Epigenetic and genetic discovery of novel adipose tissue targets for obesity therapeutics

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Background

Obesity is a widely prevalent disease that has profound consequences on systemic health, including increased rates of cardiovascular disease, diabetes, cancer and other conditions. While incretin-based therapies promote weight loss and have altered the landscape of obesity interventions, challenges remain as these treatments can be poorly tolerated, cause loss of lean muscle mass, and may not achieve clinically desired levels of durable weight loss. Therefore, there remains an important need to develop novel obesity therapeutics to address these limitations. We sought to address this through the identification of novel adipose tissue targets for obesity therapy by combining epigenetic analysis of human adipose tissue with large scale human genetics analysis to identify genes strongly associated with obesity. We generated novel adipose-targeted siRNAs for several of these genes, and target inhibition *in vivo* demonstrated efficacy to reduce body weight and fat mass in DIO mice, while maintaining lean muscle mass.

Methods

For epigenetic analysis, primary human white adipose tissue-derived adipocytes and preadipocytes from healthy, overweight and obese BMI donors were subjected to whole-genome DNA methylation analysis. Brown adipocytes cultured from human supraclavicular brown adipose tissue depots were also analyzed. Differentially methylated regions were identified and catalogued. Genes with human genetic support were identified through mapping genome-wide association study loci across a comprehensive panel of metabolic traits. Approaches used to map variants to genes include spatial proximity, colocalization with expression quantitative trait loci, identification of protein coding variants, and cross referencing with gene burden tests. Intersection of genes identified through epigenetic and genetic analysis, along with confirmation of gene expression in adipose depots, resulted in identification of novel putative adipose targets for obesity therapeutics. We next conducted screening campaigns to identify high-efficiency siRNA sequences to target these genes in rodents. Modification and conjugation of efficient siRNA sequences to mediate adipose-tissue targeting was performed, and adipose-targeted siRNA constructs were tested for *in vivo* silencing efficacy and therapeutic activity in diet-induced obesity (DIO) models.

Epigenetic profiling reveals underlying features of adipose tissue in health and disease

