Facioscapulohumeral muscular dystrophy (FSHD), one of the most common forms of muscular dystrophy, is a rare monogenic disease that currently has no effective treatment. It is caused by deletions of the microsatellite D4Z4 repeats at a subtelomeric region of chromosome 4q35. Ethnic-specific numbers less than 1 within this region cause chromatin re-arrangement resulting in stochastic and aberrant expression of the embryonic transcription factor DUX4 in skeletal muscle. The expression of DUX4 is dysregulated and results in muscle atrophy in facial muscles and around the shoulder, eventually progressing to lower extremity and truncal weakness. While the root cause of this disease has been elucidated, the molecular and cellular microenvironmental underpinnings that drive the loss of muscle mass remain unexplored.

Here we describe the first characterization of human FSHD skeletal muscle biopsies using single nuclear RNA-seq (snRNA-seq). Our goal was to understand the dynamic FSHD microenvironment in greater resolution. Analysis of these snRNA-seq profiles suggested an increase in relative populations of myoblasts and fibroblasts within the FSHD muscle microenvironment. In addition to expanded intercellular heterogeneity within the FSHD microenvironment, we also saw a significant increase in transcriptionally undefined cells within diseased muscle. Characterization of these FSHD muscle biopsies will help us to understand the processes contributing to FSHD pathogenesis that will hopefully lead to new target/therapeutic hypotheses.

1. Background

(A) In FSHD, loss of repression of the D4Z4 locus leads to expression of DUX4 in skeletal muscle. p38α has been shown to regulate DUX4 expression in FSHD myotubes (23).

(B) Aberrant expression of DUX4 leads to a disease cycle characterized by discrete hallmarks of disease driven by altered cellular communication within dystrophic tissue.

2. Approach

3. Experimental Design/Methods

A. FSHD needle biopsies from human skeletal muscle

B. Single Nuclear Analysis

Initial Processing: We used Seurat v3 (24) to process single nuclear RNA-seq data. This analysis yielded more than 250 genes and less than 500 genes were retained in the analysis. Additionally, we filtered out cells with more than 5% mitochondrial genes. In order to merge FSHD and healthy biopsies we used the CCA algorithm as implemented in the Seurat v3 package.

Cell Type Annotation: For each biopsy, we clustered the snRNA-seq profiles and identified differentially expressed genes between clusters using a non-parametric Wilcoxon rank sum test. We then used an OR rule for sets of genes to determine if we could identify any ligand-receptor interactions that drive these cell-to-cell interactions.

Pseudotime trajectory: In order to understand differences between healthy and disease FAP cells we first looked at all healthy cells from (2) and co-clustered them with disease FAP cells from each biopsy using the CCA algorithm to perform batch correction. Based on these clustering results, we then identified healthy cells that were most similar to the disease FAPs. Finally, we performed gene enrichment analysis (GO, Reactome pathway enrichment) on the lists of marker genes for clusters we couldn’t annotate with evidence. Any remaining unannotated clusters were labeled as undefined and enriched for genes associated with it.

4. Results

A. FSHD snRNA-seq profiles

B. Relative Cell Type Composition

C. FAP cell state in healthy and disease biopsies

5. Discussion

We report a first-in-class single nuclear profiling of the dystrophic FSHD microenvironment which is characterized by an expansion of FAPs, endothelial-like cells, and myofibroblast cell populations, as well as a decrease in myonuclei in FSHD muscle compared to healthy muscle biopsies. Further analysis to characterize cell-cell interactions via ligand receptor pairs expressed in these expanded cell populations in FSHD will allow us to understand how dysregulation of healthy skeletal muscle composition and tissue state may alter the FSHD micro-environment leading to dystrophic pathology.

References


6. Conclusion

We will use these interactions as positive controls when assessing predicted cell-to-cell interactions in FSHD and healthy biopsies. Differences in relative cell type abundances between dystrophic and healthy muscle can effect these interactions, and contribute to the presence of inflammatory infiltrates and adipocytes in muscle, as well as to unaired ECM remodelling.

(B) One hypothesis is that receptor-ligand interactions between cells in healthy and dystrophic muscle could be contributing to a disease phenotype in the healthy state. We hypothesize that interactions between endothelial and smooth muscle cells are common in all FSHD but not healthy biopsies, as well as several cell-to-cell interactions that are common in severe but not FSHD biopsies. By using single nuclear data to predict cell-to-cell interactions and receptor-ligand interactions between those cells, we can start to understand what interactions may be associated with a disease phenotype.

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