Skeletal Muscle Cell Populations in Symptomatic SOD1^{G93A} ALS Mice

Isha Pema[1,2], Cristhian Montenegro[1,2], Angelina Heminway[1,2,3], Arnav Rao[1,2,4], Justin Lau[1,2], Nesa Milan[1,2], Brian Feeley[1,2], Xuhui Liu[1,2]
[1]San Francisco Veterans Affairs Health Care System, [2]Department of Orthopaedic Surgery, University of California, San Francisco, [3]Swarthmore College, Swarthmore, PA, [4]Case Western Reserve University, Cleveland, OH

INTRODUCTION: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease in which motor neuron degeneration causes muscle weakness and eventual paralysis. Muscle dysfunction is one of the first symptoms in all patients and progressive degeneration has a severe impact on muscle function and overall life quality. Our recent study looked at muscle pathology in SOD1G93A (Sod1) mice, a widely-used model displaying a neurodegenerative phenotype similar to ALS in humans. These mice develop symptoms including body weight loss, muscle weakness and limb paralysis starting from about 4 months of age. The symptoms progress fast and the mice die shortly after the development of limb paralysis. In this study, we aimed to explore the cell populations in skeletal muscle of Sod1 mice with symptoms of limb weakness in comparison to age-matched littermate wildtype mice.
METHODS: Symptomatic Sod1 mice with hindlimb paralysis (n=2) and their littermate wildtype controls (n=2) were sacrificed at 5 months of age. The gastrocnemius and hamstring muscles from both legs were digested into a single cell suspension via mechanical and chemical means. Cells were submitted to the UCSF Genomics Core for single cell RNA sequencing. FASTQ files were generated using CellRanger and subsequently analyzed for clusters and differentially-expressed genes using Seurat. Cell data from all four mice was combined, normalized, analyzed for variable features, and scaled before undergoing principal component analysis. Elbow plots were used to determine the number of dimensions to include (40-50), and UMAP reductions were run following feature clustering. A UMAP plot was generated and clusters were labeled using cell marker genes. A second UMAP plot was generated by grouping based on mouse cell origin (Wildtype and Sod1) and the plots were compared. To explore differences within fibroadipogenic progenitor cells (FAPs), the FAP population was extracted, re-clustered, and a UMAP plot was generated. Animal protocols were approved by IACUC.
RESULTS: Single cell sequencing clustering analysis and UMAP plots led to the labeling of 9 cell populations using the following genes: Cd3e for T-cells, Pdgfra for FAPs, Pax7 for satellite cells, Acta1 for myofibers, Cd19 for B-cells, Pecam1 for endothelial cells, Scx and Col1a1 for tenocytes and fibroblasts, Cd68 for macrophages, Tnf for neutrophils, and S100b and Pmp22 for Schwann cells. A total of 1092 Wildtype and 1226 Sod1 mouse muscle cells were analyzed. As expected, populations of immune cells, including T-cells, B-cells, and neutrophils were present in Sod1 muscle to a greater extent than in the wildtype controls (Liu and Wang, 2017). In addition, macrophages (mostly M1) were much more common in Sod1 muscle in comparison to wildtype. Gene expression was used to divide the population of FAPs into four major subpopulations: pre-adipogenic FAPs (Mme+), mechanosensitive FAPs (Piezo2+), pre-fibrogenic FAPs (Cd55+), and activated FAPs (Gli1+). Although there were no patterns or differences in these populations between Wildtype and Sod1 groups, cells within each cluster were observed to congregate by origin and were unevenly distributed.
DISCUSSION: As published previously, greater numbers of immune cells in Sod1 mice relative to their wildtype controls were expected, in line with increased inflammation due to the disease progression. In addition, M1 pro-inflammatory macrophages were also noted in Sod1 mice, falling in line with the state of elevated inflammation in Sod1 mice. The FAP population was analyzed further, as these mesenchymal-like progenitor cells are associated with muscle fibrosis (Cd55+) and fatty infiltration (Mme+) in disease, as well as mechanosensitivity (Piezo2+) (Garcia 2023). All of these subpopulations were present in the mice studied, in both Wildtype and Sod1 mouse muscle. However, looking at the top 25 differentially expressed genes between Wildtype and Sod1 FAPs in this dataset indicate some differences in gene expression but no definitive patterns. It is worth noting that the FAP subpopulation consisted of 758 total cells (423 Wildtype and 355 Sod1), making this a very small batch of cells to draw conclusions from. These preliminary data may suggest that in addition to an increase in immune response in early symptomatic Sod1 mice, there may be further differences in FAP pre-adipogenic and pre-fibrogenic subpopulations that can be parsed out in future studies with FAP-enriched sorts and Sod1 mice further along in disease progression.
SIGNIFICANCE/CLINICAL RELEVANCE: These data support the increase in immune cell activity in the Sod1 mouse model of ALS. Further, the data gives direction for future studies to analyze the changes in FAP subpopulations and their effect on skeletal muscle as ALS progresses, leading to a potential therapeutic target.
REFERENCES: [1] Liu J, & Wang F (2017). Role of Neuroinflammation in Amyotrophic Lateral Sclerosis: Cellular Mechanisms and Therapeutic Implications. *Frontiers in immunology*, *8*, 1005. https://doi.org/10.3389/fimmu.2017.01005. [2] Garcia SM, Diaz A, Lau J, Chi H, Lizarraga M, Davis M, Liu X, Feeley BT (2023). Distinct human stem cell subpopulations drive adipogenesis and fibrosis in musculoskeletal injury. bioRxiv; 2023. DOI: 10.1101/2023.07.28.551038.
ACKNOWLEDGEMENTS: We would like to acknowledge the UCSF Genomics Core for their help and guidance.

