

Introduction

Summary

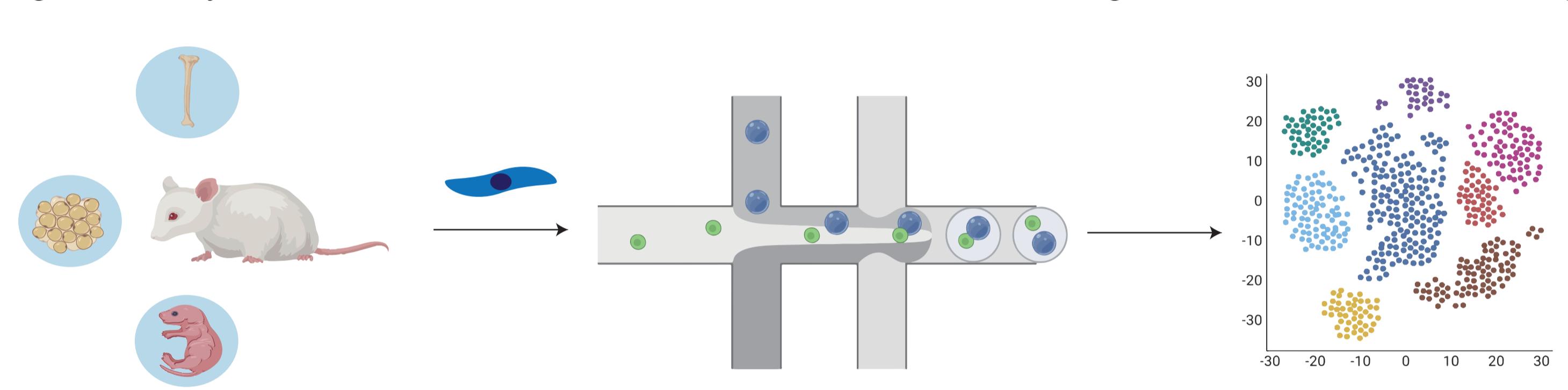
- The medical potential of mesenchymal stem/stromal cells (MSCs) has attracted the attention of numerous researchers for years; yet, application difficulties arise from the intercellular heterogeneity.
- The intercellular heterogeneity may be exposed using single-cell RNA sequencing.
- In this study, we collected 494997 cells by integrating data from 17 research. There are 18 subsets of MSCs and their gene markers are found.

Definition, application and current research status of MSC

Mesenchymal stem/stromal cells (MSCs) are an essential type of pluripotent stem cell because they can self-renew and develop into osteoblasts, adipocytes, and chondrocytes¹. Furthermore, mesenchymal stem cells secrete proteins that promote angiogenesis and wound healing, regulate the inflammatory milieu, and assist neuronal development. MSCs are a popular stem cell product in clinics, with promising applications in cell therapy and regenerative medicine².

Single-cell sequencing approaches can fully characterize cell type diversity by directly detecting various molecular signatures in hundreds to millions of individual cells. These measures reveal gene expression processes that influence cellular identity and heterogeneity among cell populations³.

The intercellular heterogeneity is partially shown in earlier studies that applied single-cell sequencing technologies to study MSCs from various tissues; however, more extensive integration of these data is still required.



