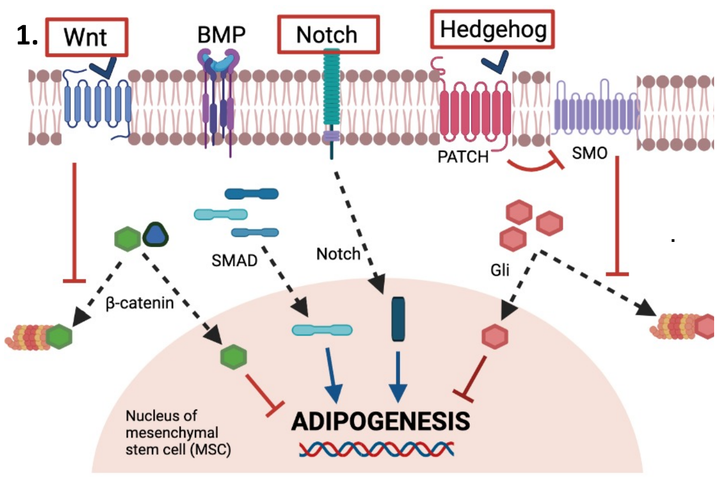
Identifying potential therapies for muscular dystrophies in the Wnt/Hh/Notch library

Vellia Justian [200769921], Esther Fernández-Símón, Jordi Díaz-Manera
John Walton Muscular Dystrophy Research Centre, Newcastle University | ***v.justian2@newcastle.ac.uk***

I. INTRODUCTION

Muscular dystrophies are characterised by muscle fibre damage, necrosis, as well as replacement of muscle fibres with fat tissue and fibrosis. These changes result in the **loss of muscle functionality** and **progressive damage**. Cells that are responsible for adipogenic and fibrogenic replacement of the muscle are the **fibroadipogenic progenitors cells (FAPs),** which are muscle interstitial mesenchymal stem cells that can differentiate into adipocytes and fibroblasts. Its differentiation is **influenced by various signalling** in the microenvironment, such as the **Wnt/Hh/Notch pathway.**



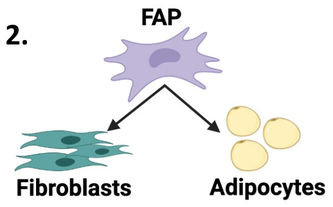


Figure 1. The Wnt, Notch, and Hedgehog (Hh) signalling pathways in the adipogenesis of mesenchymal stem cells. **Figure 2.** Differentiation of FAPs.
Created with BioRender.com

Taken together, modulation of the adipogenic differentiation of FAPs can be a potential therapeutic approach to halt the progression of muscle damage in muscular dystrophies.

II. AIM

To identify pharmacological compounds of the Wnt/Hh/Notch library that have the potential to modulate the adipogenic differentiation of FAPs.

III. METHODS

A. Selection Stages and Criteria

1
High-throughput Screening (Single Replicate)

0.1μM and 1μM

Selection criteria 1:
Minimum 30% perilipin-I inhibition

2
Validation of Pre-Selected Compounds (Triplicate)

Selection criteria 2:
Perilipin-I inhibition without promoting fibrogenesis

3
IC50 and Viability Assay (Triplicate)

Tested in descending doses from 2200nM to 0nM

Selection criteria 3:
Noncytotoxic maximum inhibitory dose

Future Studies

B. Treatment Process: In-Cell Western and Viability Assay

Plate immortalised human FAPs into 96-well plates in 5000cm² density

24 hours

ADIPOGENIC DIFFERENTIATION		FIBROGENIC DIFFERENTIATION
Positive control Adipogenic medium (StemPro) +DMSO 0.01%	Treatment	**Positive control** Fibrogenic medium (TGFβ1) + DMSO 0.01%
Negative control DMEM+Glutamax + 2% FBS + 1% Pen/Strep		**Negative control** DMEM+Glutamax + 10% FBS + 1% Pen/Strep
Pharmacological Compound Diluted in adipogenic medium		**Pharmacological Compound** Diluted in fibrogenic medium

6 days — For stage 3: Viability Assay (PrestoBlue™) — 3 days

In-Cell Western
Fixative: 0.4% PFA
Blocker: Casein in PBS

Anti-Perilipin-I — Primary Antibody — Anti-Collagen-I

24 hours

Goat anti-Rabbit (800) + CellTag (700) in Casein — Secondary Antibody — Donkey anti-Goat (800) + CellTag (700) in Casein

