Single-cell Transcriptional Profiling Supports GP Stem Cell Hierarchy

Yiwei Kong¹, Yu Zhou², Shreya Kumar¹, Shannugam Muruganandan¹, Dan Bita¹, Shobana Sekar¹, Rachel Pierce¹, Hannah Alberico¹, Andrew Piasecki¹, Dori Woods², Andrea M. Ionescu³
¹Northeastern University, Boston, MA, ²Boston Children’s Hospital, Boston, MA, ³Los Altos High School, Los Altos, CA

kong.yiw@northeastern.edu

INTRODUCTION: We recently discovered a FoxA2+ long-term skeletal stem cell (LTSSCs) population at the top of growth plate (GP) resting zone (RZ), with higher clonogenicity and longevity than the previously identified PTHrP+ short-term skeletal stem cells (STSSCs)²–⁴. To validate the newly discovered GP stem cell hierarchy, we performed a comprehensive single-cell RNA sequencing analysis on GP cells isolated from P18 C57B6 mice.

METHODS: Single-cell RNA Sequencing. The single-cell suspension from GP cartilage of P18 C57B6 mice was loaded on a Chromium Controller (10x Genomics). Library construction was performed using Chromium Single Cell 3’ GEM, Library & Gel Bead Kit v3 (10x Genomics) following the manufacturer’s protocol and sequenced using Illumina NovaSeq 6000 platform.

scRNA-seq Data Analysis. Alignment of scRNA-seq data to the mouse genome (mm10) and gene counting was completed utilizing the Cell Ranger. A filter was applied to remove low-quality cells. Then R package “Seurat”, “Monocle 3” were applied to perform downstream analysis and visualization.

RESULTS: We separated clusters positive for chondrocyte markers (Acan, Col2a1) from erythroid cells, B cells, neutrophils, osteoblasts, macrophages, endothelial cells, NK cells and monocytes. To further investigate the chondrocyte population, we performed sub-clustering of the chondrocytes based on their gene expression pattern, yielding 1444 cells separated in 9 clusters (0-8). High expression level of Col2a1 was observed in all clusters (A1). Cluster 6 was enriched for proliferation markers Mki67 and Cdk1 (A2-3). Clusters 2, 3, 6 showed enrichment for columnar chondrocytes marker Gdf10 and hypoxia-associated gene Cox4i2 (A4-5). Cluster 4 has high levels of hypertrophic chondrocytes markers Col10a1 and Ihh (A6-7). Cluster 5 was enriched for S100a7a and Cdkn1c (p57) (A8-9), expressed in pre/hypertrophic chondrocytes. Cluster 1 expressed markers of late hypertrophic chondrocytes Mmp13 and Bmp7 (A10-11). The remaining clusters (0, 8, 7) were annotated as RZ cells positive for skeletal stem cell markers Pdpn, Xrcc2 and Srp9 (A12-13). Consistent with GP geography, hypoxia-induced gene, Mif was highly expressed in all clusters except for Cluster 8 (RZ FoxA2+ layer - at the top of the GP) and Cluster 1 (late hypertrophic chondrocytes - at the bottom of the GP) (A15). Lastly, PTHrP expression is mapped to RZ cluster 0, consistent with specific localization of PTHrP+ cells at the bottom of the RZ. Future investigations need to determine whether RZ cluster 7 are progenitor cells. These results confirm a distinct separation of FoxA2+ and PTHrP+ cells at the bottom of the GP.

DISCUSSION: GP RZ was characterized as a stem cell rich region, which is essential for the endochondral bone development and GP regeneration after injury. We recently discovered FoxA2+ LTSSCs, which can differentiate into the PTHrP+ STSSCs overtime⁵. With scRNA-seq analysis, we identified the GP stem cell hierarchy, and we found that FoxA2+ LTSSCs and PTHrP+ STSSCs are independent of each other in transcriptomic level.

SIGNIFICANCE/CLINICAL RELEVANCE: Our study expands our knowledge of the GP at a single-cell level. A better understanding of the GP stem cell hierarchy is developing, with the aim of contributing to further research on the cartilage regeneration upon GP injury.

REFERENCES:

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