CASE STUDY

Assessment of brown fat activation to prevent obesity and metabolic syndrome

Using optical imaging of CRISPR/cas9 engineered adipose tissue to study obesity prevention

Introduction

Obesity is a global epidemic that is the fifth leading cause of death worldwide[1-3]. In the United States (US) alone, nearly 85% of adults are expected to be overweight or obese by 2030. In addition to the increased risk of overall mortality, obesity is associated with an increased risk for morbidities, such as metabolic disorders, and pose a significant burden to health care systems. Estimates of obesity costs in the US range from $147 - $210 billion per year, which represents roughly 21% of annual US health care expenditures[2]. Therefore, the development of preventative and therapeutic strategies for obesity and subsequent morbidities is of great interest to researchers and the health care community[1,3].

Dr. Chih-Hao Wang and Dr. Matthew Lynes are part of a leading metabolism research team at the Joslin Diabetes Center in Boston, Massachusetts. Their team's focus is on the mechanisms underlying the regulation of energy balance to develop potential therapies for combating obesity and related diseases. One particular approach they are interested in, is harnessing the thermogenic potential of activated brown and beige fat to prevent or treat obesity and obesity-related metabolic disorders. The team takes a broad-based approach to explore the mechanisms that underlie brown and beige fat in physiological and pathophysiological conditions, including cellular and molecular analysis, multi-omics profiling, transgenic models, and *in vitro* and *in vivo* imaging.

This case study demonstrates how Dr. Wang, Dr. Lynes, and their team utilized preclinical bioluminescence optical imaging to visualize and quantify uncoupling protein 1 (UCP1) activation in CRISPR/Cas9 engineered white adipose tissue (WAT) for the prevention of obesity and obesity-related metabolic disorders.

Our challenge

"Brown adipose tissue (BAT) is recognized as a potential target to prevent obesity and subsequent metabolic disorders; therefore, imaging methods that allow for the assessment of BAT activation are of great importance to the research community. A major challenge to our research is that imaging modalities commonly used to image BAT (e.g., PET/CT and MRI) are typically expensive, require long scan times and can be low throughput."

Dr. Chih Hao Wang

The challenge

There are two types of fat present in mammals: white adipose tissue (WAT) and brown adipose tissue (BAT). Despite differences in their anatomy, morphology, and functions, both WAT and BAT contribute to systemic energy homeostasis[3]. WAT is the main site for excess energy storage, while BAT plays a pivotal role in thermogenesis. Further, BAT activity is inversely correlated with body mass index and fat mass. Therefore, an understanding of the BAT thermogenic differentiation program and the factors that activate this thermogenic circuit represent a novel therapeutic approach to combat obesity and its related metabolic disorders[1,3].

Evidence suggests that activation of the thermogenic circuit is mediated by uncoupling protein 1 (UCP1). UCP1 is a proton leak channel found in the inner mitochondrial membrane that dissipates energy in the form of heat by uncoupling the proton motive force from ATP production. Interestingly, UCP1 expression is restricted to BAT under basal conditions, however evidence supports that stimulation can not only increase UCP1-mediated thermogenic capacity in BAT, but it can also activate the recruitment of brown-like beige adipocytes in WAT that express *UCP1* to produce heat in a process called browning. Considering the abundance of WAT relative to BAT, induced browning of WAT may hold great potential for preventing or treating obesity and obesity-related metabolic disorders[1,3].

Non-invasive *in vivo* preclinical imaging tools that enable longitudinal assessment of biological changes or efficacy of pharmacological interventions for brown and beige adipocyte recruitment are still lacking. PET/CT and MRI are the most common methods to visualize BAT localization and activation. However, PET employs radioactivity and requires either cold or norepinephrine pretreatment to activate BAT metabolism. MRI can image BAT by exploiting the water to fat ratio but suffers from poor adipose tissue signal contrast from surrounding internal structures. Further, these modalities are expensive, low throughput, and require long scan times[4,5].