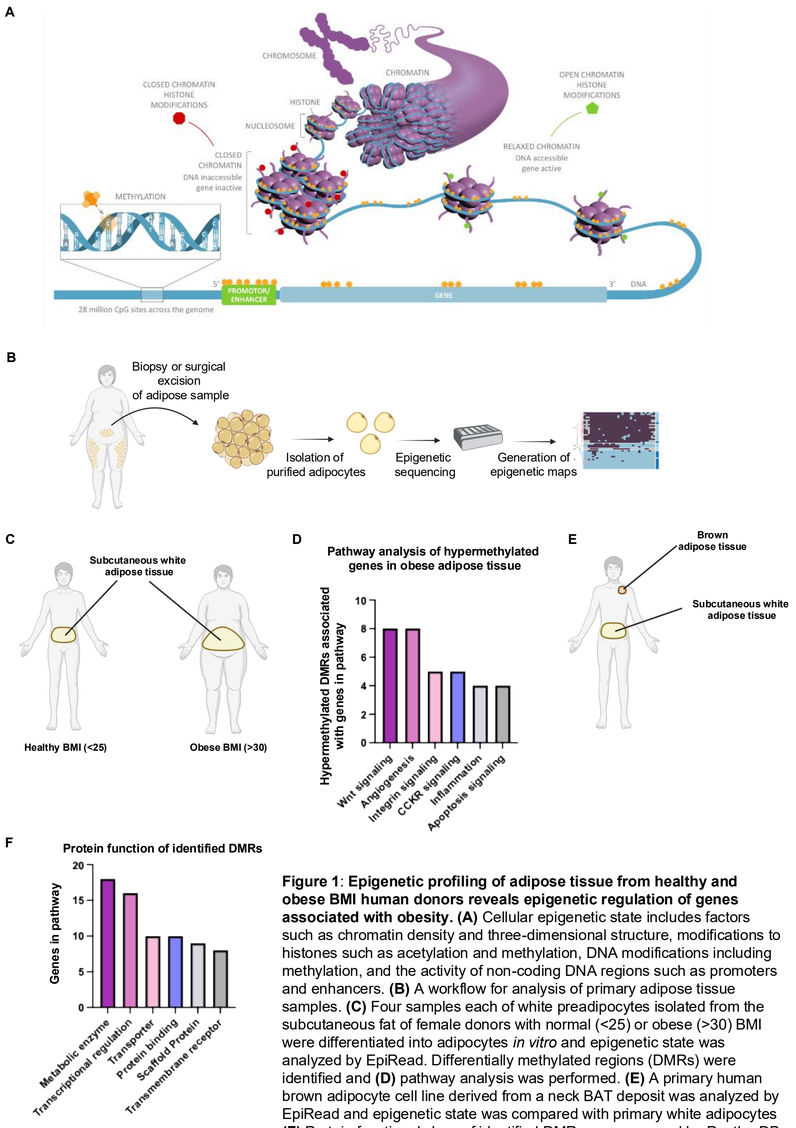

Figure 1: **Epigenetic profiling of adipose tissue from healthy and obese BMI human donors reveals epigenetic regulation of genes associated with obesity. (A)** Cellular epigenetic state includes factors such as chromatin density and three-dimensional structure, modifications to histones such as acetylation and methylation, DNA modifications including methylation, and the activity of non-coding DNA regions such as promoters and enhancers. **(B)** A workflow for analysis of primary adipose tissue samples. **(C)** Four samples each of white preadipocytes isolated from the subcutaneous fat of female donors with normal (<25) or obese (>30) BMI were differentiated into adipocytes *in vitro* and epigenetic state was analyzed by EpiRead. Differentially methylated regions (DMRs) were identified and **(D)** pathway analysis was performed. **(E)** A primary human brown adipocyte cell line derived from a neck BAT deposit was analyzed by EpiRead and epigenetic state was compared with primary white adipocytes **(F)** Protein functional class of identified DMRs was assessed by PantherDB classification.

Human genetics analyses identify genes associated with obesity and cardiometabolic disease phenotypes

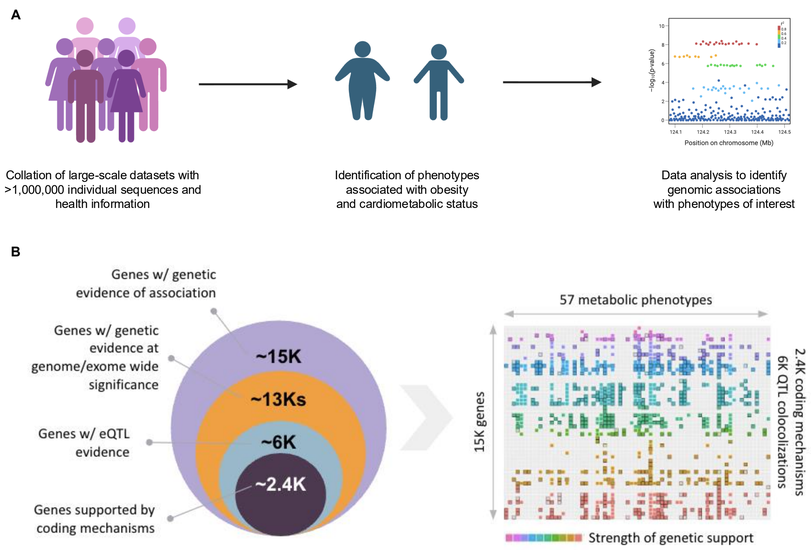

Figure 2: **Large scale genomics analysis of human datasets associates genetic variants with metabolic phenotypes. (A)** In collaboration with Genscience, 748 genetic association studies were analyzed for genetic evidence supportive of gene links with 57 metabolic phenotypes. **(B)** Genome wide analysis uncovered approximately 15,000 genes with evidence of association with metabolic attributes. Of these, approximately 13,000 genes had significant association at a genome or exome wide level. More stringent analysis using expression quantitative trait loci (eQTL) analysis revealed approximately 6,000 genes with robust evidence of metabolic phenotype associations. Finally, approximately 2,500 genes had evidence of coding mechanisms associating with metabolic traits in human subjects.

Combining epigenetic and genetic analyses identifies novel candidate targets for obesity therapy

