Mapping human bone marrow cellular heterogeneity by single-cell transcriptomic and proteomics

Michael P. Duffy[1] (michael.duffy@Pennmedicine.upenn.edu), Shovik Bandyopadhyay[2], Kyung Jin Ahn[3], Charles Nelson[1], Kai Tan[3], Ling Qin[1]
[1]Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA [2]Cellular and Molecular Biology Graduate Group, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA [3] Division of Oncology and Center for Childhood Cancer Research, Children's Hospital of Philadelphia, Philadelphia, PA

Disclosures: The authors declare they have no conflicts of interest.

INTRODUCTION: Bone marrow (BM) is a complex organ primarily made of mesenchymal and hematopoietic lineage cells that functions to form new bone and to produce blood. Despite extensive work studying single human hematopoietic cells (e.g., Human Cell Atlas - Immune Cell Atlas), non-hematopoietic cells, particularly mesenchymal lineage cells, have not been mapped due to their rareness. It is well known that mesenchymal cells provide niche support for hematopoiesis. In the study, we developed a robust protocol to isolate cells from human femoral head and mapped them at a high level of heterogeneity using single-cell omics approaches.

METHODS: Specimens –Non-human subject research determination was made by University of Pennsylvania IRB. Surgical waste from total hip arthroplasty was collected at Penn Presbyterian Medical Center. A dental biopsy core was used to collect samples for CODEX (co-detection by indexing) and the distal bone marrow tissue cut into about 1 mm cubes and subjected to 1 hour of digestion (PBS + 2 mg/mL Collagenase I + 4 mg/mL Dispase II) at 37 ˚C to isolate a single cell suspension (Fig 1A, B). Single cell RNA-sequencing (scRNA-seq) – Using EasySep system (StemCell Tech.), cells were first RBC-depleted and then separately enriched for non-hematopoietic (CD45 depletion) and hematopoietic progenitor (CD34 selection) cells, and then mixed with RBC-depleted unenriched BM cells at 10:3:3 ratio for scRNA-seq using 10x genomics. Cell culture –scRNA-seq defined markers were used to sort four MSC populations – Fibro, Thy1, Adipo, and Osteo. Cells were seeded for CFU-F, proliferation, and differentiation studies using MesenCult Proliferation Kit (StemCell Technologies). Population doubling level was calculated weekly for eight weeks while controlling seeding density (n=6-8). Osteogenic (AlphaMem with 10% FBS, 0.1 mM LAA, 2 mM Glutamine, 10 nM DMSO and 1.8 mM KH_2PO_4), adipogenic (AlphaMem with 15% FBS, 0.1 mM LAA, 2 mM Glutamine, 0.5 mM isobutylmethylxanthin, 60 µM indomethacin, 0.5 µM hydrocortisone and 10 µg/mL insulin) and chondrogenic (DMEM High Glucose, 10 ng/mL TGF-beta1, 10 mM sodium pyruvate, 40 ug/mL L-proline, 1% ITS+ Premix, 100 nM DMSO, 50 ug/mL LAA) media were used. Alizarin Red S, Oil Red O and Alcian Blue staining was performed after 21-28 days of induction (n=3). CODEX – A custom antibody panel was used to spatially profile the proteome of 12 samples using Akoya Phenocycler system and whole slide images segmented using Mesmer before cell annotation.

RESULTS: From 12 human bone specimens (age 52-74, median 65±8), we profiled 53,417 hematopoietic and 29,325 non-hematopoietic cells at 3,117 genes/cell and identified 35 cell clusters of hematopoietic, mesenchymal, endothelial, and smooth muscle cell origin (Fig 1C). Further analyzing mesenchymal cells yielded Fibro-mesenchymal stromal cells (MSCs), Osteofibro-MSCs, Osteo-MSCs, Osteoblasts, Adipo-MSCs, and THY1+ MSCs with distinct markers (Fig. 2A). Fibro-MSCs, marked by canonical mesenchymal stem markers, were identified as the most primitive population by CytoTRACE. Both THY1+ and Adipo-MSCs highly expressed adipogenic markers, mimicking an adipogenic precursor population we previously identified in mouse bone marrow (PMID 32286228). Sorting Fibro-, Thy1+-, Osteo-, and Adipo-MSCs demonstrated that Fibro-MSCs generated highest rate of CFU-Fs (Fig. 2F-G) and have the highest proliferative ability over long-term culture (Fig 2B). All subgroups showed trilineage differentiation ability in culture (Fig. 2C-E). While all mesenchymal and endothelial cells produced hematopoietic supportive factors, Adipo- and THY1+-MSCs expressed the highest amount of CXCL12 and KITL to regulate HSPCs, IL7 to regulate lymphopoiesis, and Csf1 to regulate myelopoiesis (Fig. 3A). To define BM single-cell anatomy, we employed whole-slide CODEX multiplexed imaging of 12 specimens. By computationally annotating 803,132 cells, we identified 32 cell types, including Adipo- and THY1+-MSCs and 6 major BM structures (adipocytes, arterioles, bone, macrophages, sinusoids, and CXCL12+ stroma). By implementing point pattern analysis, we found that primitive, SPINK2+, hematopoietic stem and progenitor cells (HSPCs) were localized primarily close to adipocytes (Fig 3B and 3C).

