Master's thesis in Bioinformatics

CHARACTERIZING HUMAN BROWN ADIPOCYTES IN WHITE ADIPOSE TISSUE WITH SINGLE-CELL TRANSCRIPTOMICS

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Abbreviations

- **APC**: Adipocyte Progenitor Cells.
- **ATP**: Adenosine Triphosphate.
- **ASW**: average silhouette width.
- **AUC**: area under the recovery curve.
- **BMI**: Body Mass Index.
- **BAT**: brown adipose tissue.
- **BMP**: bone morphogenetic proteins.
- **cAMP**: cyclic Adenosine Monophosphate.
- **CCA**: canonical correlation analysis.
- **C/EBP**: CCAAT-enhancer binding protein.
- **cLISI**: cell-type Local Inverse Simpson's Index.
- **EC**: endothelial cells.
- **FA**: fatty acids.
- **FAP**: fibroblasts and adipocyte progenitors.
- **FDG-PET**: [18F]-fluodeoxyglucose positron emission tomography.
- **FDR**: False Discovery Rate.
- **FFA**: free fatty acids.
- **GTP**: Guanosine Triphosphate.
- **iLISI**: integration Local Inverse Simpson's Index.
- **LEC**: lymphatic endothelial cells.
- **MSL**: mesothelial cells.
- **NE**: norepinephrine.
- **NST**: non-shivering thermogenesis.
- **PC**: principal component.
- **PCA**: Principal Component Analysis.
- **PET**: Positron Emission Tomography.
- **PPARA**: Peroxisome Proliferator-Activated Receptor Alpha.
- **PPARG**: Peroxisome Proliferator-activated Receptor gamma.
- **PPARGC1A**: PPARG coactivator 1 alpha.
- **PRDM16**: PR Domain Containing 16.
- **PPGL**: pheochromocytoma and paraganglioma.
- **rPCA**: reciprocal Principal Component Analysis.
- **SCENIC**: Single-Cell rEgulatory Network Inference and Clustering.
- **scIB**: single-cell Integration Benchmarking.
- **scRNA-seq**: single cell RNA sequencing.
- **scVI**: single cell Variational Inference.
- **snRNA-seq**: single nuclei RNA sequencing.
- **SNP**: Single Nucleotide Polimorfism.
- **SVF**: stromal vascular fraction.
- **TF**: transcription factor

- **UCP1**: Uncoupling Protein 1.
- **UMAP**: uniform manifold approximation and projection.
- **UMI**: Unique Molecular Identifier.
- **Vireo**: Variational Inference for Reconstructing Ensemble Origins.
- **VEC**: vascular endothelial cells.
- **VEGF**: Vascular Endothelial Growth Factor.
- **WAT**: white adipose tissue.
- **WHO**: World Health Organization.

Declaration of GAI tools

I used generative artificial intelligence (GAI) to complete this project, specifically OpenAI GPT-5.3-mini, Perplexity AI (2026) and Anthropic Claude Opus 4.8.

1. Perplexity AI and OpenAI GPT-5.3-mini were used for feedback on own text and alternative ways of formulating text: rephrasing sentences and restructuring paragraphs.
2. Anthropic Claude Opus 4.8 was used for programming tasks.
3. Perplexity AI was additionally used to support me in my reading process by summarizing papers on key statements.

There has been no other usage of GAI for this project besides than for the purposes stated above.

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ABSTRACT

Obesity is a chronic multifactorial disease associated with numerous comorbidities, including cardiovascular disease, type 2 diabetes, and several forms of cancer. Despite the availability of lifestyle interventions, pharmacological treatments, and bariatric surgery, long-term weight management remains challenging. Brown adipose tissue (BAT) has emerged as a promising therapeutic target due to its ability to increase energy expenditure through non-shivering thermogenesis mediated by uncoupling protein 1 (UCP1). Understanding the molecular mechanisms underlying brown adipocyte differentiation and activation may contribute to the development of new obesity treatments.

This study aimed to characterize brown adipocytes in human adipose tissue and identify molecular features associated with the differentiation of brown adipocytes. Single-nucleus RNA sequencing (snRNA-seq) was performed on adipose tissue samples collected from five patients diagnosed with catecholamine-secreting paraganglioma, a condition known to promote BAT recruitment through chronic adrenergic stimulation. Samples were obtained from three adipose tissue depots: subcutaneous, visceral (particularly, omental), and perirenal. Publicly available snRNA-seq datasets from non-paraganglioma donors were incorporated as controls. Following quality control, demultiplexing, doublet removal, and batch-effect correction, cell populations were identified through clustering and were annotated following different approaches. Downstream analyses included compositional analysis, differential gene expression, gene-set enrichment, trajectory inference, gene regulatory network reconstruction, and cell-cell communication analysis.

A comprehensive cellular atlas of human adipose tissue was generated, revealing the presence of multiple immune, vascular and adipocyte populations across depots. Within the adipocyte compartment, distinct adipocyte subpopulations were identified, including a population displaying transcriptional features consistent with brown adipocytes. These cells exhibited expression patterns associated with thermogenic activity and mitochondrial metabolism such as *UCP1*. Functional analyses suggested enrichment of marker genes related to glycolysis, oxidative phosphorylation, and fatty acid metabolism. Trajectory inference indicated the existence of transitional cellular states starting in control-specific adipocytes and ending in brown-like adipocytes. Regulatory network analysis identified candidate transcriptional regulators potentially involved in brown adipocyte identity, while cell-cell communication analyses highlighted signalling pathways that may contribute to adipocyte differentiation.

Overall, this study provides a detailed single-nucleus transcriptomic characterization of brown adipocytes in human adipose tissue. The findings contribute to the understanding of brown adipocyte biology and identify candidate molecular pathways involved in the differentiation of brown adipocytes. These results may support future efforts to promote thermogenic adipocyte recruitment as a therapeutic strategy for obesity, after experimental validation.