Preclinical optical imaging has emerged as an alternative imaging tool to visualize brown and beige adipocyte recruitment and activation that can potentially circumvent the challenges associated with other imaging modalities. In this study, Dr. Wang, Dr. Lynes, and their team took a cell-based approach using autologous cell transfer to activate the thermogenic circuit as a potential way to prevent obesity and ameliorate metabolic syndrome. Specifically, the team created human brown-like (HUMBLE) cells by engineering human white preadipocytes using a CRISPR-Cas9–SAM–gRNA system to express UCP1. Importantly, these HUMBLE cells also expressed a luciferase reporter driven by the human *UCP1* promoter, which allowed longitudinal monitoring of *UCP1* activation in the transplanted cells *in vivo* via non-invasive, high-throughput bioluminescence optical imaging on the IVIS® platform.

Seeing is believing

Preclinical imaging is recognized as a powerful tool for monitoring cellular and molecular events, and a variety of imaging technologies are being investigated as tools for studying gene expression in living subjects. For example, optical imaging has been paired with genetic engineering techniques, such as CRISPR/Cas9, to visualize changes in expression and activation of genes of interest. Specifically, genome editing allows for the specific integration of a luciferase gene that is under promotor control of a gene of interest. Therefore, luciferase expression and subsequent light output depends on endogenously driven expression and activation of that gene of interest.

The objective of the current study was to transform white adipocytes into HUMBLE cells in order to examine the potential of utilizing HUMBLE cells for the prevention of obesity and obesity-related disorders. To do this, CRISPR/Cas9 genome editing was utilized to increase endogenous UCP1 expression in human white adipocytes. Importantly, these cells also expressed a luciferase reporter driven by the UCP1 promoter in order to longitudinally monitor and quantify UCP1 activation *in vivo* via bioluminescence optical imaging on Revvity's IVIS platform.

In order to evaluate the potential long term-metabolic benefits of HUMBLE cells, immune-compromised nude mice were placed on a high fat diet (HFD) for 2 weeks to mimic diet-induced obesity. After 2 weeks, mice were transplanted in the thoracic-sternum region with white control preadipocytes, HUMBLE cells or brown control preadipocytes (Figure 1A). Body weight was monitored throughout the study and glucose tolerance tests (GTT) were performed at 4-, 8- and 12-weeks post transplantation. UCP1 expression was also monitored throughout the study via IVIS imaging.

Results demonstrate that mice transplanted with HUMBLE or brown control cells gained less weight over the course of the study and had improved glucose tolerance compared to mice transplanted with white control cells at 4-, 8- and 12-weeks post-transplantation[3]. Mice transplanted with HUMBLE or brown control cells also had increased UCP1 activation throughout the study, relative to mice transplanted with white control cells, as indicated by IVIS bioluminescence optical imaging (Figure 1B).