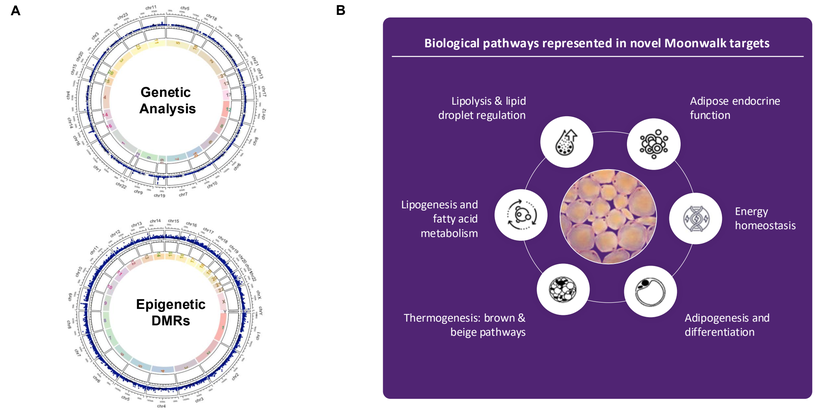

Figure 3: The integration of genomics analysis with epigenetic datasets enables the identification of potential novel targets for therapeutic intervention in metabolic diseases (A) Representations of genomic loci associated with metabolic phenotypes through genetic analysis across the genome, along with the presence of DMRs between healthy and obese BMI adipocytes. **(B)** After analysis of epigenetic and genetic evidence, and consideration of adipose tissue gene expression, we identified novel adipose targets for therapeutic interventions in obesity. These targets were functionally diverse, playing roles in adipose tissue pathways including lipolysis, lipogenesis, thermogenesis/browning, adipose endocrine function, tissue energy homeostasis, and adipogenesis and differentiation.

An adipose-targeting siRNA platform mediates efficient and selective silencing of target genes in fat depots

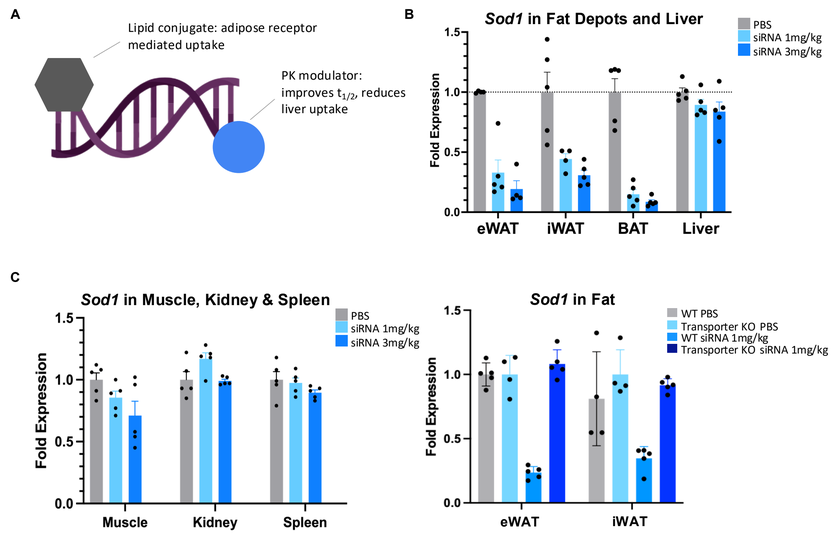

Figure 4: Lipid-conjugation of siRNA constructs, in conjunction with PK modulation, enables high efficiency silencing of adipose tissue targets. (A) Overview of adipose-targeting siRNA platform. siRNAs are conjugated to a lipid, which enables adipose tissue-specific uptake mediated through a cell surface receptor, along with a PK modulator which improves siRNA half-life and reduces liver uptake. **(B-C)** A *Sod1*-targeting siRNA construct was generated in the adipose-targeting format and administered to mice via subcutaneous injection at 1 or 3 mg/kg. After 14 days, indicated tissues were harvested and qPCR analysis of *Sod1* expression levels was performed. **(D)** Wildtype (WT) or cell-surface transporter knockout (KO) mice were treated with PBS or 1 mg/kg *Sod1*-targeting siRNA via subcutaneous injection. After 14 days, eWAT and iWAT were collected and qPCR analysis of *Sod1* expression levels was performed.

Adipose-targeted siRNAs directed against novel adipose targets mediates weight loss with maintenance of lean mass and absence of toxicity in DIO model