MSCs From Different Tissues

Single-cell RNA sequencing

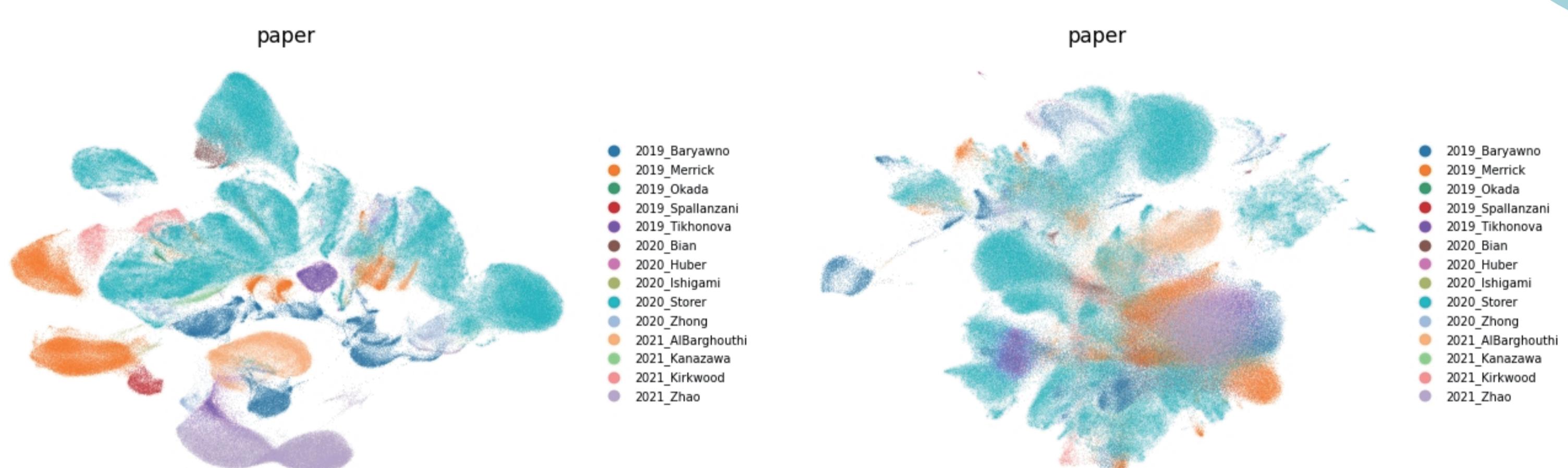
Data analysis

Results

mMSC
Atlas

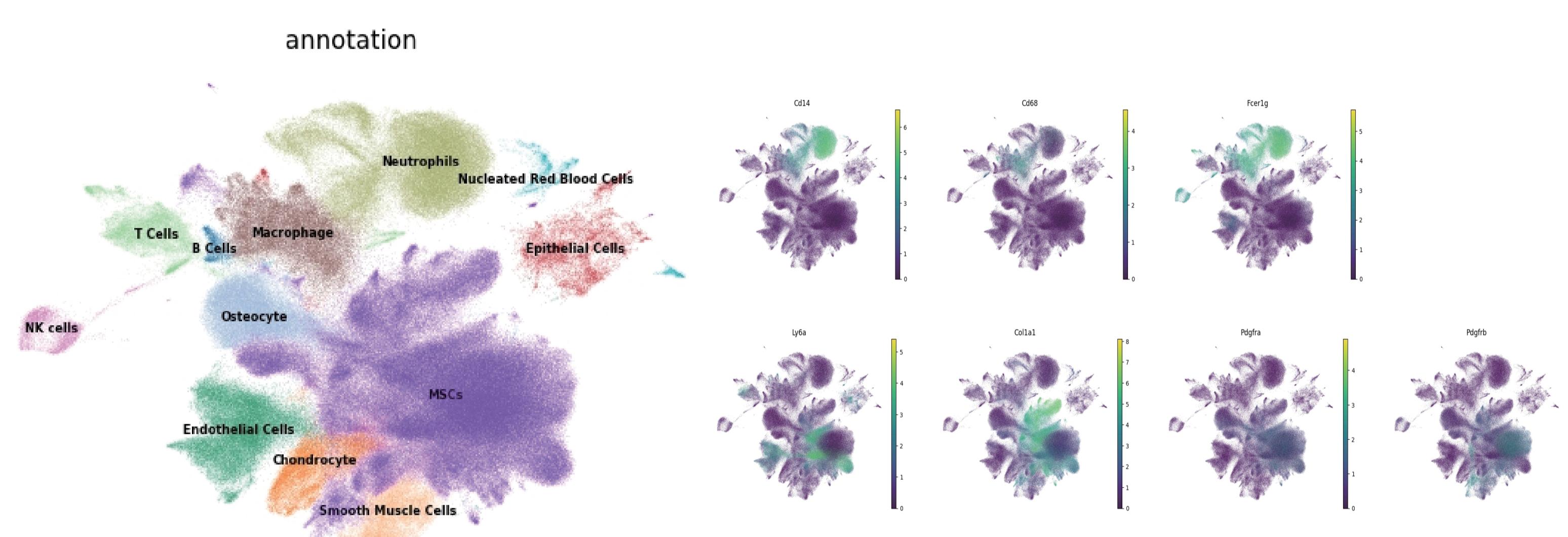
• UMAP figures of the integrated data after batch effect removing

