Abstract

White adipocytes play a role in energy homeostasis through endocrine signaling and serve important functions such as accumulation of lipids to maintain an energy reservoir in times of food scarcity. In addition, white adipose tissue can be induced to undergo browning in response to stimuli such as cold exposure, whereby brite adipocytes arise in white depots. Brite adipocytes can create heat and dissipate energy by non-shivering thermogenesis, and browning of white adipose depots thereby represent a potential therapeutic strategy for combating the world-wide rise in obesity.

In this work, we utilised genome-wide sequencing technologies to investigate the thermogenic capacity of white adipocytes (Part I) and the transcriptional regulation of adipocyte differentiation (Part II).

In the first part of this work, we established 3D cell cultures of *in vivo* differentiated white adipocytes isolated from mouse epididymal and inguinal white adipose tissue (eWAT and iWAT) depots and investigated the cell autonomous browning capacity in response to stimulation. We find that iWAT derived white adipocytes are capable of transdifferentiation to a brite phenotype both in response to elevation of cAMP levels by isoproterenol (iso) treatment as well as peroxisome proliferator-activated receptor γ (PPARγ) activation by rosiglitazone (rosi) stimulation. In contrast, eWAT derived white adipocytes are resistant to PPARγ stimulation while brite characteristic genes are induced in response to iso treatment although to a lesser degree compared to iWAT derived mature adipocytes. Interestingly, we find that iso and rosi induce different metabolic gene programs with a stimulation of glucose metabolism related genes in response to iso treatment and an induction of fatty acid metabolism and TCA cycle related genes in response to rosi stimulation. Using this 3D cell culture model system of isolated *in vivo* differentiated white adipocytes, we demonstrate adipose depots specific effects in response to stimulation of browning. This model system better mimics the *in vivo* environment of adipocytes and circumpass the need for *in vitro* differentiation. 3D cultures of isolated adipocytes thereby holds promise for future studies investigating adipocyte function and cell autonomous response to stimulation.

In the second part of this work, we focused on the transcriptional regulation of adipocyte differentiation by the lysine demethylase 5 (KDM5) family of histone demethylases. The KDM5 family is generally considered co-repressors through demethylation of the activating histone mark H3K4me3 at promoter regions. However, by genome-wide investigation of the transcriptome in response to KDM5 knockdown, we find that gene regulation is deregulated with approximately equal numbers of up- and downregulated genes. We demonstrate a role of the KDM5s during early adipocyte differentiation and find that the KDM5s are necessary for stimulation of the mitotic clonal expansion by induction of a subset of cell cycle regulatory genes. We thereby demonstrate a dual mechanism for the KDM5s in regulating gene expression both as co-repressors and co-activators during early adipocyte differentiation. In addition, we find that global

H3K4me3 levels at promoters increase significantly in response to KDM5 knockdown, but this does not elicit a strong effect on gene expression indicating a weak causal strength of the H3K4me3 mark in direct gene activation. Future studies into the regulatory mechanism of the KDM5s would further contribute to elucidate the dual mechanism of gene regulation by the KDM5s.