

Epigenetic mapping of adipose tissue in obesity uncovers targets for therapeutic intervention in metabolic disease

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Abstract

Experimental and clinical evidence demonstrates that adipose tissue plays a key role in regulating systemic metabolic state; however, a detailed understanding of the mechanisms and role of epigenetic regulation of genes and biological pathways in adipose-related metabolic control remains elusive. We have generated a whole genome epigenetic atlas of primary human adipose tissue that identifies epigenetic alterations associated with obesity. Bioinformatics and machine learning analysis of our epigenetic atlas identified differentially methylated regions (DMRs) in WAT isolated from obese donors (BMI>30) compared to WAT derived from healthy individuals (BMI<25). Comparisons of WAT and BAT within individuals also revealed epigenetic features defining each cell type. We then integrated our adipocyte epigenetic datasets with large scale genomics datasets correlating functional genomic variants at loci across 57 defined metabolic phenotypes to identify potential therapeutic targets for obesity-related disease. A number of putative target genes are now being characterized in preclinical and translational models of obesity.

Taken together, we have established a fully integrated platform for the discovery, validation and therapeutic targeting of disease-relevant adipocyte genes in obesity, MASH and other cardiometabolic indications.

Epigenetic regulation of chromatin controls gene expression, defining cell and tissue state

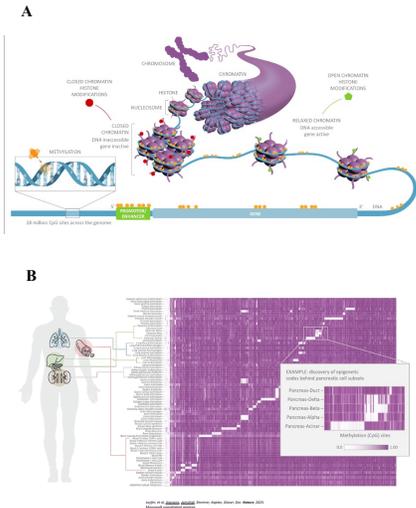


Figure 1: A key factor influencing the tissue specification and functionality of cells is the epigenetic state of the cell DNA. (A) Cellular epigenetic state includes factors such as chromatin density and three dimensional structure, modifications to histones such as acetylation and methylation, DNA 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.

Results

Epigenetic analysis reveals impact of obesity on white adipocyte epigenetic state

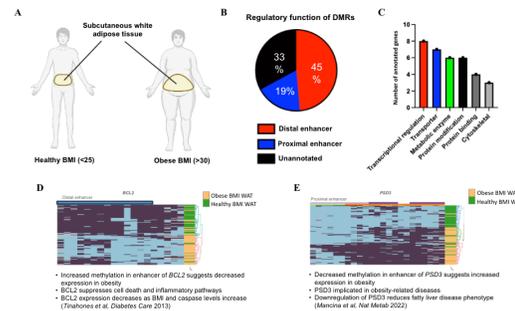


Figure 2: Assessing the epigenetic impact of obesity on white adipocytes. (A) Four samples each of white preadipocytes isolated from the subcutaneous fat of female donors with normal (<25) or obese (>30) BMI were differentiated into adipocytes *in vitro* and epigenetic state was analyzed by EpiRead. (B) Differentially methylated regions (DMRs) were identified and the regulatory function of DMR genomic coordinates was analyzed for ENCODE designed enhancer regions. Regulatory functions of DMRs are charted. (C) Protein functional class of identified DMRs was assessed by PantherDB classification. Representative DMRs in the (D) *BCL2* and (E) *PSD3* enhancer regions are depicted.

Integrating genomics analysis with epigenetic profiling identifies putative targets for therapeutic intervention in metabolic disease

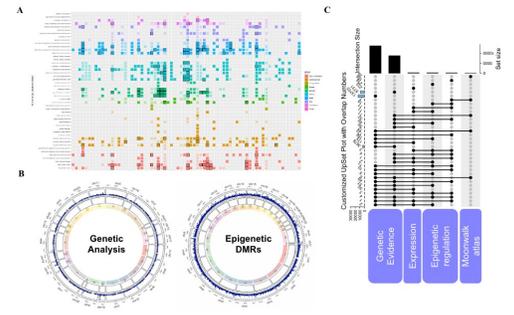


Figure 5: The integration of genomics analysis with epigenetic datasets enables the identification of potential novel targets for therapeutic intervention in metabolic diseases (A) Table summarizing the genetic evidence for associations with metabolic phenotypes for top identified genes. Each column 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 diseases. In general, combining genetic evidence with tissue specific expression data, along with epigenetic information, allows for identifying genes with increased probability of function roles in disease biology.

