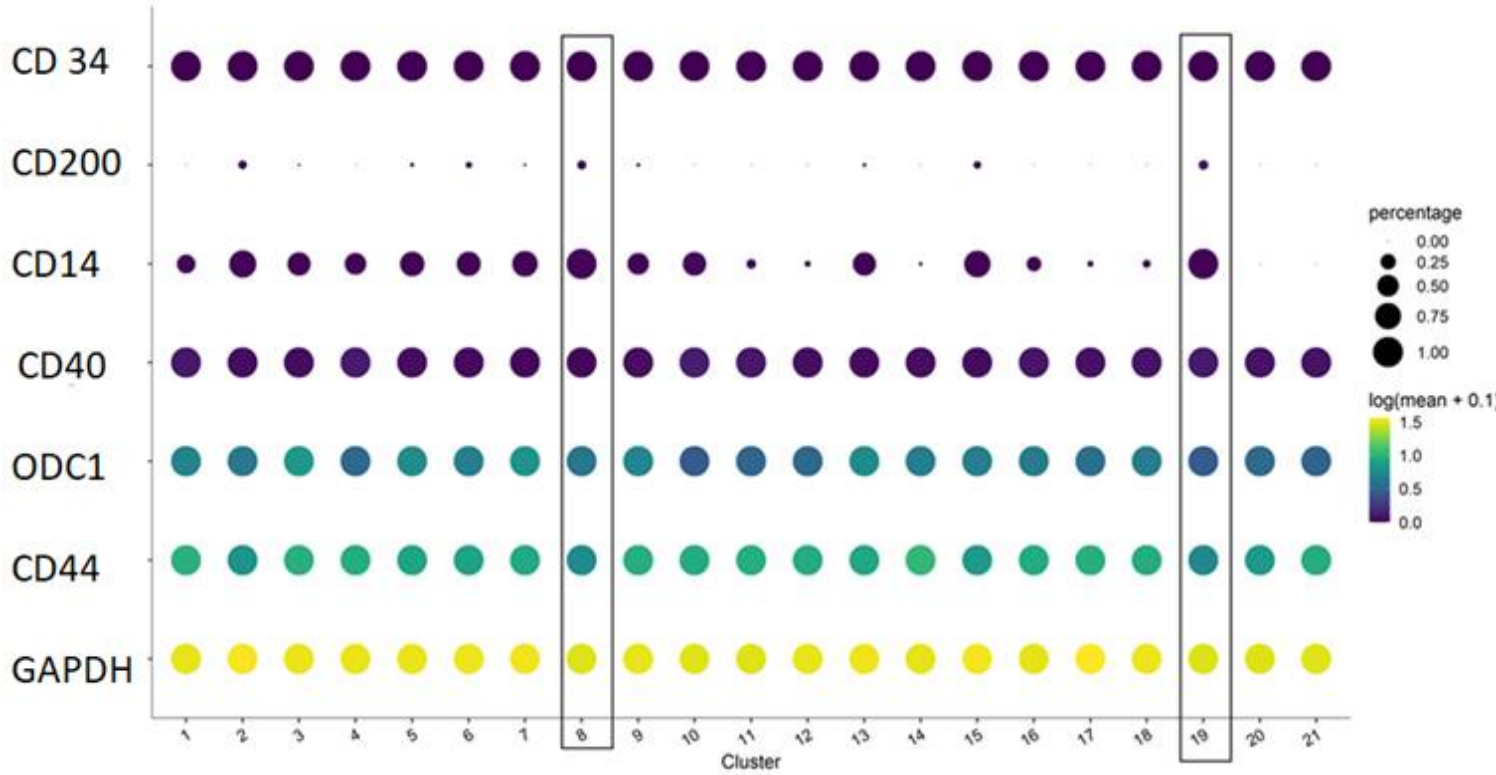
There were both bone marrow- and adipose-specific clusters



FastMNN batch-corrected samples followed by Monocle3 processing resulted in 21 unique clusters. Clusters 8/19 were bone marrow-specific, while clusters 14/17/20/21 were adipose-specific.

The transcriptome profile of two clusters were bone marrow-specific



CD200 (bone marrow marker) and other known markers for MSCs were shown for all the clusters. We identified 2 populations of cells (clusters 8 and 19) that were seen only in bone-marrow derived samples.

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