Understanding how Fibro-Adipogenic Progenitor Cells React to Obesity/Injury and its Effects on Muscle Health

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Introduction

Millions of people worldwide suffer from obesity, commonly described as adipose tissue expansion due to a build-up of lipids from excess nutritional intake. Adipose tissue expansion can impede the functions of vital organs causing various types of metabolic disorders such as type 2 diabetes and cardiovascular conditions like coronary heart disease. However, adipose tissue expansion not only occurs in obesity but also in contexts of skeletal muscle injury. During muscle regeneration after injury, the formation of intramuscular adipose tissue (IMAT) occurs within the fibers of skeletal muscles. In obesity, lipid accumulation in IMAT can lead to decreased muscle strength and inflammation.[1] Adipocytes that comprise IMAT are derived from fibro-adipogenic progenitor cells (FAPs). FAPs are present in uninjured skeletal muscle tissue, and distinctly marked by the expression of platelet-derived growth factor receptor alpha (PDGFRa).[2] FAPs have adipogenic capabilities as well as provide support to muscle stem cells, which are myogenic precursors. However, FAPs are not capable of differentiating into muscles. It has been shown that FAPs from muscle tissue are capable of adipogenesis *in vitro* and lead to the formation of IMAT *in vivo*.[3] However, the absence of PDGFRa+ FAPs results in decreased IMAT production in healthy muscle tissue, but FAPs do differentiate into IMAT in injury and obesity conditions. This suggests that there is an unknown mechanism within healthy muscle tissue inhibiting FAP differentiation and a separate mechanism in injured muscle tissue that stimulates FAP differentiation. Our project aims to identify what is behind these mechanisms as well as understand how FAPs react to injury and obesity and how this may affect muscle health.

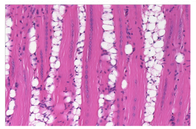


Figure 1. Image of IMAT

Materials and Methods

Gene expression in lean and obese mice: Wild-type C57BL6/J mice were kept on high fat (60% kCal fat) or chow diet for 22-24 weeks and muscle tissue was harvested. RNA was extracted using Trizol reagent and gene expression was measured with quantitative reverse transcription PCR, using *Tbp* as an internal normalization control.
Glycerol injury: 50uL of 50% glycerol/PBS was injected into the tibialis anterior of WT CD1 mice. Tissues were collected 5 days post-injury.
Magnetic-activated cell sorting: Primary FAPs and satellite cells (MuSCs) were isolated using magnetic activated cell sorting. Mice were killed via CO2 inhalation and tissues were harvested and minced until homogenous and enzymatically digested in collagenase type 1 and dispase II. Resulting cell suspensions were quenched in DMEM containing 10% bovine serum, filtered through 100um filters, and centrifuged. Erythrocytes were removed from the cell population, which was filtered again through 40um filters. Sorting was then performed using magnetic bead-conjugated antibodies and filtration through Miltenyi LS columns. FAPs: negative selection against CD45, positive selection against PDGFRa. MuSCs: negative selection against Miltenyi satellite cell isolation cocktail, positive selection against integrin alpha 7 (ITGa7).
Immunofluorescent imaging: Cells were fixed in 4% paraformaldehyde (PFA). Cells were permeabilized in 0.2% Triton X-100, and blocked in 1% BSA in PBS. Cells were incubated with primary antibody against Myosin Heavy Chain 1 (MyH1, DSHB clone F59) overnight at 4˚C, and with secondary antibody (anti-mouse 549, BioLegend) for 2 hours at room temperature. Finally, cells were stained with Hoechst (nuclei) and/or Bodipy (lipid) for 10 minutes at room temperature.

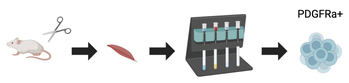


Figure 2. Process of magnetic bead sorting PDGFRa+ FAPs

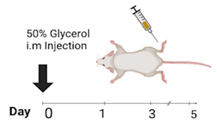
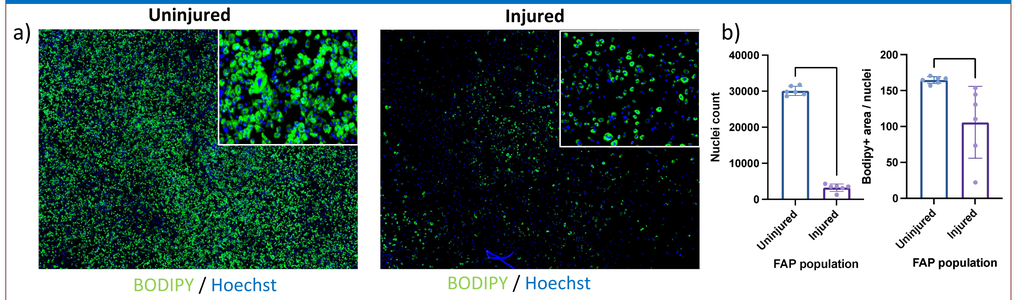


