Single-cell RNAseq identifies heterogeneity in myoblasts from older adults with differences related to muscle mass and function

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Introduction

- Sarcopenia, the age-related decline in muscle mass and function, is associated with adverse outcomes including frailty, falls and mortality [1].
- Environmental factors such as physical inactivity, diet and obesity are implicated in the causation of sarcopenia, linked to cellular and molecular changes within skeletal muscle, but why individuals experience greatly different rates of muscle decline remains elusive [2].
- To date, most studies investigating gene regulation within muscle dysregulation have focused on bulk transcriptomic analysis.
- Many studies have shown an age-related decline in the number of satellite cells (SCs) (Myoblasts) and/or an age-related loss of satellite cell function within muscle dysregulation, however, the precise role that SCs play in the loss of muscle mass and strength during older age remains controversial [3].
- It is now becoming clear with the advent of single cell transcriptomics which deconvolutes cellular heterogeneity at the tissue or cellular level, that muscle satellite cells are not a homogeneous population of cells [4].
- However, to date, there have been limited studies in humans investigating the transcriptional heterogeneity of myoblasts and how this may vary in individuals with low muscle mass and function compared to healthy aged individuals.

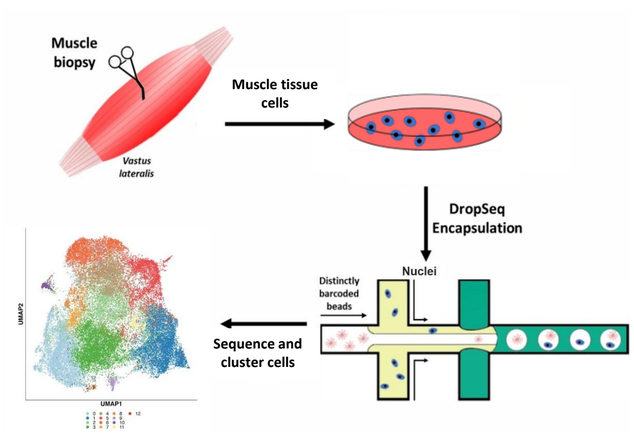
AIMS: ***To determine the transcriptional heterogeneity of human myoblasts isolated from older individuals we investigated single cell gene expression profiles of cultured myoblasts isolated from vastus lateralis of participants aged 72-83 from the Hertfordshire Sarcopenia Study extension (HSSe). In addition, we examined the transcriptional profile of myoblasts with respect to appendicular lean mass index (ALMi) and grip strength, two clinical parameters which contribute to the definition of sarcopenia.***

Methods: Myoblast Culture, Dropseq, Sequencing and Single Cell Discovery Pipeline

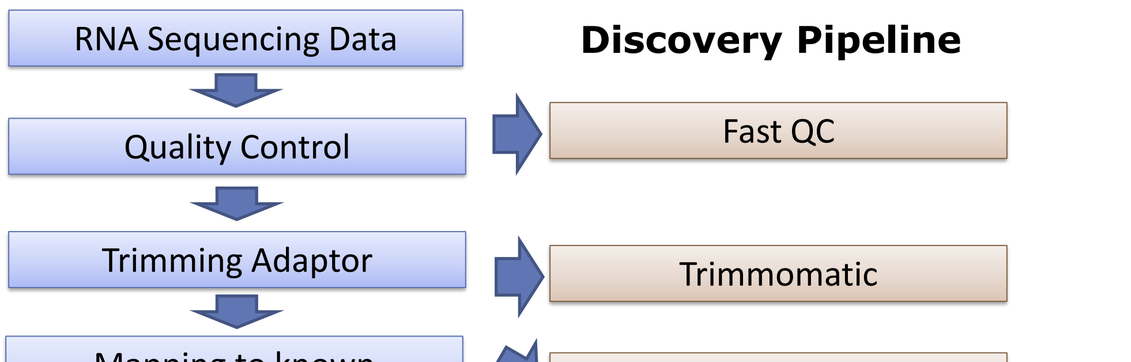
Study Design

Human primary myoblasts were cultured from muscle biopsies taken from 132 participants of the Hertfordshire Cohort Study Extension (HSSe). Isolation of myogenic cells was confirmed by immunocytochemistry against CD56. Cells were collected at passage 4 for single cell RNAseq (scRNAseq).

