Targeting Pathogenic Fibro-Adipogenic Progenitor Cell Differentiation to Promote Muscle Repair

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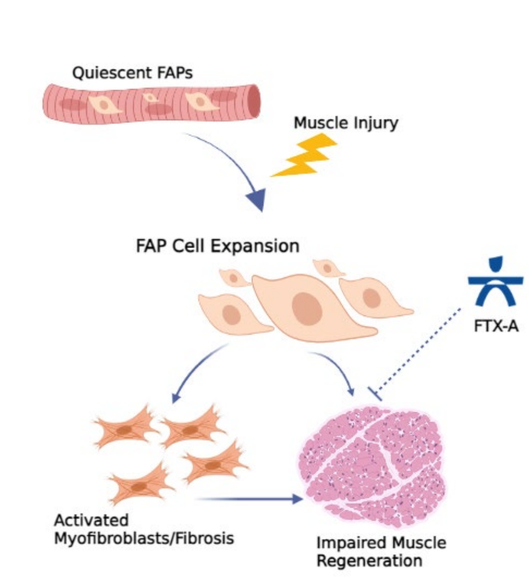
Abstract

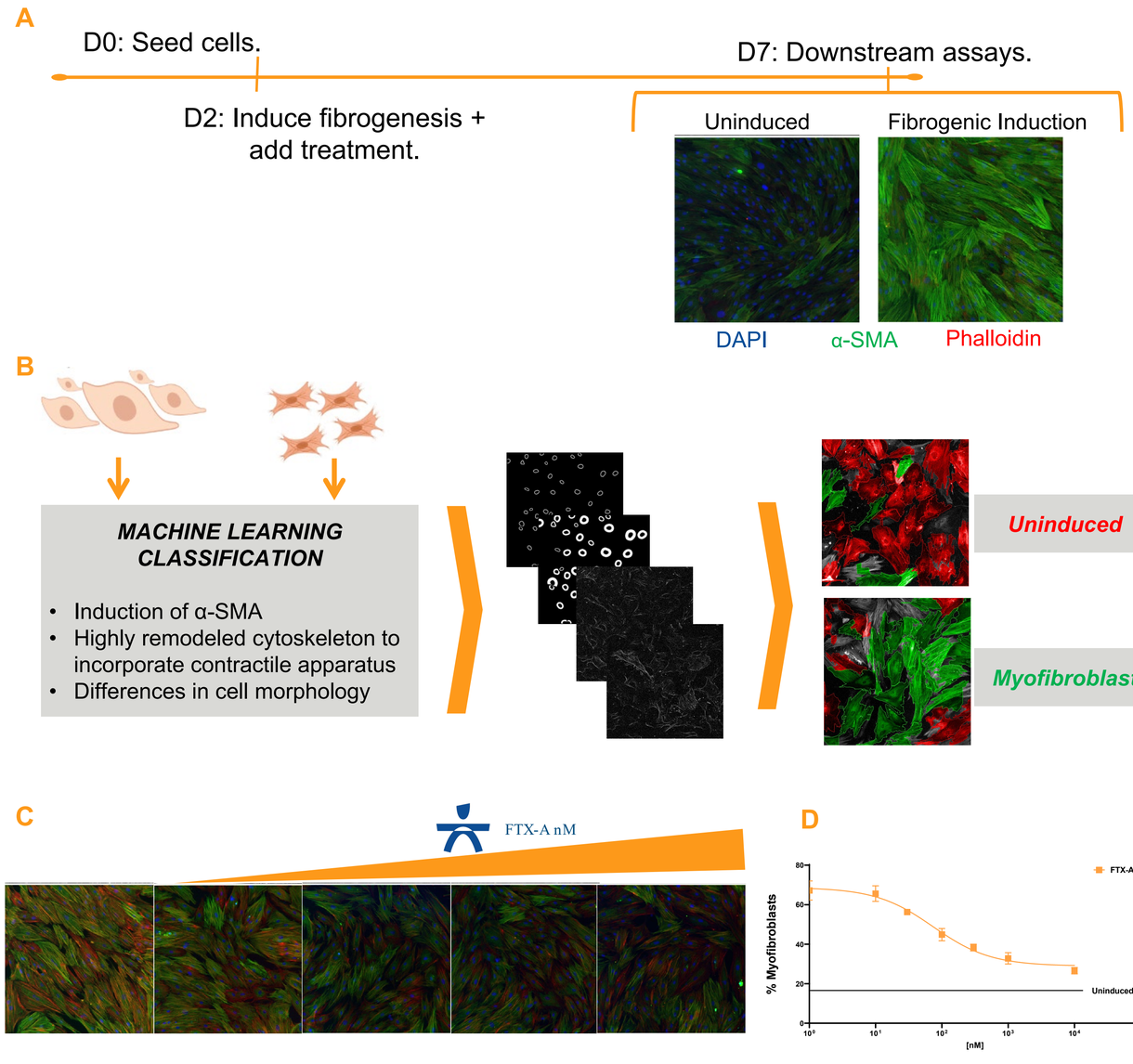
In skeletal muscle, replacement of contractile myofibers with non-contractile fibro-fatty infiltrate is a hallmark of severe muscular dystrophies. Fibro-adipogenic progenitor cells (FAPs), a group of tissue-resident mesenchymal progenitors, exist on a tight balance, acting as major promoters of muscle regeneration during acute injury but also as a major source of pathogenic muscle replacement during chronic injury due to their ability to differentiate into myofibroblasts and adipocytes. Novel studies within the muscle field have shown that chronic muscle damage and inflammation in muscular dystrophies favor the persistence of FAPs in a pathologic state, hindering regeneration and leading to muscle fibrosis, adipogenesis, and osteogenesis. We have shown via snRNAseq that pathogenic regions of FSHD biopsies are characterized by expansion and dysregulation of FAPs, and their expression profiles strongly suggest differentiation toward a fibrogenic lineage that characterizes the active stages of disease. Optimization of a cellular model and read-outs of fibrogenic differentiation has led to *in vitro* biological validation of a fibrogenic differentiation modulator. We tested compounds across varying chemotypes and putative structurally similar negative controls, to show prevention of myofibroblast differentiation via inhibition of Target A. Future work aims at *in vivo* validation to assess impact on muscle function and regeneration in dystrophic mouse models. This ongoing work suggests that promotion of a pro-myogenic FAP cell state represents a significant opportunity to not only modulate muscle fibrosis but promote repair in dystrophic muscle to generate novel treatments targeting the muscle micro-environment to help patients with devastating muscle disorders.

