EP.321

Revolutionizing drug discovery in genetically-defined muscle disease using single-cell and high-dimensional datasets

L. Alejandro Rojas, Ayla Ergun, Caroline Sartain, Anthony Accorsi, Richard Lieberman, Anugraha Raman, Joe Maglio, Erin Valentine, Mary Wertz, Elizabeth Townsend, Damon Polioudakis, Ivan Efremov, Lorin A. Thompson, Serena J. Silver, Christopher M. Moxham

Fulcrum Therapeutics. 26 Landsdowne Street, Cambridge, Massachusetts, USA.

Abstract

Our vision at Fulcrum Therapeutics is to transform the future of patients living with genetically-defined diseases. Our approach to drug discovery avoids the pitfalls that have plagued our industry and harnesses recent technological advances that allow us to forge a new path for patients. Using the latest advances in single cell methods, we can look deeper into diseased tissue to generate powerful insights into the drivers of disease and identify new ways to re-balance the cellular composition and dysregulation of pathological microenvironments. Our FulcrumSeek platform brings together the deep understanding of tissue biology elucidated from the hypothesis-generating combination of snRNA-seq and spatial transcript/proteomics studies of human samples, with the latest technologies in cellular modeling and comprehensive phenotypic screening using high-throughput RNA-seq and high content imaging. We are applying the power of machine learning together with the creative and inventive spark of human intelligence to unravel the richness of this data and revolutionize drug discovery in muscular dystrophies. Using this approach, we have characterized biopsies from dystrophic muscle to understand the cellular composition, interactions, and processes that drive muscle degeneration and loss of function. Now, we are creating a database of cellular profiles generated in response to our highly curated and annotated chemical library and functional genomics tools to identify novel targets that can modulate these drivers of disease. Here, we demonstrate that by applying this integrated target discovery approach in FSHD patient biopsies as well as patient-derived myotubes in vitro, we can rapidly rediscover previously known targets in FSHD, as well as identifying novel targets. This platform has the potential to fundamentally change the way drugs are discovered and developed for patients with devastating muscle diseases.

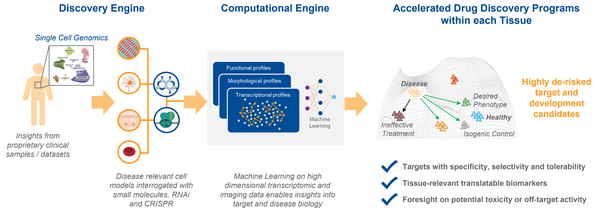
Approach to Target Discovery at Fulcrum Therapeutics

FulcrumSeek Platform identifies targets that have the potential to rebalance monogenic, polygenic, and heterogenous diseases

- Our core focus is on patients with diseases of high unmet need
- We use the most relevant cellular models to study a given disease
- We systematically employ target & modality agnostic screening approaches to enable rapid identification of high-quality targets

Fulcrum's patient-centric approach systematically & rapidly pinpoints multiple novel intervention points

FulcrumSeek™: Accelerating Discovery of Disease-Modifying Therapies



FulcrumSeek™ Product Engine enables intelligent drug discovery in disease-relevant models through high-dimensional data and machine learning

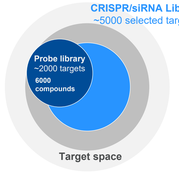
Chemical Biology Approach for the Rapid Identification of Tractable Targets

Small molecule probe library

- ~6000 hand-selected small molecules from literature, patents and commercial sources
- Constantly being updated with newly synthesized compounds of interest
- ~2000 targets with diverse mechanisms, including kinase, chromatin modifiers, nuclear hormone receptors, GPCRs, ion channels and other enzymes.

