Intersecting an Adipocyte Epigenetic Atlas with Functional Interrogation in Primary Human Adipocytes

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Abstract

Background

Adipocyte dysfunction is associated with epigenetic changes and recent evidence suggests obesity imprints epigenetic memory that mediates increased rebound weight gain following cessation of anti-obesity treatments. Therefore, the identification and therapeutic modulation of key epigenetically regulated genes may be a durable and robust approach to treating obesity. Previously, we reported on our generation of an epigenetic atlas of human adipocytes sourced from white preadipocytes differentiated into adipocytes and from brown adipocyte cell lines. We now extend these analyses with additional samples and integrate the resulting epigenetic analyses of adipocytes with detailed genomic analysis identifying human gene variants associated with cardiometabolic disease phenotypes to generate candidate gene targets for therapeutic interventions. We demonstrate that targeted perturbation of selected genes in primary human adipocyte models and murine adipose depots improves cellular phenotypic and molecular profiles. These data support novel therapeutic approaches for treating obesity and related comorbidities.

Methods

WAT and BAT were extracted during surgical procedures from human donors and underwent whole genome DNA methylation sequencing. In some instances, preadipocytes and mature adipocytes were isolated for analysis as purified cell populations. We generated genome-wide epigenetic maps and identified differentially methylated regions (DMRs) in each sample group. In parallel, human genetics datasets were analyzed using GWAS, eQTL, burden tests, and coding variant analyses to identify genetic loci associated with a panel of phenotypes associated with obesity and cardiometabolic disease. Genetic targets were derived from the integration of these datasets and underwent genetic perturbation using *in vitro* primary human adipocyte models. The *in vivo* role of selected targets was examined through siRNA inhibition. The *in vitro* and *in vivo* transcriptional and phenotypic impacts of perturbations were then measured.

Results

Our adipose tissue atlas identified epigenetic alterations associated with obesity phenotypes and also provides features distinguishing human brown and white adipocytes. Combining our epigenetic atlas and genetic analysis led to the identification of potential drug targets. Genetic perturbation experiments in human adipocytes validated functional roles for several of these targets, with inhibition resulting in increased expression of catabolic genes associated with enhanced energy expenditure. *In vivo* inhibition of selected targets also mediated transcriptional reprogramming of adipocytes towards increased lipolysis.

Conclusions

The integration of genetic and epigenetic analysis in adipocytes provides a robust platform for identifying genes with functional roles in promoting adipocyte dysfunction. Genetic perturbation of these target genes established a mechanistic understanding of their role in obesity and provides support for further investigation as potential therapies for obesity and related diseases.

Epigenetic mapping reveals defining characteristics of cell state

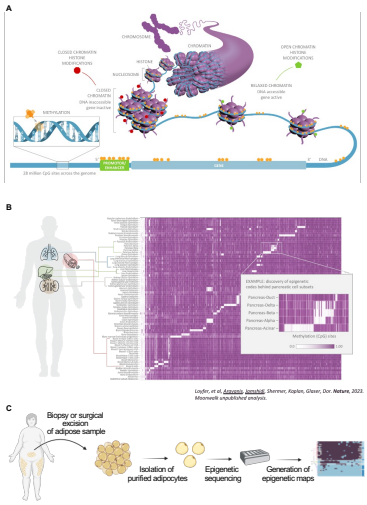
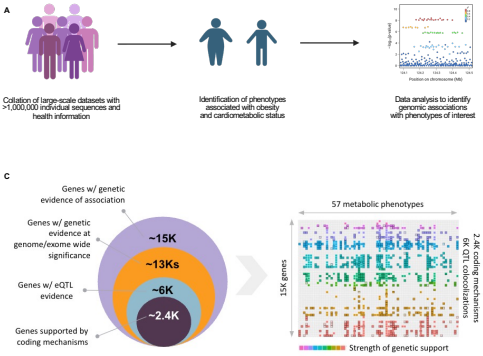


Figure 1: A key factor influencing the tissue specification and functionality of cells is the epigenetic state of the cell DNA. **(A)** Cellular epigenetic state includes factors such as chromatin density and three dimensional structure, modifications to histones such as acetylation and methylation, DNA modifications including methylation, and the activity of non-coding DNA regions such as promoters and enhancers. While each form of epigenetic regulation plays an important role in controlling cell states, **(B)** recent work has demonstrated that DNA methylation states are highly determinative of cell and tissue specification and can be used as a high-resolution classifier of cell and tissue states. **(C)** Workflow for analysis of primary adipose tissue samples.

Large-scale genomics analysis identifies gene coding variants associated with obesity and metabolic disease



Genetic finding	Definition	Strength of Evidence
Coding mechanism	Genetic variant in coding region that is associated with phenotype	Highest
Burden Test	Cumulative loss of function variants mapped to a gene and association with phenotype	High
eQTL colocalization	Co-localization of genetic variant that alters gene expression and are associated with phenotype	High

Figure 4: Large scale genomics analysis of human datasets associates genetic variants with metabolic phenotypes. In collaboration with Genscience, 748 genetic association studies were analyzed for genetic evidence supportive of gene links with 57 metabolic phenotypes. 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.

