Project Description

Title: DGAT1/2 Knockout Cell Characterization
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Scientists know that the enzyme DGAT is involved in the formation of cells that store fat in the body and that the ability to control fat-cell formation might help clinicians fight the current obesity epidemic in America. Nicholas studied mouse fat cells that had DGAT removed, hoping its removal would prevent fat storage cell formation. While he found that removing DGAT did not fully prevent the formation of fat cells, he was able to gain insight into how DGAT affected these fat cells.

Purpose

- Is it possible to create a brown adipocyte from adipocyte precursors?
  - White adipocytes store fat in triglycerides → obesity
  - Brown adipocytes burn fat → solution to obesity?

- Want to alter the triglyceride pathway through an enzyme knockout (KO) in hopes of forcing a brown phenotype

Background

- DGAT: Catalyzes the final step of triglyceride formation
- Previous KO studies in vivo
  - DGAT1: Protected from obesity
  - DGAT2: Die shortly after birth
- Previous KO studies in vitro
  - KO cells have more mitochondria than WT cells
  - Brown adipocytes have more mitochondria than white adipocytes
- Is a DGAT1/2 KO cell actually a brown adipocyte?

Hypotheses

- The DGAT1/2 KO cell will resemble an adipocyte
  - Adipocyte genes – expected to have higher expression levels in the differentiated cell than the undifferentiated cell, as verified by qPCR
- The DGAT1/2 KO cell will resemble a brown adipocyte in terms of gene expression, as compared to the WT and verified by qPCR
  - Brown adipocyte-specific genes – expected to be higher in KO
  - White adipocyte-specific genes – expected to be lower in KO
  - Mitochondrial genes – expected to be far higher in KO
- Characterize the DGAT1/2 KO cell
  - Other genes for overall characterization (hormones, transcription factors, triglyceride formation genes, etc.)
- Compare gene amplifications in KO and WT to brown adipocytes (BAT) and white adipocytes (WAT)

Results

- 36 of 55 genes total had higher expression in the Diff. compared to Undiff. (KO condition, p<0.05)
- Higher expression in KO, compared to WT (Diff. condition, p<0.05)
  - 7 of 10 brown adipose genes (1 lower)
  - 2 of 5 white adipose genes (1 lower)
  - 3 of 7 mitochondrial genes
- 0.4 to 0.9 fold induction (avg) in KO compared to WT, regardless of gene association (graph, right)

Conclusions and Acknowledgements

- KO cell has an adipocyte gene expression profile
  - Diff. induced most genes tested, as compared to Undiff.
- KO cell is a “mixed” adipocyte – part white, part brown
  - White adipocyte and brown adipocyte genes induced equally
  - Mitochondrial gene induction was not significantly different than any other genes
- Overall characterization confirms adipocyte expression profile
- Many thanks to Dr. Charlie Harris, my mentor throughout the project
- Thanks to Bob Farese and Dr. Dan Rader for overseeing the project, both at UCSF and at UPenn

References