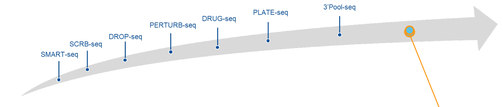
CRISPR gRNA and siRNA libraries

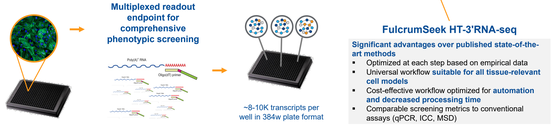
- ~5000 prioritized targets including mechanisms of interest such as signaling pathways, chromatin modifiers, transcription factors.
- Opportunity for identification of targets with no chemical matter available
- Scalable miniaturized delivery protocols optimized for challenging cell models in arrayed screening formats



Developing Novel Technologies for Phenotypic Screening

High-throughput transcriptomic profiling



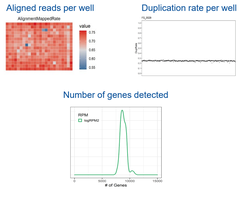


FulcrumSeek HT-3'RNA-seq

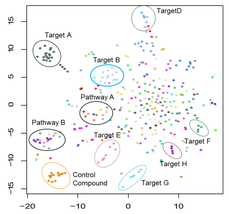
Significant advantages over published state-of-the-art methods

- Optimized at each step based on empirical data
- Universal workflow **suitable for all tissue-relevant cell models**
- Cost-effective workflow optimized for **automation and decreased processing time**
- Comparable screening metrics to conventional assays (qPCR, ICC, MSD)

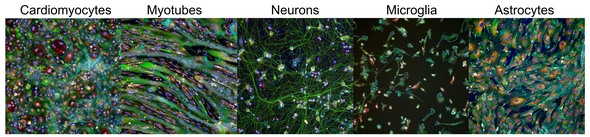
384w plate level visualization of QC metrics used before data analysis



Transcriptional changes induced by compound treatments cluster by target and pathways

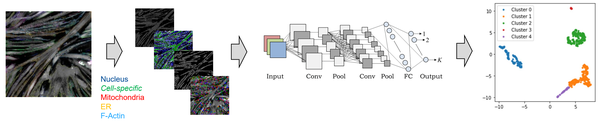


High-content imaging for morphological profiling

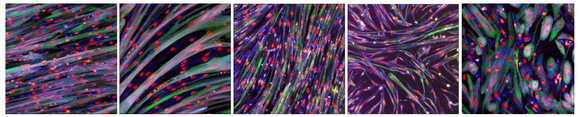


Nucleus *Cell-specific* Mitochondria Endoplasmic Reticulum F-Actin

Segmentation and extraction of cellular features for incorporation of visual, feature, and expression data into computational pipeline to characterize phenotypic changes induced by perturbations

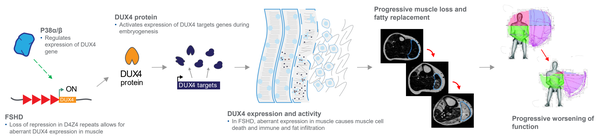


This approach allows for the identification and characterization of phenotypic changes in myotubes caused by small molecule, functional genomic perturbations, and disease states.



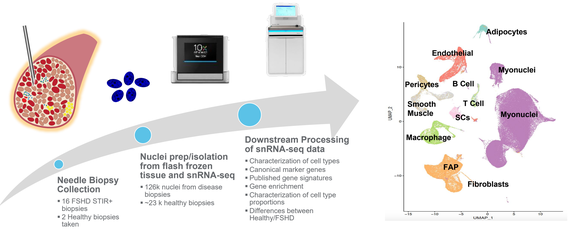
Wildtype myoblasts were treated with indicated compounds and differentiated for 7 days in vitro before fixation and staining

Facioscapulohumeral Muscular Dystrophy (FSHD)

