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Identifying Key Transcription Factors in Chondrocyte Dedifferentiation by single-cell multiomic analyses

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Articular cartilage plays a crucial role in joints by acting as a shock absorber and facilitating smooth movement. However, its avascular nature significantly limits its capacity for self-repair. Osteoarthritis (OA), which affects over 10% of the U.S. adult population, highlights the challenges of cartilage regeneration. Autologous Chondrocyte Implantation (ACI) is a promising therapeutic approach, yet the in vitro expansion of chondrocytes often results in dedifferentiation, leading to a loss of phenotype and reduced therapeutic efficacy. To address this challenge, we identified several chromatin regulators as key factors influencing chondrocyte dedifferentiation. Specifically, we utilized single-nuclei multiome sequencing to explore the dynamics of gene expression and chromatin accessibility across three passages (P0, P3, and P6) during the in vitro expansion of human chondrocytes. Our transcriptome analyses revealed that progressive passaging was associated with a decrease in genes linked to cartilage repair potential and an increase in genes related to the transition from cartilage development to extracellular matrix remodeling. Additionally, applying ChromVAR to analyze single-nuclei chromatin accessibility allowed us to identify transcription factor (TF) motifs that were transiently accessible during the early stages of the single-cell trajectory. We further narrowed our focus to five transcription factors (AHR, NRF1, STAT1, STAT2, and TEAD1) based on their correlation with corresponding gene expression levels. Future work will involve applying inhibitors targeting these factors to cells in vitro between passages P0 and P2. Moreover, we will conduct clinical validation using *Sus scrofa* ACI to evaluate the impact of these factors on cartilage therapy.