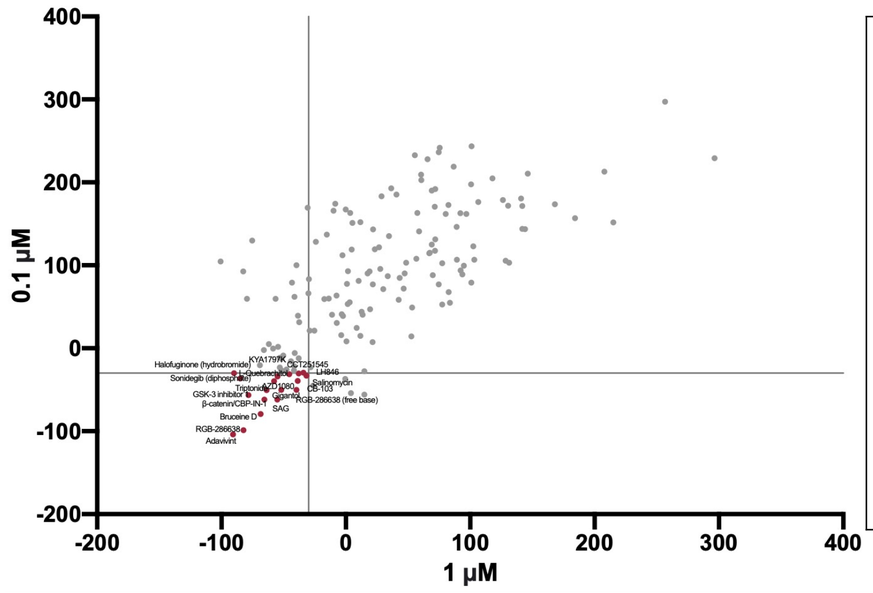
1 hour

Imaging acquisition and quantification of perilipin-I and collagen-I

Figure 3. Flow of study. **(a)** Selection stages and its criteria. (**b)** Treatment processes in the study, including in-cell western and viability assay.

IV. RESULTS

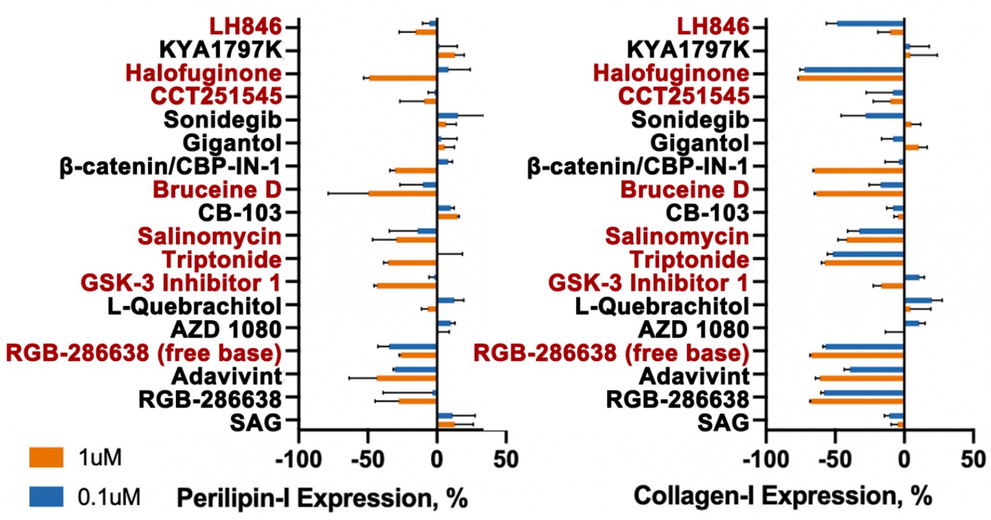
1. High-throughput Screening of 187 pharmacological compounds resulted in the pre-selection of **18 compounds**.



Step 1 pre-selected compounds with ≥30% perilipin-I inhibition in high and low dose:

- LH846
- KYA1797K
- Halofuginone
- CCT251545
- Sonidegib
- Gigantol
- β-Catenin
- Bruceine D
- CB-103
- Salinomycin
- Triptonide
- GSK-3 Inhibitor 1
- L-Quebrachitol
- AZD 1080
- RGB-286638 (free base)
- RGB-286638
- Adavivint
- SAG