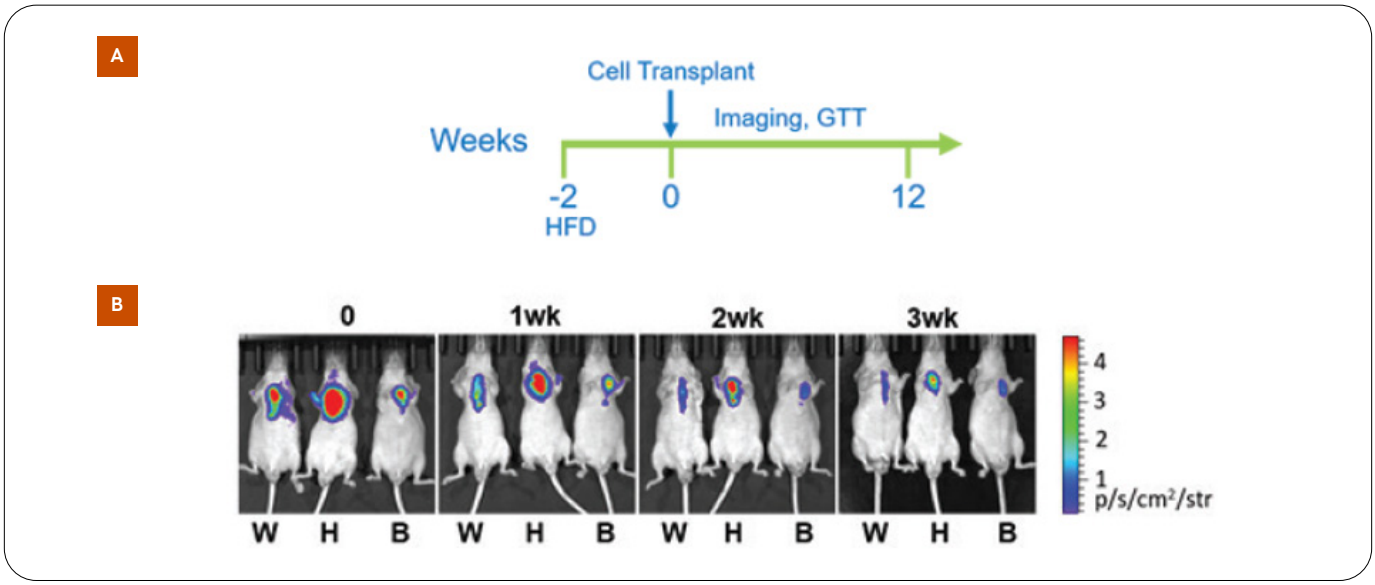

Figure 1: Visualizing long term metabolic benefits of HUMBLE cell transplantation in mice. (A) Study design for transplantation of bioluminescent cells into nude mice. (B) Longitudinal bioluminescence imaging of white control (W), HUMBLE (H) or brown control (B) cells using an IVIS imaging system, or brown control preadipocytes into nude mice. (B) BW change in mice more than 12 weeks after transplantation with white control, HUMBLE, or brown control cells; n = 8 mice per group.

What is optical imaging and why use it for BAT imaging?

Dr. Matthew Lynes

"Optical imaging is a tool that allows for the sensitive detection of photons emitted from bioluminescent and fluorescent reporters. Here it has been adapted to longitudinally and non-invasively visualize and quantify BAT activation by driving the luminescent reporter with a BAT-specific promoter. Importantly, optical imaging is cost-effective, requires short scan times and is high-throughput relative to other imaging techniques."

The outcome

Using preclinical bioluminescent optical imaging with Revvity's IVIS platform, Dr. Wang, Dr. Lynes, and their team were able to non-invasively visualize and quantify UCP1 activation longitudinally in obese mice transplanted with white control cells, HUMBLE cells or brown control cells. They found that obese mice transplanted with either HUMBLE cells or brown control cells demonstrated increased UCP1 activation compared to mice transplanted with white control cells. Further, obese mice transplanted with either HUMBLE cells or brown control cells exhibited less weight gain and more glucose tolerance compared to mice transplanted with white control cells. Collectively, these results strongly support that genetic engineering can be used to activate the thermogenic circuit in order to induce browning of WAT and can potentially be used to prevent or treat obesity and obesity-related metabolic disorders. Dr. Wang, Dr. Lynes, and their team will continue to use optical imaging to identify the underlying mechanisms that increase UCP1 expression and subsequent browning of WAT for the prevention and treatment of obesity and obesity-related metabolic disorders.

Conclusion

The ability to non-invasively visualize and quantify the activation and recruitment of brown and beige adipocytes *in vivo* is of significant interest to researchers who aim to develop treatments for obesity and obesity-related metabolic disorders. PET/CT and MRI are the traditional preclinical imaging modalities used to visualize BAT localization and activation; however, both suffer from technical challenges that limit their utility. This case study demonstrates that optical imaging is an alternative approach that can be easily utilized to visualize and quantify brown and beige cell recruitment and activation for the prevention or treatment of obesity and obesity-related metabolic disorders.

Optical imaging on the IVIS platform offers:

- High sensitivity 2D bioluminescence and fluorescence imaging
- 3D tomographic reconstruction
- High throughput
- Fast image acquisition
- Standalone optical systems or optical integrated with x-ray or CT

References

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Dr. Chih-Hao Wang China
Medical University Graduate Institute of Biomedical Science, Taiwan

Dr. Matthew Lynes Joslin
Diabetes Center Boston, Massachuetts USA