Development of an in-vitro muscle atrophy model to evaluate small molecule treatments targeting peroxisome proliferator-activated receptors (PPAR)

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Background

- Muscle atrophy occurs when proteolysis begins to take place, and contractile proteins and organelles are removed, which ultimately shrinks muscle fibers. This can happen for various reasons, including but not limited to; aging, muscular dystrophies, cancer, diabetes, and myopathies(1).
- On a molecular level, muscle atrophy is caused by decreased use of muscle. When this happens, the transcription factor FOXO, a member of the "forkhead box" proteins, is activated which in turn upregulates the transcription of MAXbf and MuRF-1, the E3 ubiquitin ligases. These genes place strings of ubiquitin on proteins to mark them for

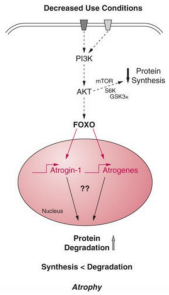

Figure 1: Atrophy Signaling Pathway (2)
Role of the transcription factors, receptors, and signaling pathway in the atrophy response. Most important factor is FOXO, which enters the nucleus and activates the antigens which are the genes that increase protein degradation. The red factors represent the activated proteins or genes.

- Currently, there is no effective drug to treat muscle atrophy in humans. The goal of this research is to develop and atrophy model to treat with small molecules, but an optimal hypertrophy model needs to be developed first to effectively atrophy the myotubes.
- Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptors that include three subtypes that have been identified to date: PPARα, PPARγ, and PPARβ. It is currently known that the activation of these receptors can produce anti-inflammatory effects in different types of cells such as macrophages, smooth muscle cells, and endothelial cells(3). The PPARs are involved in energy homogeneity through the control of lipids and metabolism(4). PPAR alpha in particular is noted to have caused a decrease in the expression of ubiquitin ligases(4).

Objective: Determine the optimal concentration of Fetal Bovine Serum (FBS) in media to produce the most effective hypertrophy results in C2C12 myoblasts.

Methods

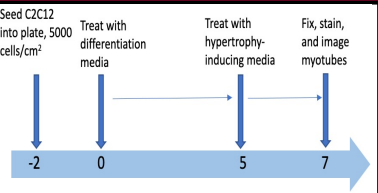

Figure 2: Timeline of Hypertrophy Experiment
Each number on the timeline indicates the number of day each action occurs in the experiment. Each dark blue line indicates a new action, and each thin, light blue line indicates a media change each day in between. C2C12 cells were brought up and split twice at around 80% confluency before seeded into a 48 well plate in media containing Dulbecco's Modified Eagle medium (DMEM), 10% Fetal Bovine Serum(FBS), and 1% Penicillin Streptomycin (PS). Two plates were used, the first only using three wells, and the second using nine wells. After the first two days in the plate, media was extracted from each well and replaced with 250 microliters of differentiation media in each well: 2% Horse Serum, DMEM, and 1% PS. Media was warmed to 37 degrees Celsius. Media was changed in the wells each day for five days. After the fifth day, plate one was fixed, stained, and imaged for analysis to determine average width of myotubes. Plate two was treated with hypertrophy inducing media: wells A1-A3 continued with differentiation media as a control, wells B1-B3 were treated with 10% FBS, DMEM, and 1% PS media, and wells C1-C3 were treated with 20% FBS, DMEM, and 1% PS media to induce hypertrophy. Media was changed the next day, and on day seven plate two was fixed, stained, and imaged for analysis of hypertrophied myotubes.

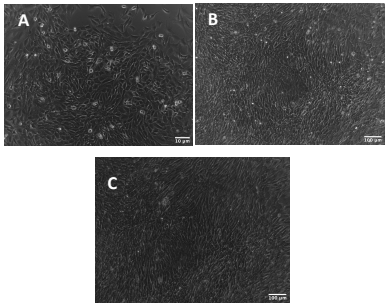

Figure 3: Differentiation of C2C12 cells
Progression of the differentiation of C2C12 cells in plate 2, well A1. Figure 3A is day 0, right before treatment with differentiation media. Figure 3B shows day 3, after being treated with 2 days of differentiation media before. Figure 3C shows day 5, after being treated with differentiation media for 4 days prior.

Results

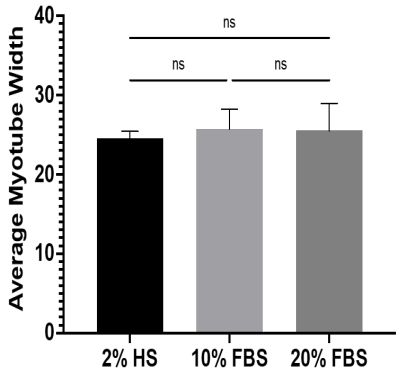

Figure 4: Comparison of Myotube Width after Hypertrophy Stage
There was no statistical difference between the average width size of the myotubes treated with media containing 10% Fetal Bovine Serum (FBS) and the myotubes treated with media containing 20% Fetal Bovine Serum (FBS). There is also no difference in width compared with the media containing 2% Horse Serum (HS).

Conclusions

- According to our data from the hypertrophy experiment, there was no statistical difference between the width of myotubes treated with 10% Fetal Bovine Serum (FBS) and DMEM media, 2% Horse Serum (HS) and DMEM media, and 20% Fetal Bovine Serum (FBS) and DMEM media. It is important to note that of all three data points, 10% Fetal Bovine Serum media has the greatest width, being 25.6939549 micrometers.
- There is no significant data to say that 10% FBS media creates significant increase in width to the myotubes during the hypertrophy data. The average width of myotubes before hypertrophy was calculated to be 23.5994593 micrometers, which concludes that there was only approximately an increase of 2 micrometers during the hypertrophy stage.

Future Directions

- For future directions of this research, I plan to perform another experiment comparing the effects of 10% fetal bovine serum and 20% fetal bovine serum for hypertrophy to be able to confidently say there is no significant difference.
- I also plan to see the percentage of atrophy that is produced after treating with serum starvation, which is 2% horse serum media. This treatment is taken from a previous model performed by Nicolas Dumonte and Jerome Frenette (5).
- Dexamethasone (DEX) will also be a treatment of interest to perform atrophy. It has been noted in literature that DEX induces protein degradation and generally suppresses the synthesis of proteins(6).
- After completing an optimal atrophy model in the C2C12 cells, treatment with the small molecule fenofibrate will be performed with the hypothesis that targeting the PPAR alpha pathway will inhibit atrophy in the myotubes.

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