Single Cell Microfluidics and Transcriptomic Sequencing



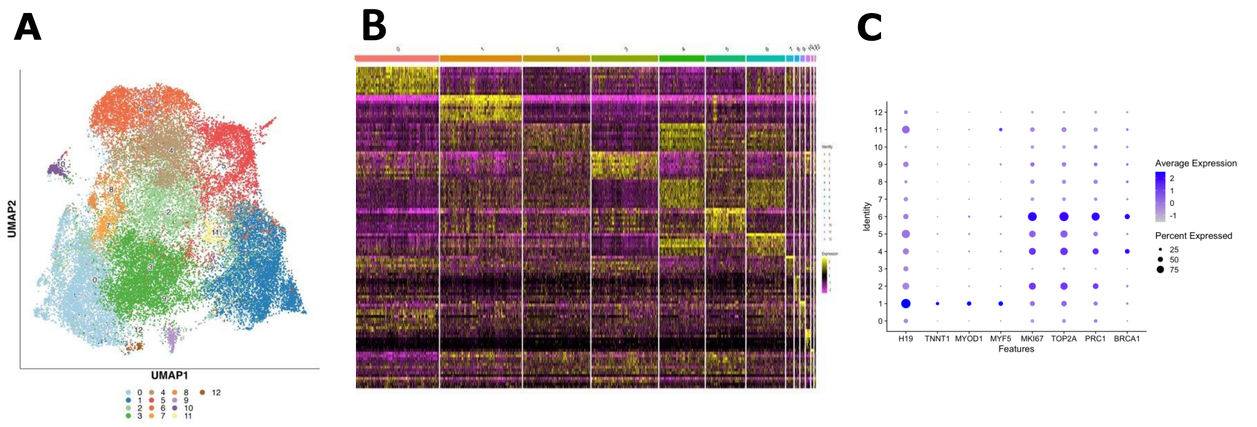
- Cells were grown (~60% confluence), harvested and run through a microfluidic device (Dropseq) with barcoded Macosko beads and droplet generation oil.
- Droplets were broken, beads collected and attached RNA was reverse transcribed.
- Beads were treated with exonuclease, and cDNA amplified by PCR.
- PCR product was tagmented and libraries purified.
- Libraries were pooled to a final concentration of 250pM and sequenced on an Illumina NovaSeq 6000.



Results: scRNAseq Cell Clustering

scRNAseq of actively proliferating human myoblasts identifies cellular heterogeneity in gene expression

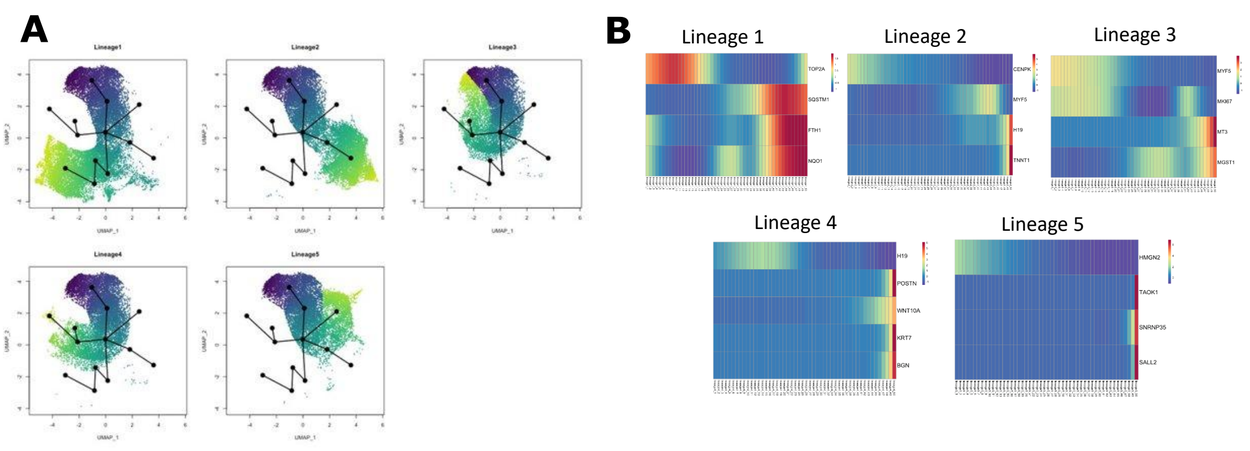
- Uniform Manifold Approximation and Projection (UMAP) analyses identified **13 subpopulations** of cells (C0-C12) based on their gene expression profiles.
- Each cluster was found to have a unique transcriptomic fingerprint with heterogeneous gene expression.



Images: (**A**) UMAP single cell clustering. (**B**) Heatmap showing top 10 differentially expressed genes for each cluster. (**C**) Dot plot showing expression of myogenic genes within clusters.

Results: Pseudotime Trajectory Analysis

Actively proliferating myoblasts exist along five different pseudotime lineages



Images: (**A**) Pseudotime lineages. (**B**) Heatmap showing differential expression of genes of interest within each lineage

Conclusions

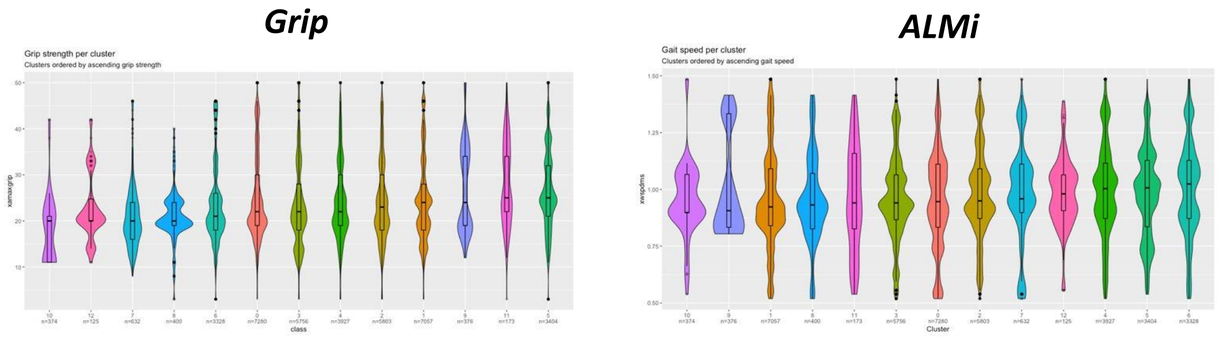
In this study we carried out single-cell RNAseq (scRNAseq) on human primary myoblasts grown *in vitro*, isolated from *vastus lateralis* biopsies of participants from the HSSe cohort.