The dimensionality reduction of integrated data using UMAP, and scVI model was used for batch effect removal.



• Clustering and annotation

After clustering by leiden algorithm, some classical gene markers were used to annotate each cluster.

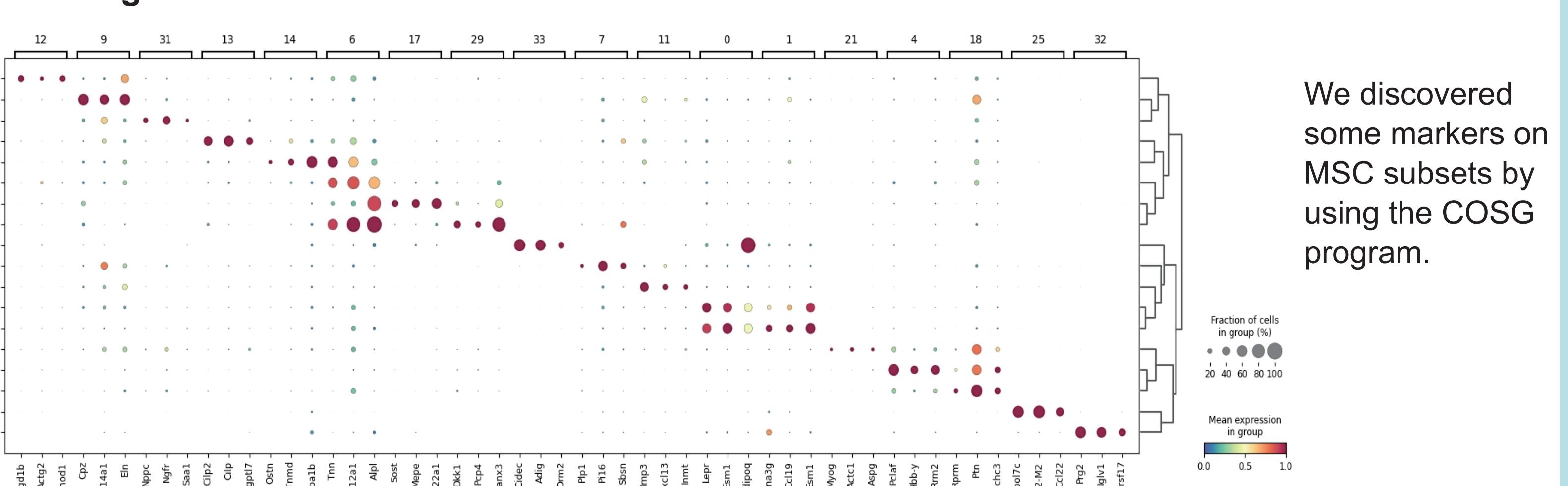


• PAGA analysis of MSCs subsets

The MSC subgroup was chosen for further research after annotation.

The association between the various clusters is revealed by PAGA analysis across all MSC subsets, and the diverse groups are revealed by PAGA analysis on a few well-known and classic MSC gene markers.

