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Single-cell RNA sequencing reveals the heterogeneity of adipose tissue-derived mesenchymal stem cells under chondrogenic induction

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This study investigated how adipose tissue-derived mesenchymal stem cells (AT-MSCs) respond to chondrogenic induction in vitro using droplet-based single-cell RNA sequencing (scRNA-seq). We analyzed 37,219 high-quality transcripts from control cells and cells induced for 1 week (1W) and 2 weeks (2W). Four distinct cell clusters (0-3), undetectable by bulk analysis, exhibited varying proportions. Cluster 1 dominated in control and 1W cells, whereas cluster 3, 2, and 0 exclusively dominated in control, 1W, and 2W cells, respectively. Furthermore, heterogeneous chondrogenic markers expression within clusters emerged. Gene ontology (GO) enrichment analysis of differentially expressed genes unveiled cluster-specific variations in key biological processes (BP): (1) Cluster 1 exhibited upregulation of GO-BP terms related to ribosome biogenesis and translational control, crucial for maintaining stem cell properties and homeostasis; (2) Additionally, cluster 1 showed upregulation of GO-BP terms associated with mitochondrial oxidative metabolism; (3) Cluster 3 displayed upregulation of GO-BP terms related to cell proliferation; (4) Clusters 0 and 2 demonstrated similar upregulation of GO-BP terms linked to collagen fibril organization and supramolecular fiber organization. However, only cluster 0 showed a significant decrease in GO-BP terms related to ribosome production, implying a potential correlation between ribosome regulation and the differentiation status of AT-MSCs. Overall, our findings highlight heterogeneous cell clusters with varying balances between proliferation and differentiation before and after chondrogenic stimulation. This provides enhanced insights into the single-cell dynamics of AT-MCSs during chondrogenic differentiation.