Uncovering the epigenetic biology of human brown adipocytes

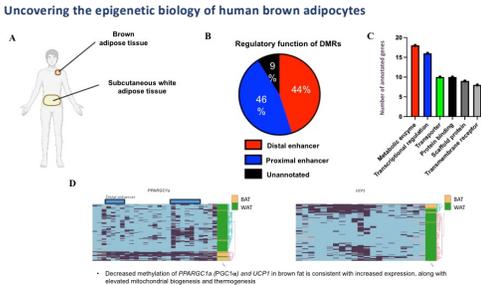


Figure 3: Assessing the epigenetic features of brown adipocytes (A) A primary human brown adipocyte cell line derived from a neck BAT deposit was analyzed by EpiRead and epigenetic state was compared with primary white adipocytes. (B) DMRs were identified and the regulatory function of DMR genomic coordinates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

Genomics datasets allow for identification of putative coding variants associated with metabolic phenotypes

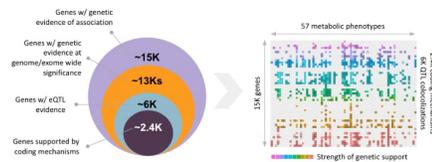


Figure 4: Large scale genomics analysis of human datasets associates genetic variants with metabolic phenotypes. In collaboration with Genescreen, 748 genetic association studies were analyzed for genetic evidence supportive of gene links with 57 metabolic phenotypes. Genome wide analysis uncovered approximately 15,000 genes with evidence of association with metabolic attributes. Of these, approximately 13,000 genes had significant association at the 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.

Genetic perturbation of identified adipocyte candidate gene target improves metabolic phenotype

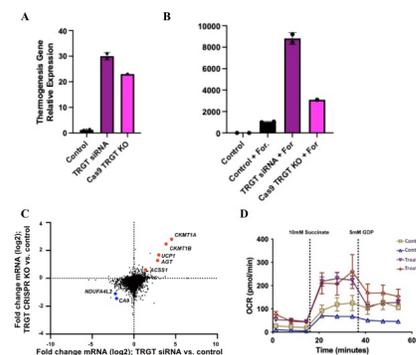


Figure 6: Perturbation of identified adipocyte target drives increased thermogenic gene expression. (A) Primary human adipose capillary cells were treated with siRNA targeting a candidate target gene or underwent CRISPR/Cas9 KO of gene expression. Cells were differentiated into adipocytes and expression of genes associated with thermogenesis were measured by qPCR in basal state or (B) after induction of browning metabolic program through treatment with forskolin. (C) mRNA sequencing was performed on siRNA and CRISPR KO cells compared with controls and differentially expressed genes were plotted. (D) *In vivo* delivery of mRNA encoding a thermogenic regulatory protein to hepatocytes was performed via lipid nanoparticle (LNP) and viable mitochondria were isolated and profiled for uncoupled mitochondrial respiration by Seahorse Extracellular Flux analysis.

Summary

- Moonwalk Biosciences is generating an epigenetic atlas of primary human adipose tissue subsets
- Epigenetic analysis of human adipose tissue uncovers epigenetic regulation associated with cell type specification and systemic metabolic health
- Integration of epigenetic and genomic analysis allows for identification and prioritization of potential novel targets for therapeutic intervention in patients with metabolic diseases
- Perturbation and functional assessment of an identified target demonstrated an improved metabolic phenotype in human adipose tissue models
- Moonwalk Bio is currently pursuing several novel targets for adiposity and obesity-related disease

Financial Disclosures

- R.J.K., A.C., S.B., S.G., P.M., N.-C.W., J.T.L., A.J., J.V. and A.A. are employed by and hold equity in Moonwalk Biosciences