• Find gene markers between MSCs subsets



We discovered some markers on MSC subsets by using the COSG program.

Research Gap

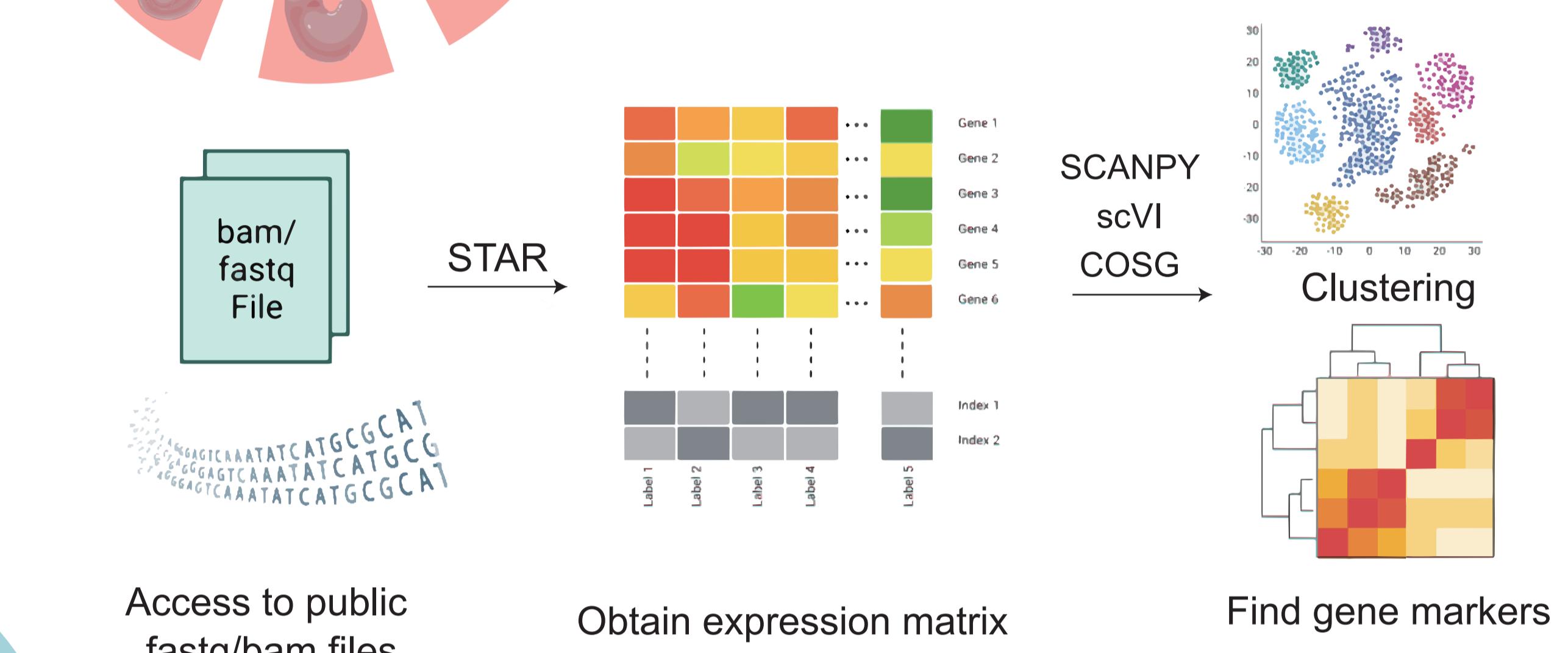
- What is the **cell composition** of MSCs?
- What are the **features** of cell subsets of MSCs?
- Are there **specific markers** that can classify and purify functional subsets of MSCs?

• Study summary and workflow



Our data source for this investigation was gathered from 17 published publications, all of which focused on isolated MSCs in mice. And 9 distinct starting tissues for sampling are used in these studies.

