Mechanistic investigation of a circadian clock enhancer in treatment of obesity

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Project Outline

Background, hypothesis and aims: We aim to elucidate the molecular mechanisms by which the epigenetic reader, bromodomain BRD4, confers circadian rhythmicity on to downstream metabolic pathways to result in weight loss. Most organisms have evolved a circadian clock to anticipate and prepare for the variations in environment associated with the 24h light-dark cycles. The circadian clock in mammals is a transcription/ translation feedback loop, which orchestrates the timed expression of approximately 30% of the genome. The circadian clock temporally coordinates metabolism directly by regulating glucose metabolism/ energy partitioning, but also indirectly through timing the rest/activity cycle and hormone secretion. Strengthening the circadian clock improves metabolic outcomes and conversely, circadian disruption leads to metabolic pathologies [1,2]. To highlight the impact of circadian regulation on metabolism; a single night of sleep-deprivation or jet lag [2,3] is sufficient to make a healthy individual appear pre-diabetic. Similarly, isocaloric meals at wrong times of day leads to circadian desynchrony and reprograms metabolic pathways [4,5]. Indeed, these observations are supported by numerous epidemiological and pre-clinical studies [6].

Based on this, we recently tested the hypothesis that pharmacologically strengthening circadian rhythms in animal models with metabolic disorder, induced by diet induced obesity (DIO) would ameliorate their symptoms. We found that inhibition of bromodomain 4 (BRD4), a reader of histone acetylation marks, leads to strengthening of circadian rhythms, and that the BRD4 is key to conferring circadian and metabolic regulation. Indeed, the circadian clock relies on chromatin remodelling to effect rhythmic changes in gene expression and accomplishes this through a suite of histone modifiers[7]. In an *in vivo* mouse model of DIO, we found BRD4 inhibition (daily oral drug for 12 weeks) eliminates weight-gain, lowers liver steatosis, without altering food intake and enhances weight loss in already obese mice (~10% in 4 weeks) and this effect is mirrored in lipid accumulation *in vitro* assays with adipocytes following BRD4 knockdown. However, BRD4 inhibition causes an acute increase in blood glucose (basal and clearance) despite increasing insulin sensitivity (30% increase in glucose clearance by exogenous insulin). Thus, *the central hypothesis of this project is that there are distinct pathways under circadian control governed by BRD4 that regulate fat loss and glucose uptake. The identification of these distinct mechanisms will allow the selective development of targets that underpin weight loss.* Our aim is to describe the molecular underpinnings of the separate effects of BRD4 inhibition /knockout in **a)** adipocytes (assess lipolysis and differentiation) and **b)** skeletal muscle/ hepatocytes/ pancreatic islets (assess glucose uptake, sensitivity / insulin release). These studies will

enable the selective identification of novel targets that cause fat loss without the negative effects on glucose clearance.

Approach: To profile the differential chromatin landscape associated with BRD4 loss of function that underpins weight loss, we will conduct H3K27ac and BRD4 ChIP-Seq in DFAT cells (human adipocytes) after BRD4 inhibition/knockout over a 24h, every 4h. The former will identify the rhythmic histone acetylation landscape, and the latter, the aspects of this landscape read by BRD4, and how these pertain to weight-loss. To assess the impact of chromatin changes on gene expression /protein levels, we will also perform RNA-seq and quantitative proteomics. We will identify up to 50 gene targets that are differentially associated with decrease in fat accumulation in a BRD4 dependant manner. The choice of these 50 will also be informed by human genomic resources such as Open Targets (https://www.opentargets.org). We will then validate each of these targets using knock-in or knock-out experiments in the BRD4 KO and control cells, with adipocyte lipolysis and differentiation as functional outcomes. To eliminate targets that cause changes in glucose uptake and insulin release, we will assess these targets in human skeletal muscle cells and hepatocytes (for glucose uptake) and pancreatic islets (for glucose sensing/ insulin release) to ultimately identify targets that could mediate weight loss without affecting glucose sensing and uptake. Follow on studies from this project will assess up to two targets in mouse models of DIO to ultimately validate our target.

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