DISCUSSION: Our novel sample processing enabled the creation of the first human bone marrow scRNA-seq dataset to capture the heterogeneous mesenchymal cell population along with the full hematopoietic lineage. scRNA-seq analysis identified several mesenchymal progenitor cells, and our *in vitro* experimentation supported the CytoTrace data that predict Fibro-MSCs as the primitive mesenchymal progenitors. Using CODEX imaging with a novel 53-antibody panel, we discovered an adipocytic, but not endosteal or perivascular, niche for HSPCs.

SIGNIFICANCE: Our novel findings provide a critical resource for understanding bone marrow physiology in an aged population and present a foundation to study hematopoietic cell regulation by mesenchymal lineage cells.

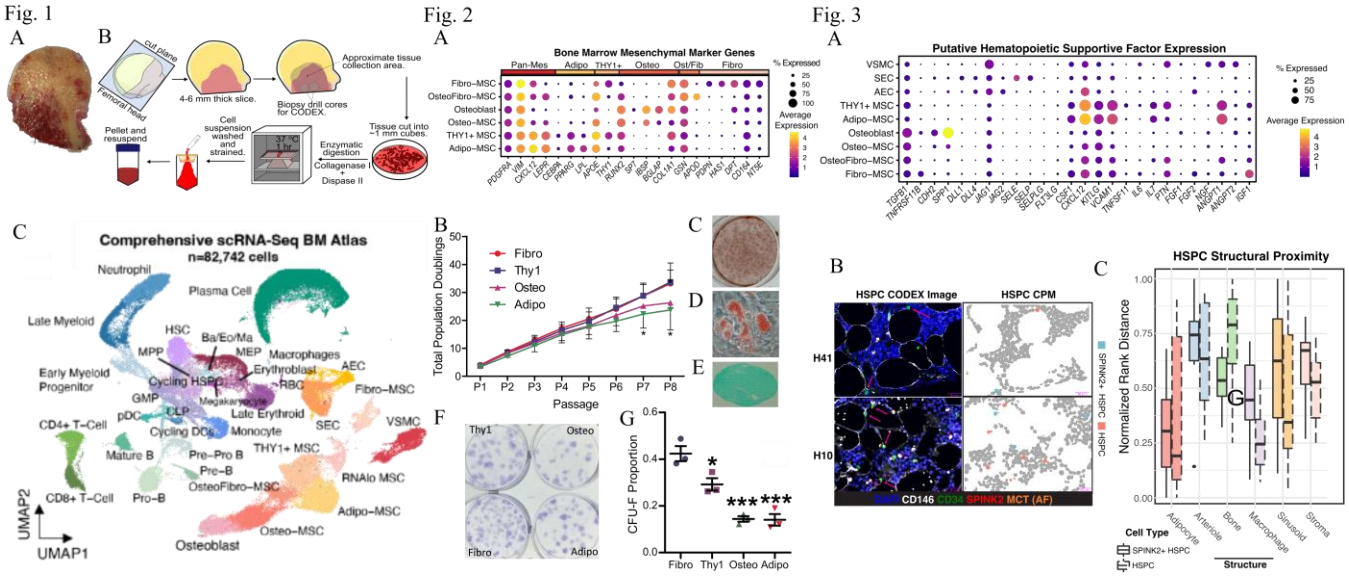

Fig 1. Capturing heterogenous bone marrow scRNA-seq data. A. Typical sample section collected. B. Single cell capture workflow. C. UMAP of 35 cell clusters from 12 hip samples. **Fig 2. Identification of Fibro-MSC as primitive bone marrow mesenchymal cell** A. Characteristic gene expression for mesenchymal clusters. B. Cumulative population doublings showing slowed proliferation in Osteo- and Adipo-MSCs. C/D/E. Demonstrated osteo-, adipo-, and chondrogenic potential (Fibro-MSCs shown). F. CFU-F staining of sorted cells. G. CFU-F proportion per sample is higher in Fibro-MSCs. **Fig 3. Hematopoietic regulation by MSCs and mesenchymal cells**. A. Hematopoietic support factors are highly expressed in Thy1+/- and Adipo-MSCs. B. Sample CODEX image used to define spatial patterning of primitive HSPCs. C. HSPC proximity is nearest adipocytes.