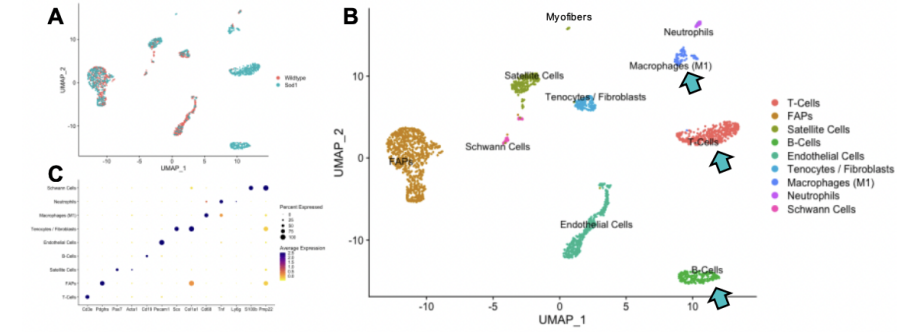

Figure 1: UMAP plot of scRNAseq clustering of skeletal muscle single cells from wildtype (n=2, 1092 cells) and 5-month-old Sod1 (n=2, 1226 cells) mice. A) Clusters labeled by group. B) Clusters labeled by cell type; arrows denote cell populations present in Sod1 but not in wildtype mice. C) Dot plot denoting genes used to identify cluster populations.

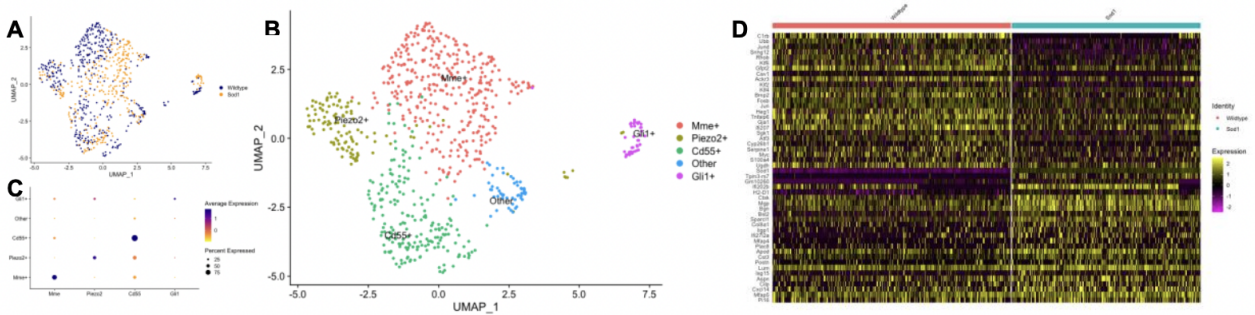

Figure 2: UMAP plot of scRNAseq clustering of skeletal muscle FAPs from wildtype (n=2, 423 cells) and 5-month-old Sod1 (n=2, 423 cells) mice. A) Clusters labeled by group. B) Clusters labeled by cell type. C) Dot plot denoting genes used to identify cluster populations. Mme+, pre-fibrogenic; Piezo2+, mechanosensitive; Cd55+, pre-adipogenic; Gli1+, activated. D) Heatmap of top 25 DEGs between wildtype and Sod1 FAPs.