TNF-related weak inducer of apoptosis as a regulator of β-oxidation and differentiation in murine adipocytes
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Introduction
Obesity is known to be strongly correlated with immunodeficiency, which is particularly problematic for obese cancer patients with cancers typically responsive to immunotherapy. Ways to circumvent obesity-linked immunodeficiency are vital in cancer treatment for such individuals.

A promising approach to combating obesity-linked immunodeficiency is through reducing obesity itself through medication. Another approach is through reintroducing cytokines involved in activation, differentiation, or growth of immune cells.

Tumor necrosis factor (TNF) related weak inducer of apoptosis, also known as TWEAK or TNFSF12, is such a cytokine. It not only has downstream signaling pathways that promote immune cell proliferation/differentiation but also has large-scale effects as an inflammatory cytokine of the TNF family; thus, it is a therapeutic agent of interest in the low-grade inflammation present in obesity.

Moreover, obesity research often involves rat adipocyte models, for which there are straightforward differentiation cocktails to utilize. These consist of 3 main molecules, which bind to receptors that induce transcription of adipogenic genes. Many papers have started to use another differentiation agent, Rosiglitazone, to speed up the process; however, studies comparing differentiation levels with and without are inconclusive or suggest no difference across cell lines.

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Thus, we aim to observe the effects of TWEAK in a murine adipocyte model to determine if it has any lipolytic and anti-differentiation effects that would cause anti-obesity effects.

Hypotheses
1. TWEAK antagonizes differentiation and growth of pre-adipocytes and adipocytes
2. TWEAK will be expressed at lower levels in obese mice as compared to those at normal weights
3. Rosiglitazone and other thiazolidinediones are not effective in promoting differentiation

Methodology
Cell culture: 3T3-L1 pre-adipocyte cells were sourced from ATCC (ID: CL-173). Cells culture medium comprised of 10% Bovine Calf Serum and 90% DMEM with 4.5 g/L glucose as per ATCC recommendation. The cells were cultured in 100 mm culture plates in a cell culture incubator set to 37 degrees Celsius and 5% CO2. Cells were frozen immediately upon arrival for 1 week, then thawed and subcultured near confluency.

Oil Red O Staining: Oil Red O staining was done using a StainLab kit (ID: KIT0R). Stained cells were cultured in Lab-Tek II Chamber Slides. After medium was aspirated, plates were washed with PBS, fixed with 10% formalin, then stained with Oil Red O solution and modified Mayer’s Hematoxylin solution. Slides are then removed from chamber and observed; photos of slides were taken on an Olympus microscope with digital camera.

RT-qPCR (primer selection): Target genes were decided through literature analysis intended to find markers of adipocyte differentiation, fatty acid synthesis, β-oxidation, and lipolysis that are widely expressed 3T3-L1 cells or white adipocyte tissues. Primers were found on PrimerBank, and primers were used to design specific primers. β-actin was the housekeeping/reference gene used.

RT-qPCR

TWEAK ELISA Assay: Done using Invitrogen Mouse TWEAK ELISA kit (ID: EMTNF12). Results were graphed along with linear regression of the standard curve.

Results (cont.)

TWEAK ELISA Assay, NASH vs Chow-fed mice

Conclusion
TWEAK is shown to inhibit differentiation and promote β-oxidation in 3T3-L1 pre-adipocytes, where it may prove useful in combating obesity. Rosiglitazone, a thiazolidinedione PPARγ agonist that promotes growth of pre-adipocytes, is found to have similar anti-differentiation effects in adipocytes. This supports our hypothesis that increased TWEAK levels would favor differentiation.

Future Directions
Although the various effects of TWEAK in adipocytes have been laid out in this study, it is imperative that the mechanism and downstream cell signaling pathways are further researched. Indeed, if a more targeted downstream receptor or molecule exists that causes similar effects on an important downstream signaling pathway that cannot be disturbed for fear of adverse effect, it will greatly influence research on TWEAK. In that vein, the differentiated Protocol 2 cells (control and TWEAK-treated) will be sent for RNA-seq in efforts to try and elucidate possible downstream signaling pathways.