Single Cell Transcriptomics Identifies Age-related Transcriptional Heterogeneity Within Fibroadipose Progenitor Cells That Can Be Reprogrammed By Mechanical Loading In Old Rats Recovering From Disuse Atrophy

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BACKGROUND

- Older adults do not fully recover muscle mass after disuse atrophy.
- Extracellular matrix (ECM) content increases with age and during the recovery period following disuse, which likely blunts the responsiveness of muscle to mechanical stimuli in older adults.
- Understanding how age affects ECM deposition and if this process can be remodeled may give insight into therapeutic strategies for enhancing muscle mass recovery in older adults.

PURPOSE

- To identify age-related changes in the gene expression of ECM-regulating cells in skeletal muscle, and if mechanical loading can reverse these changes.

METHODS

- **Animals**: 10- and 30-month-old male F344/BN rats (n=1 per condition).
- **Mechanical Loading** (ML): ML was performed every other day (total of 4 bouts) for 30 min at 4.5 N load and 0.5 Hz.

- **Cell Isolation**: Muscles were digested in collagenase and live mononuclear cells sorted using propidium iodide and FACS.
- **Single cell RNA-sequencing (scRNA-seq)**: scRNA-seq was performed using a 10X Chromium Controller and sequenced at Novogene on a Hiseq Platform. Analyses of quality control, PCA, UMAP, and GSA were performed using Partek Flow.

SUMMARY OF FINDINGS

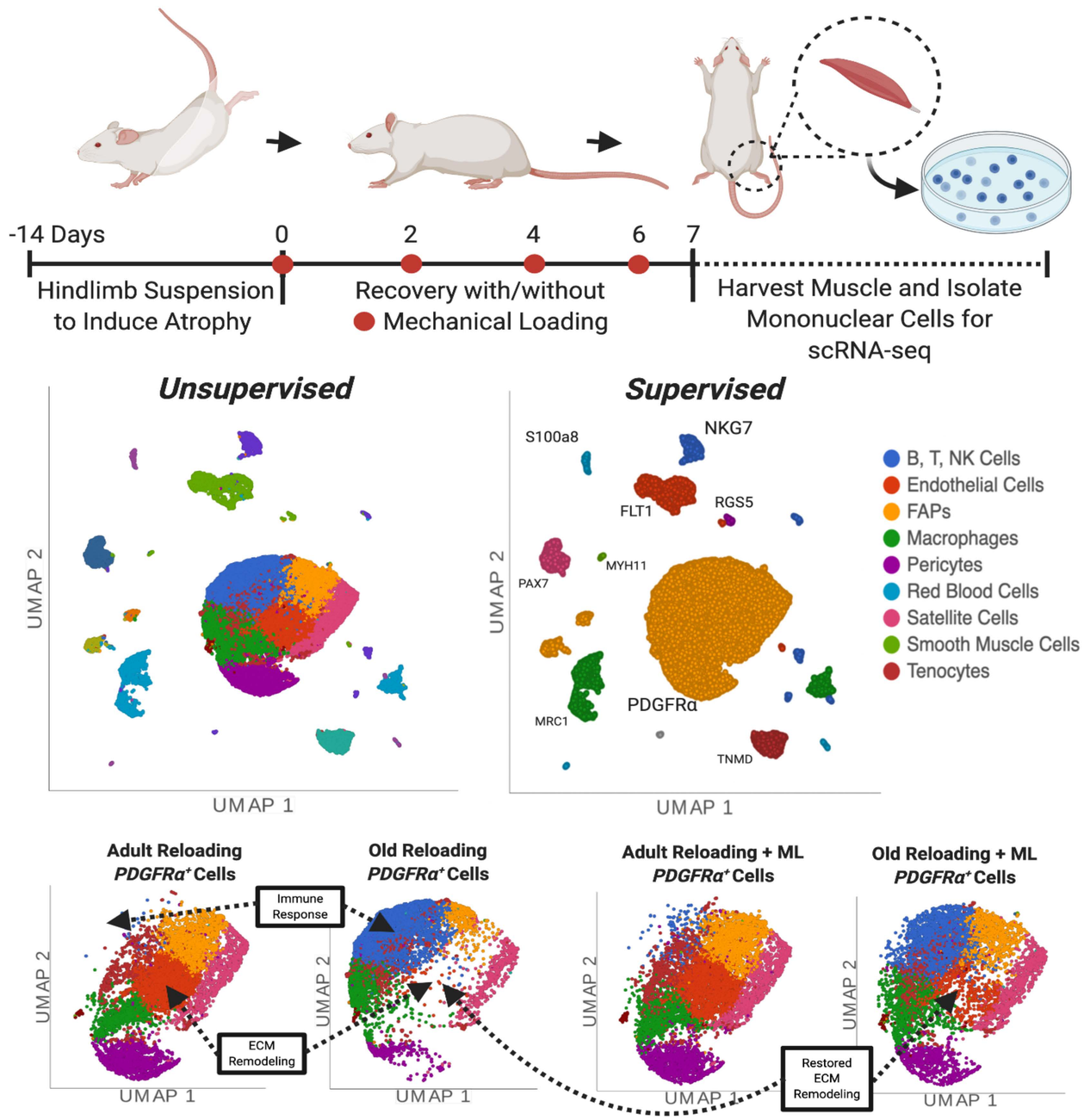

Figure 1. Summary of Findings. Following 14 days of hindlimb suspension, adult and old rats ambulated freely in their cages with or without mechanical loading every other day for a total of 7 days (4 total bouts). The right gastrocnemius muscle from each animal was harvested, live mononuclear cells were isolated and sorted via FACS, and cells underwent single cell RNA sequencing. Supervised cell classifications using known gene identifiers identified 9 cell populations, which expanded to 16 following unsupervised graph-based clustering, with the 7 previously unidentified clusters all within FAPs. GSA analysis identified age-related differences in FAPs, including an absence of ECM remodeling transcriptomes and a presence of immunomodulation. Mechanical loading reprogrammed the ECM remodeling transcriptomes in FAPs similar to that of an adult animal.

Figure 2. Mechanical loading lowers inflammation-related gene expression while also activating proliferation and ECM-remodeling-related gene expression in FAPs. RE; reloading, ML; mechanical loading.

CONCLUSION

- Single cell RNA sequencing identifies age-dependent differences in the transcriptomes of FAPs within skeletal muscle undergoing recovery from disuse.
- During the recovery after disuse, FAPs in old rats display an immunomodulatory transcriptome, instead of an ECM remodeling transcriptome observed in adult rats.
- Mechanical loading reprograms the transcriptome of FAPs by establishing an ECM remodeling transcriptome similar to that observed in adult rats.

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