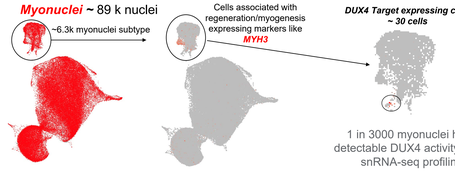


- FSHD is a debilitating, relentlessly progressive neuromuscular disease with heterogeneous muscle involvement and variable onset, severity, and progression.
- FSHD is characterized by progressive skeletal muscle weakness affecting the face, shoulders, arms, trunk, weakness of the lower extremities and pelvic girdle. Symptoms typically appear in the second decade but can begin at any age.
- FSHD is caused by the aberrant expression of DUX4 in skeletal muscle. This transcription factor is usually only expressed in early embryonic development
- Stochastic DUX4 expression results in heterogenous presentation between patients and within muscles in the same patient.

Generating a cellular atlas of skeletal muscle tissue in FSHD to identify the most relevant cell types for disease modelling in vitro



Identifying DUX4-expressing cells in FSHD skeletal muscle

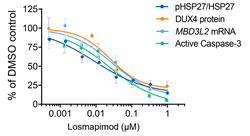


1 in 3000 myonuclei have detectable DUX4 activity using snRNA-seq profiling

An ideal target that downregulates DUX4 will decouple its expression and the myogenesis/regeneration process

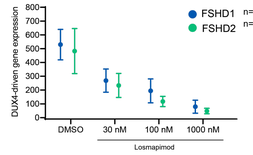
Fulcrum has previously identified the p38α/β inhibitor losmapimod as a potential therapeutic for FSHD

Losmapimod reduced p38 activity, DUX4 expression, DUX4 activity, and cell death in patient-derived FSHD myotubes



- *HSP27* is a substrate of p38 MAP kinase pathway
- *MBD3L2* is a DUX4-target gene
- **Caspase-3** is an indicator of cell death

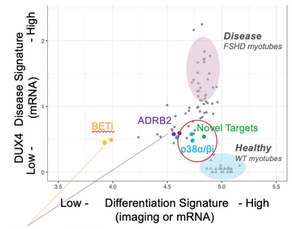
Preclinical study demonstrated a reduction of DUX4 activity across multiple patient-derived FSHD1/2 myotubes



Characterization of Novel Targets in FSHD

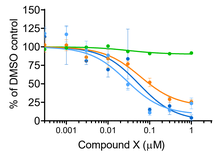
Using our high-dimensional approach we can rapidly identify and prioritize the highest quality targets that result in desired phenotypic changes and have no undesired effects in other biological processes

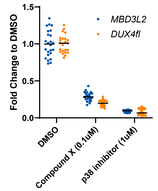
- DUX4 is lowly expressed and is not detectable by HT-3' RNAseq, but a set of DUX4 program genes can be used as a signature of DUX4 activity.
- Inhibition of p38α/β results in the inhibition of DUX4 expression and minimal effects in myogenesis.
- Inhibition of novel targets that reduce DUX4 without impacting myogenesis can be identified and quickly prioritized for further characterization and validation



Other known Targets in FSHD

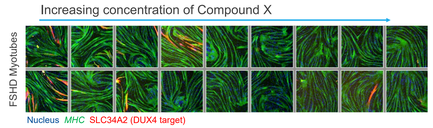
Novel Target X inhibition results in potent reduction of DUX4, its activity, and its downstream consequences





Immunofluorescence analysis of indicated endpoints after 7 days of treatment and differentiation of FSHD myotubes

RT-qPCR analysis of indicated endpoints after 7 days of treatment and differentiation of FSHD myotubes



Enabling Unprecedented Drug Discovery at Scale in Disease-Relevant Settings

Our Discovery Platform

Embodies disease context and focuses on root cause of disease

Enabled to pursue numerous indication areas: musculoskeletal, hematology, cardiovascular, CNS, pulmonary, and many others

Represents the nexus of state-of-the-art advances in high content technologies, machine learning, cellular modeling, and pharmacogenomics

Comprehensive approach to rebalance diverse disease states for disease-modifying therapies

Targets, pathways, cell types, therapeutic areas