Genomic analysis integration with epigenetic profiling of adipose tissue identifies novel targets for obesity/cardiometabolic disease therapies

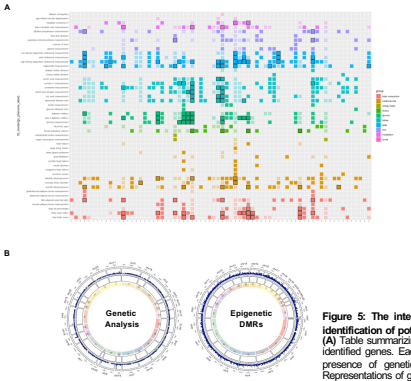


Figure 5: The integration of genomics analysis with epigenetic datasets enables the identification of potential novel targets for therapeutic intervention in metabolic diseases (A) Table summarizing the genetic evidence for associations with metabolic phenotypes for top identified genes. Each column represents an analyzed gene, with colored cells indicating the presence of genetic evidence linking the gene to the trait indicated on each row. **B)** 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 **(C)** A strategy for integrating genetic evidence with epigenetic datasets to provide support for candidate target genes for therapeutic interventions in the treatment of metabolic disease. In general, combining genetic evidence with tissue specific expression data, along with epigenetics information, allows for identifying genes with increased probability of functional roles in disease biology.

Comparison of adipocytes derived from lean and obese humans reveals epigenetic alterations associated with obesity

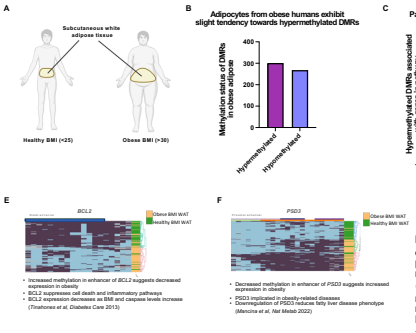


Figure 2: Epigenetic profiling of adipose tissue from healthy and obese BMI human donors. (A) Four samples each of white preadipocytes isolated from the subcutaneous fat of female donors with normal (<25) or obese (>30) BMI were differentiated into adipocytes *in vitro* and epigenetic state was analyzed by EpiRead. **(B)** Differentially methylated regions (DMRs) were identified and hyper- and hypo-methylation status in obese donor adipocytes was analyzed. **(C,D)** Pathway analysis of hyper- and hypo-methylated genes in obese donor adipocytes. **(E,F)** EpiMap of DNA methylation in the *BCL2* and *PSD3* loci demonstrates clustering of methylation state by BMI status

Comparison of adipocytes derived from human white and brown adipose depots reveals epigenetic alterations

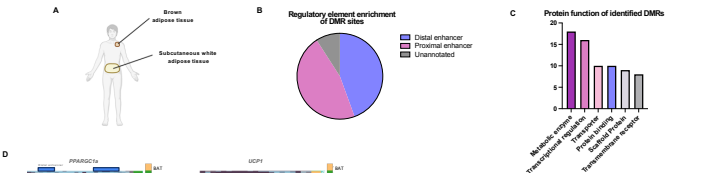


Figure 3: Epigenetic profiling of brown and white adipose(A) A primary human brown adipocyte cell line derived from a neck BAT deposit was analyzed by EpiRead and epigenetic state was compared with primary white adipocytes. **B)** Regulatory function of DMR locations was mapped for ENCODE designed enhancer regions. **(C)** Protein functional class of identified DMRs was assessed by PantherDB classification. **(D, E)** Representative DMRs in the *PPARGC1a* (encoding for PGC1α) enhancer and *UCP1* genomic regions are depicted.

Perturbation of adipose target gene induces catabolic metabolic program associated with improved metabolic health

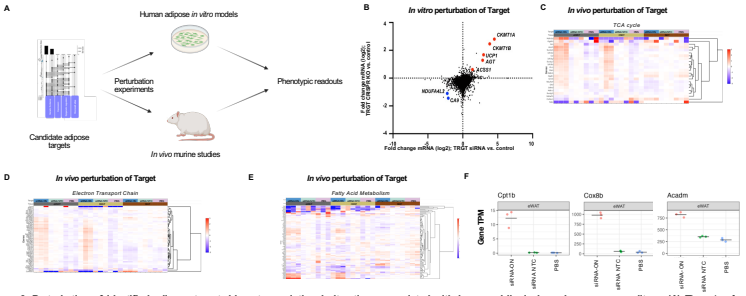


Figure 6: Perturbation of identified adipose target drives transcriptional alterations associated with increased lipolysis and energy expenditure. (A) The role of selected candidate target genes in adipose tissue was experimentally evaluated *in vitro* and *in vivo* by perturbing gene expression using siRNAs and CRISPR gene knockout. **(B)** *In vitro* inhibition of target gene expression in primary human HACAP adipocytes using orthogonal siRNA knockdown and CRISPR knockout resulted in increased expression of energy expenditure genes. **(C-E)** *In vivo* perturbation of target gene expression resulted in marked increases in adipose tissue expression of TCA cycle and electron transport chain genes, with selective increases in fatty acid metabolism genes as measured by RNAseq analysis. **(F)** RNAseq analysis of expression of *Cpt1b, Cox8b and Acadm* in eWAT.