The single cell expression matrix was obtained by aligning the fastq or bam data using **STAR** software. The primary downstream analysis tool is **SCANPY**. Following rudimentary filtering, 494997 cells remain valid. Gene markers were found using the **COSG** package, and batch effect reduction was accomplished using the **scVI** model.



Access to public fastq/bam files

Obtain expression matrix

Find gene markers

Discussion

• Advantages and limitations of this study

Advantages

- In this study, single-cell RNA sequencing data from different tissue sources were collected, it is helpful to compare the similarities and differences between MSCs from different sources.
- As the number of cells increases, more reliable conclusions on MSC subsets and their gene markers can be obtained.

Limitations

- Because all the data comes from different studies, the batch effect is still a challenge for integration. The batch effect removing result is not satisfactory.
- At present, all the conclusions have not been verified by biological experiments.
- Only MSC cells isolated from mice are not enough for practical field.

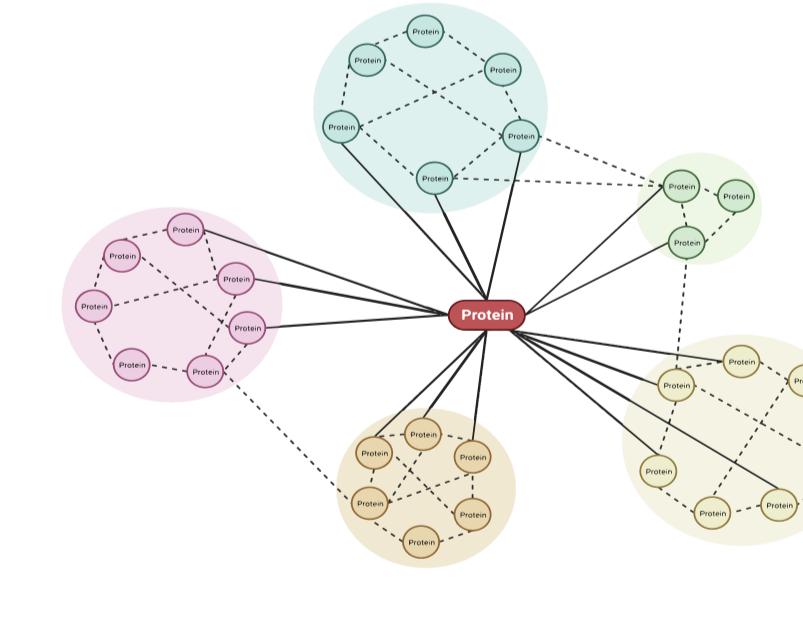
• Current results reveal some potential subpopulations of mouse MSCs

- The study's findings support some hypotheses and the findings of earlier research, including the idea that MSCs are heterogeneous. Heterogeneity appears in MSCs derived from distinct tissues as well as within MSCs originating from the same tissue. The most heterogeneous MSCs were found in adipose tissue.
- Distinct MSC subgroups express various classic marker genes differently. For instance, the expression levels of Pdgfra and Ly6a differ in two cluster groups.
- Distinct marker genes are present in each subpopulation; these genes indicate the direction, stage, and functional aspects of differentiation.

• Feature plans



Cross-species analysis



Pathway analysis



Database construction

Human sequencing data will be included in the analysis.

More in-depth and systematic research on characteristic genes should be carried out.

Make the results of markers and MSC subsets to be a user-friendly website.

Reference

- Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. *Nat Biotechnol*. 2014 Mar;32(3):252-60.
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- Zhang C, Han X, Liu J, Chen L, Lei Y, Chen K, Si J, Wang TY, Zhou H, Zhao X, Zhang X, An Y, Li Y, Wang QF. Single-cell Transcriptomic Analysis Reveals the Cellular Heterogeneity of Mesenchymal Stem Cells. *Genomics Proteomics Bioinformatics*. 2022 Feb;20(1):70-86.

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