- Unsupervised clustering of data identified 13 clusters of cells (clusters 0-12) that showed distinct transcriptional profiles, with differentially expressed genes identified between the clusters.
- Slingshot revealed 5 trajectories based on clustering, identifying cells undergoing proliferation, activation of myogenic differentiation, or cells that appear to progress towards an oxidative stressed or fibrogenic state
- Clusters ranked in terms of grip strength or ALMi suggest that increased oxidative stress and high expression of ribosomal proteins in myoblasts may reflect and/or contribute to impaired muscle mass and function.
- Pseudobulk analysis of gene expression identified genes linked with mitochondrial network remodelling and autophagy, suggestive of impaired differentiation.
- Ranking cell clusters with regards to ALMi or grip identified proportions of cells associated with greater muscle resilience and function.

Primary myoblasts from older individuals demonstrate distinct cellular heterogeneity with differences in gene expression in relation to muscle mass and function

Results: Clustering with Grip/ALMi

Of the major cell clusters, 5, 1 and 2 contained cells from individuals with the highest median grip strength or ALMi.



- Cells in Clusters 5, 11, 9 and 1 exhibited the highest median grip strength, while cells in Clusters 10 and 12 the lowest.
- Cells in Cluster 11, 5, 1 and 2 exhibited the highest ALMi, with Clusters 10 and 7 exhibiting the lowest.

Results: Pseudobulk Analysis

Low ALMi and grip strength are associated with differential gene expression within distinct cell clusters

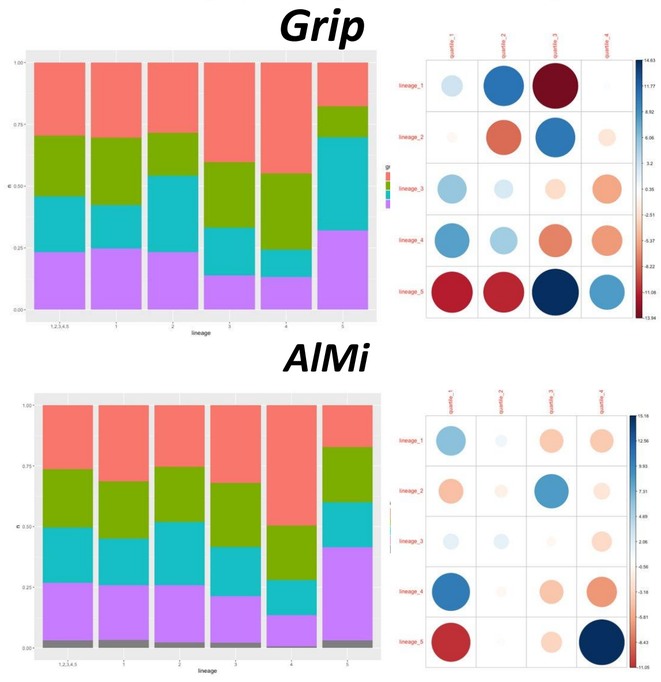
Pseudobulk analysis of gene expression differences between clusters in individuals from the highest compared to the lowest quartile of ALMi or grip strength were investigated.

ALMi

- **3 genes** differentially expressed (FDR≤ 0.05) in **Cluster 0.**
- **22 genes** differentially expressed in **Cluster 2.**
- **4 genes** differentially expressed in **Cluster 4**
- **2 genes** differentially expressed in **Cluster 5**).

Grip

- **13 genes** differentially expressed in **Cluster 1**



References

1) Walston JD. Sarcopenia in older adults. Curr Opin Rheumatol. 2012 Nov;24(6):623-7. doi: 10.1097/BOR.0b013e328358d59b. PMID: 22955023; PMCID: PMC4066461.
2) Dodds RM, Roberts HC, Cooper C, Sayer AA. The Epidemiology of Sarcopenia. J Clin Densitom. 2015;18(4):461-6.
3) Bigot A, Duddy WJ, Ouandaogo ZG, Negroni E, Mariot V, Ghimbovschi S, et al. Age-Associated Methylation Suppresses SPRY1, Leading to a Failure of Re-quiescence and Loss of the Reserve Stem Cell Pool in Elderly Muscle. Cell Rep. 2015;13(6):1172-82.
4) Zammit PS. All muscle satellite cells are equal, but are some more equal than others? J Cell Sci. 2008 Sep 15;121(Pt 18):2975-82. doi: 10.1242/jcs.019661. PMID: 18768931.