FAP Modulation Represents Opportunity to Rebalance Muscle Repair Across the Dystrophic Spectrum



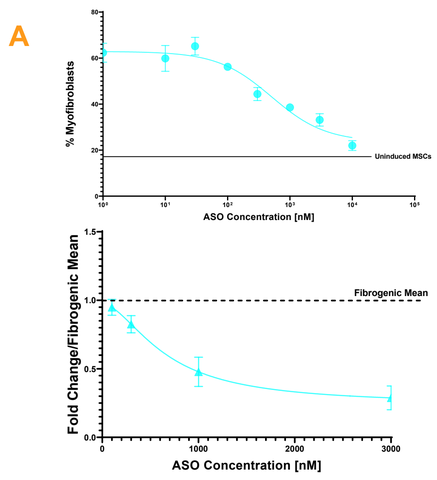
- *Fibrosis is a common hallmark across the dystrophic spectrum.*
- *Disruption of FAP homeostasis impairs muscle regeneration, leading to fibrotic degeneration of injured muscle.*
- *Reversal of FAP-mediated fibrogenesis and return to quiescent FAP cell state serves to promote muscle repair, presenting an opportunity to generate novel treatments targeting the muscle micro-environment.*

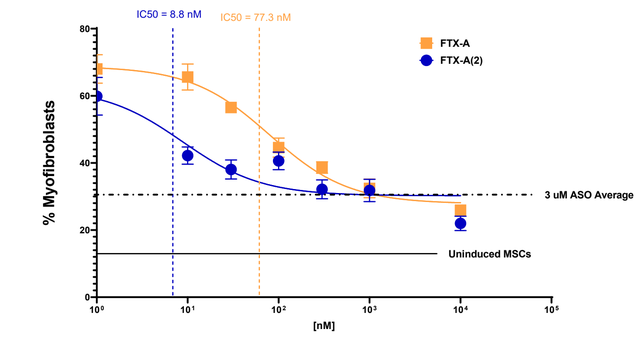
in vitro Cell Model Development, Confirmation & Perturbation



(A) Schematic of cellular assay for fibrogenic differentiation. Imaging panel includes DAPI to identify nuclei, α-SMA (alpha, smooth muscle actin) to identify myofibroblasts and phalloidin to mark the actin cytoskeleton. **(B)** Schematic of machine learning classification & feature characterization for image quantification of myofibroblast phenotype. **(C)** FTX-A reduces myofibroblast differentiation in a dose-dependent manner. **(D)** Quantification of % myofibroblasts using image classifier, running a 9-pt CRC, compared to uninduced cells.

Chemical and Genetic Inhibition of Target A Results in Prevention of Myofibroblast Differentiation