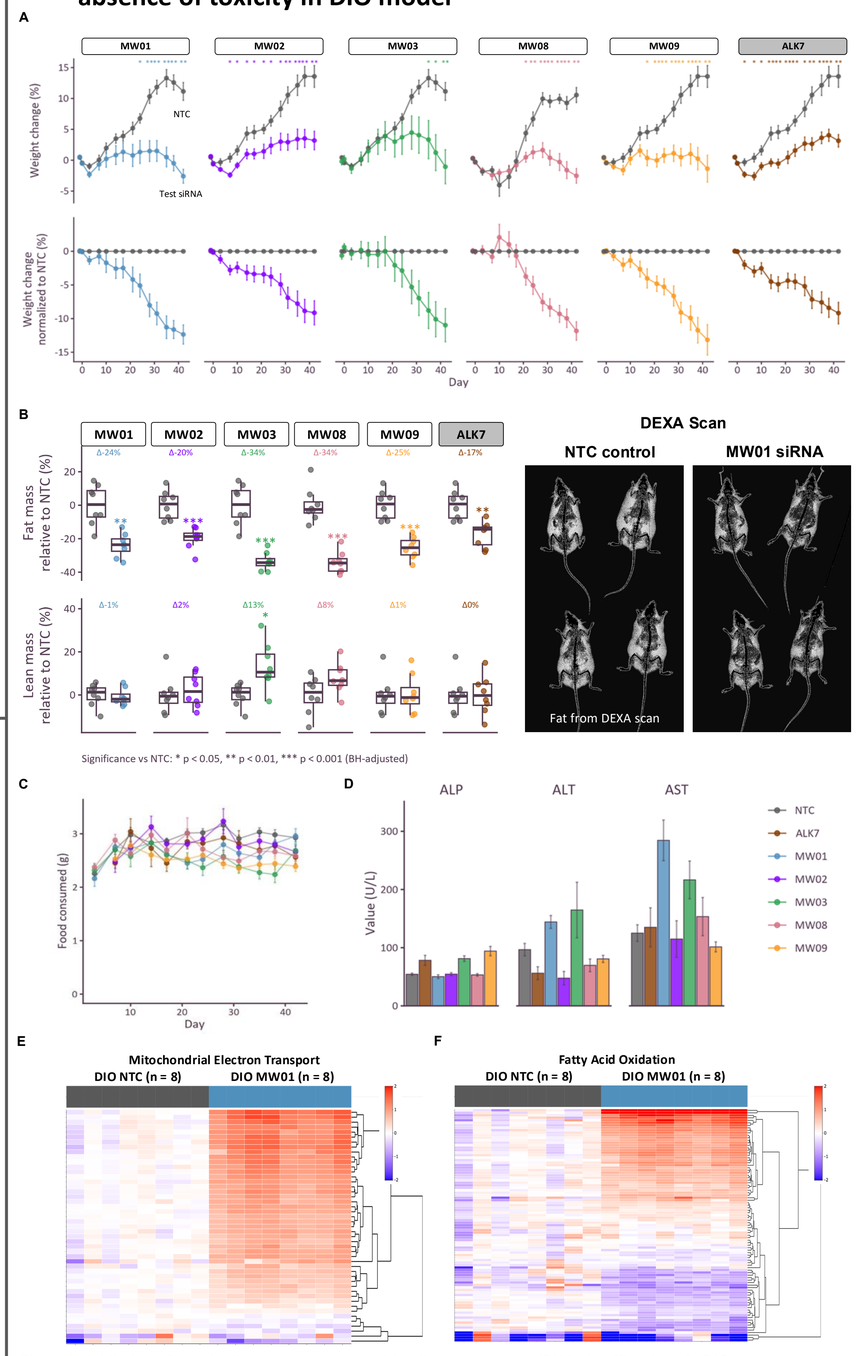

Figure 5: Inhibition of novel adipose target genes with adipose-targeted siRNAs is efficacious in DIO mouse model (A) DIO mice were treated with non-targeting control (NTC) siRNA or siRNAs targeting novel adipose targets and animal weight over time was recorded. An *Alk7*-targeted siRNA was administered for comparison. **(B)** DEXA scan was performed on treated mice to evaluate lean and fat composition, with a representative DEXA scan of NTC and MW01-treated mice shown in right panel. **(C)** Food consumption of treated mice was measured throughout the experiment. **(D)** Upon end of experiment, blood chemistry analysis of ALP, ALT and AST was performed. **(E)** After 6 weeks of treatment, samples of eWAT from NTC and MW01-treated DIO mice were collected and snap frozen. mRNA was extracted and RNAseq was performed. Expression of genes in the mitochondrial electron transport and fatty acid oxidation pathways were plotted in a heatmap.

Results

Combined epigenetic and genetic analysis identified dozens of candidate adipose targets for obesity therapeutics. We screened siRNAs targeting these genes and identified optimal siRNA sequences for both rodents and human/NHP. Efficacy studies in DIO mouse models revealed that siRNA inhibition of adipose tissue expression of several targets resulted in reduced weight with the absence of toxicity. *In vivo* mechanistic studies confirmed that inhibition was specific to the targeted molecular pathways, including marked upregulation of mitochondrial electron transport chain and fatty acid beta-oxidation pathways.

Conclusions

The identification of novel therapeutic targets for obesity may allow for improved strategies for augmenting or maintaining weight loss in patients, resulting in improved cardiometabolic health. Here, we describe the implementation of an epigenetic and genetic-based platform for target identification, along with an siRNA platform capable of mediating high-efficiency *in vivo* target silencing in murine and primate adipose tissues. Our data reveal that inhibition of several novel targets results in therapeutic activity without toxicity in murine obesity models. In addition, we have identified human-targeted siRNA constructs with favorable safety and silencing profiles that are currently being evaluated in non-human primate studies.

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