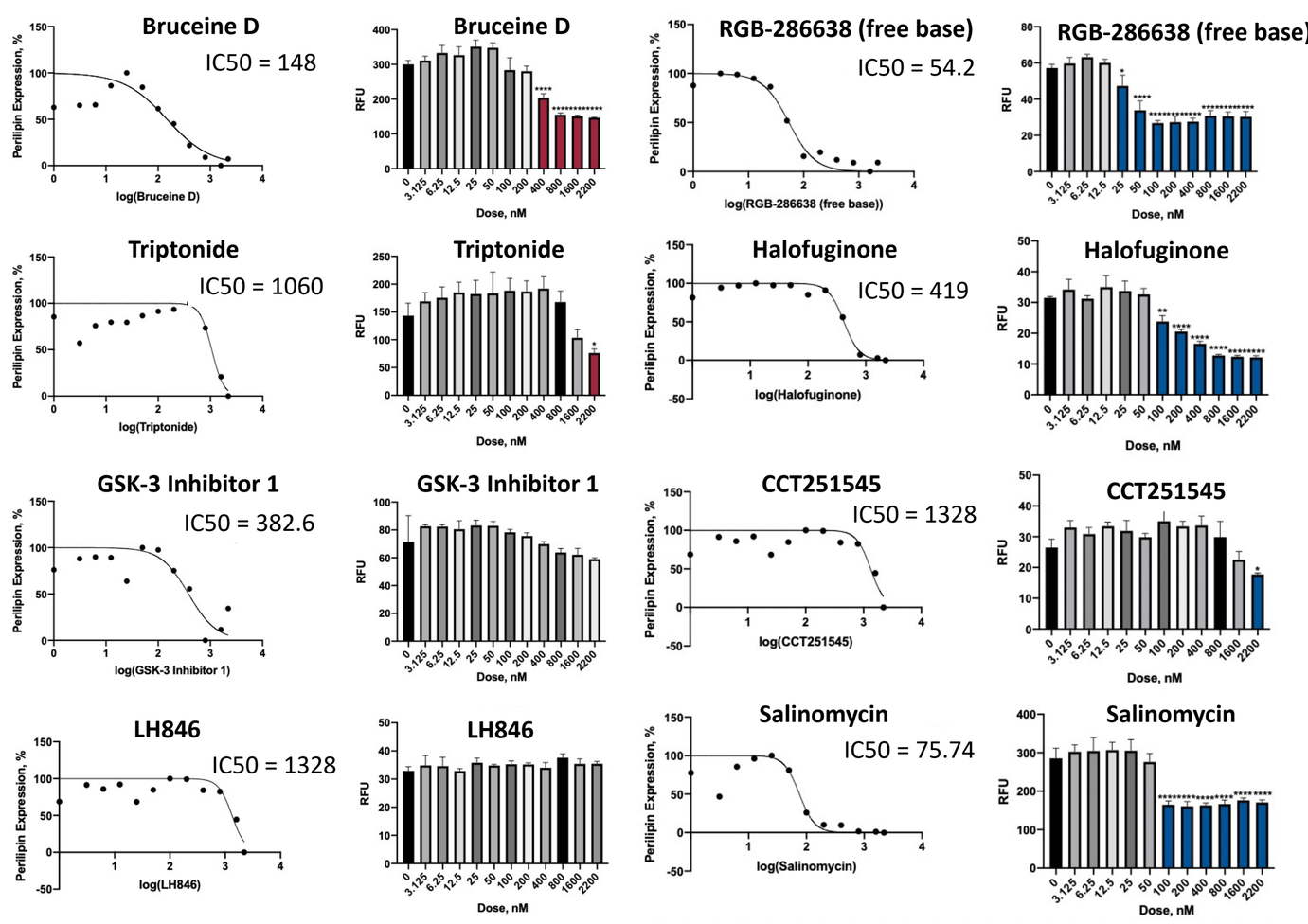
2. Validation through triplicates in adipogenic and fibrogenic differentiation narrowed down **8 compounds.**



Step 2 pre-selected compounds that showed perilipin-I inhibition without promoting collagen-I expression:

- LH846
- Halofuginone
- CCT251545
- Bruceine D
- Salinomycin
- Triptonide
- GSK-3 Inhibitor 1
- RGB-286638 (free base)

3. IC50 and Viability Assay pre-selected **4 compounds** for future studies.



Step 3 identified compounds with maximal inhibitory dose that did not significantly affect cell viability:
Bruceine D, Triptonide, GSK-3 Inhibitor, and LH846.
Test repetition on the specific maximal inhibitory dose is necessary to confirm these results.

V. CONCLUSION & FUTURE STUDIES

- Pharmacological compounds of the Wnt/Hh/Notch library have the potential to modulate adipogenic and fibrogenic differentiation in FAPs.
- Bruceine D, Triptonide, GSK-3 Inhibitor 1, and LH846 have shown an optimal maximal inhibitory dose at which adipogenic inhibition in FAPs were achieved without significantly affecting cell viability.
- Future studies will include confirming the specific maximal inhibitory dose of the pre-selected compounds and assessing its effect *in vitro*, as well as proliferation assay and migration assay. If positive results are observed, *in vivo* studies in dystrophic mice will be performed.

REFERENCES

Newcastle University · Universitas Indonesia · The John Walton Muscular Dystrophy Research Centre · NHS The Newcastle upon Tyne Hospitals NHS Foundation Trust