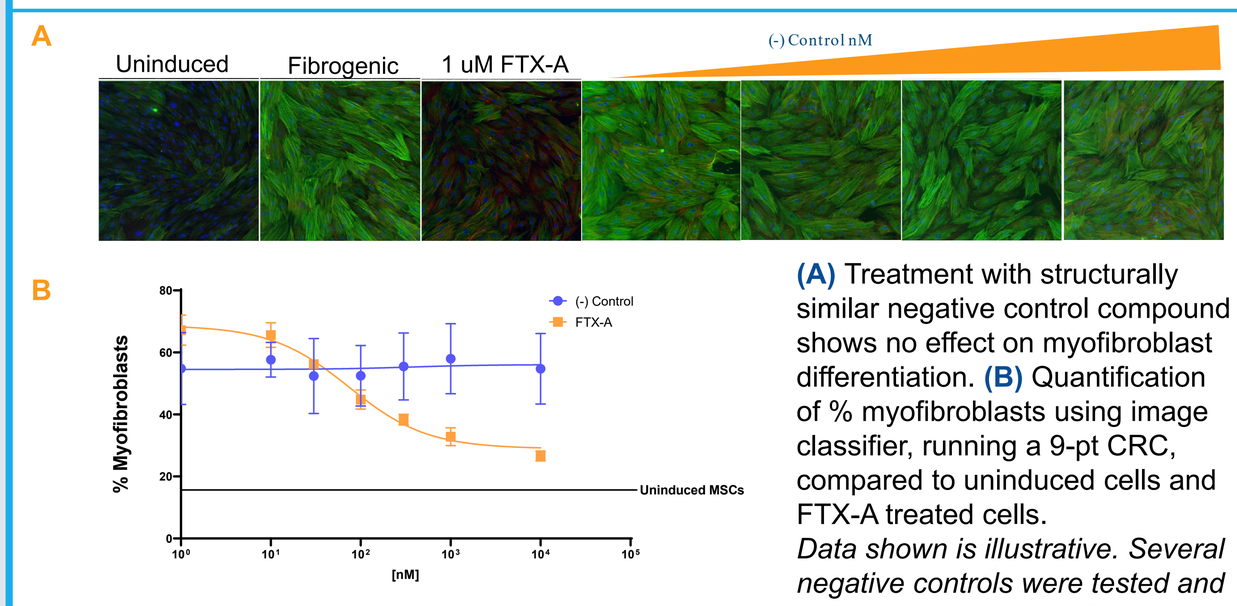
C

Compound	Reported Potency (nM)	IC50 Phenotype (nM)	IC50 Phenotype Replicate (nM)
FTX-A(2)	IC50 = 2	8.8	10.89
FTX-A	IC50 = 110	77.3	126.3

(A) ASO against Target A shows % knockdown with concurrent dose-response by measuring change in ICC phenotype (top) and in expression of Target A mRNA (bottom), compared to fibrogenic induction. **(B)** Quantification of % myofibroblasts using image classifier, running 9-pt CRCs, compared to uninduced cells and ASO treated cells. **(C)** Comparison of reported potency versus generated potency (IC50).

Data shown is illustrative of 2 compounds. Several compounds were profiled across a variety of chemotypes to generate comparative pharmacology, and to show that data was reproducible across experiments, matched reported values and showed variations in potency.

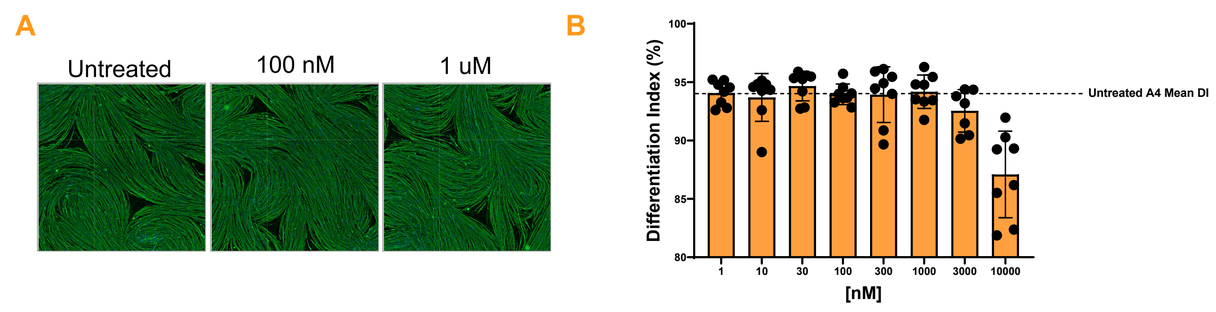
Structurally Similar Control Compounds Show No Effect on Fibrogenic Phenotype Further Suggesting On-Target Pharmacology



(A) Treatment with structurally similar negative control compound shows no effect on myofibroblast differentiation. **(B)** Quantification of % myofibroblasts using image classifier, running a 9-pt CRC, compared to uninduced cells and FTX-A treated cells.

Data shown is illustrative. Several negative controls were tested and similarly showed, no effect.

Target A Inhibition Does Not Impact Myotube Differentiation



(A) ICC images of myotubes treated with FTX-A stained for DAPI, to identity nuclei, and MHC (myosin heavy chain), to identify myotube surface area. **(B)** Quantification of differentiation index, (MHC+ nuclei)/(total viable nuclei), with increasing compound treatment, in comparison to untreated mean.

Conclusions

- We have shown optimization of a cellular model of fibrogenic differentiation including ability to reproducibly and sensitively detect perturbation of the system.
- We have shown chemical and genetic validation for Target A in prevention of myofibroblast differentiation *in vitro.*
- Maintenance of myotube differentiation coupled with inhibition of fibrogenic differentiation in FAP-like cells further suggests Target A to be a potential therapeutic, targeting the muscle micro-environment to re-balance muscle repair and replacement.