

Single-cell Transcriptional Profiling Supports GP Stem Cell Hierarchy

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DISCLOSURES: Yiwei Kong (N), Yu Zhou (N), Shreya Kumar (N), Shanmugam Muruganandan (N), Dan Bitá (N), Shobana Sekar (N), Rachel Pierce (N), Hannah Alberico (N), Andrew Piasecki (N), Dori Woods (N), Andreia M. Ionescu (N)

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)^{1,2}. 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 Srp2 (A12-14). In line with our previous studies, FoxA2 marks two distinct populations, the FoxA2+col.10- LTSSC group in the RZ (cluster 8), and the FoxA2+col.10+ HZ chondrocytes (cluster 4) (A16). 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+LTSSC and PTHrP+STSSC in the GP RZ. To further validate cluster hierarchy, we performed Monocle 3 pseudotime cell trajectory analysis, assigning cluster 8 (FoxA2+LTSSC) as a starting point. This analysis showed a continuous trajectory linking RZ chondrocytes (clusters 8, 0, 7), going through columnar chondrocytes (clusters 2, 3, 6), to terminal differentiated hypertrophic chondrocytes (clusters 5, 4, 1) (Fig. B) supporting previously identified GP stem cell hierarchy.

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 overtime¹. 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 sing-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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