Figure 3. Timeline of glycerol injury to evaluate injured muscle regeneration

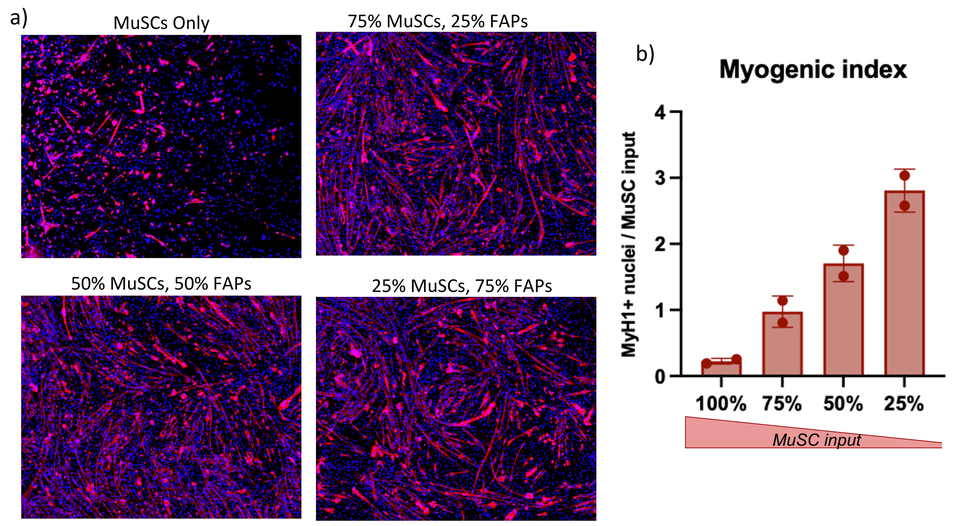
Results

FAPs derived from injured muscle are less adipogenic than FAPs derived from uninjured muscle



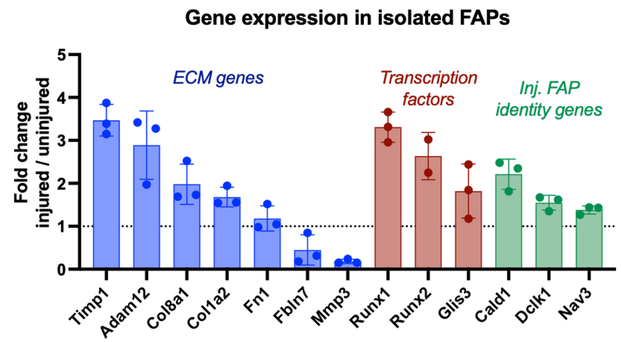
(Figure 4) Adipogenesis in Uninjured vs. Injured Muscle Tissue
a) Immunofluorescence images of uninjured and injured muscle five days after adipogenic induction. These images show that uninjured tissue has much more adipocytes compared to injured tissue.
b) Quantification of the number of nuclei present in each respective well, where uninjured tissue had a significantly larger number of nuclei than injured tissue. Normalized data shows that the uninjured tissue had statistically significantly more BODIPY than the injured tissue.

Myogenesis is enhanced by even small amounts of FAPs



(Figure 5) MuSCs and FAPs Co-Cultures
Cells were plated in 96-well plates at 20,000 cells per well. Cells were maintained in growth medium (DMEM, 20% FBS) until cells reached 100% confluency, and then switched to differentiation media (DMEM, 2% Horse serum). All cells were collected after 6 days in culture.
a) representative immunofluorescence images of co-culture wells.
b) quantification of a) showing MyH1+ nuclei normalized to MuSC input at time of plating.

Injury alters gene expression in FAPs



(Figure 6) qPCR on Uninjured vs. Injured FAPs
a) PDGFRa+ FAPs were isolated from uninjured and injured (5 days) female TAs via FACS. Gene expression was normalized to TBP as a housekeeping gene. Fold change represents injured vs. uninjured.

Conclusions

- As seen in Figure 3, adipogenesis is greater in uninjured compared to injured muscle tissue. This may indicate some sort of cell exhaustion in the injured tissue which can explain the lower rate of adipogenesis.
- Even small amounts of FAPs can significantly impact the level of myogenesis.
- FAPs increase gene expression of many ECM-related genes after injury, suggesting that FAPs could be involved in ECM deposition during muscle regeneration.
- Overall, FAPs are less adipogenic in injured conditions but have differential expression of many genes, suggesting a role in ECM modification to promote injury recovery. Additionally, even a small amount of FAPs have a great effect on myogenesis.
- **Future Directions:** qPCR could be rerun on male tissue. The co-culture experiment can be run again based on number of days instead of different ratios of MuSCs and FAPs. More investigation will need to be done to determine why FAPs are less adipogenic under injured conditions.

Acknowledgments

I would like to express special thanks to my mentor, Natalie Moore, for her mentorship and guidance on this project. Thank you to Dr. Merrick and the members of the Merrick Lab for the hospitality I received during my time in the lab. I would also like to thank PURM for giving me the opportunity to conduct summer research and funding.

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