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ADLUNG LAB

Inference of metabolic properties and transcriptional programmes across altered adipocyte/immune cell ratios

Background

Over 1.9 billion adults world-wide were overweight in 2016 [1]. Finding ways to reduce obesity and constrain metabolic diseases is an ongoing challenge of today's society. Single-cell/nucleus mRNA-sequencing technologies have shed light on the pathophysiological processes in adipose tissue. However, mechanistic understanding, taking massive changes in cells' abundance during adipose tissue remodelling into consideration, remains yet elusive.

Objectives

The goals of this tudy were to study LAM-Adipocyte interactions and behaviour on a single-cell resolution in different states of obesity and to develop a new tool for alignment of single-cell samples in a biological meaningful order.

Methods

We obtained snRNA-seq Seurat objects of mouse adipocytes and immune cells [2,3], as well as human adipocytes and immune cells [2,4] from publically available datasets. Mouse datasets contained both low fat diet (LFD) and high fat diet (HFD) treated mice. We subclustered immune datasets based on annotation for lipid-associated macrophages and validated the LAM cluster with a previously described mouse LAM associated signature [5] using R v4.2.3 and Seurat v4.3.0. Gprofiler2 v0.2.1 was used, for pathway analysis. LAM/Adipo ratio was calculated both on a per animal and per sample level. It was calculated by dividing cell counts of LAM and cell counts of adipocytes. Cell-cell interactions were calculated with CellChat v1.6.1. A p-value of < 0.05 was considered as significant for our study. Code will be available upon publication. 24,082 cells were included.

Results

We identified a LAM cluster and two distinct adipocyte populations (Fig. 1 A). Cluster specific marker genes were determined (Fig. 1 B). In HFD, more inflamed than normal adipocytes were observed, the contrary was the case in LFD. Upregulated pathways confirmed this fibro-inflammatory switch. More inflamed adipoytes compared to normal were found, when comparing tissue depots, in inguinal adipose tissue (ING). The opposite was true for perigonadal adipose tissue (PG). With the transition to HFD the number of LAM cells increased. The LAM HFD signature showed an upregulation of phagocytosis and oxidative phosphorylation. The LFD signature of LAM was proliferative and pro migration. These results were confirmed with an cell-cell interaction analysis. The correlation between mouse weight in gram and the LAM/Adipo ratio was calculated both per animal and per sample. The animal weight strongly correlated with the LAM/Adipo ratio (Fig. 2). On a per sample basis two animals disrupted the linear trendline. These were the animals with the highest weight. They have diminished counts of LAM in ING while still maintaining a high amount of LAM in PG. While LAM in severe adiposity were still conducting phagocytosis in PG, they followed apoptotic transcriptional programs in ING. We validated our findings in other mice, as well as human datasets.

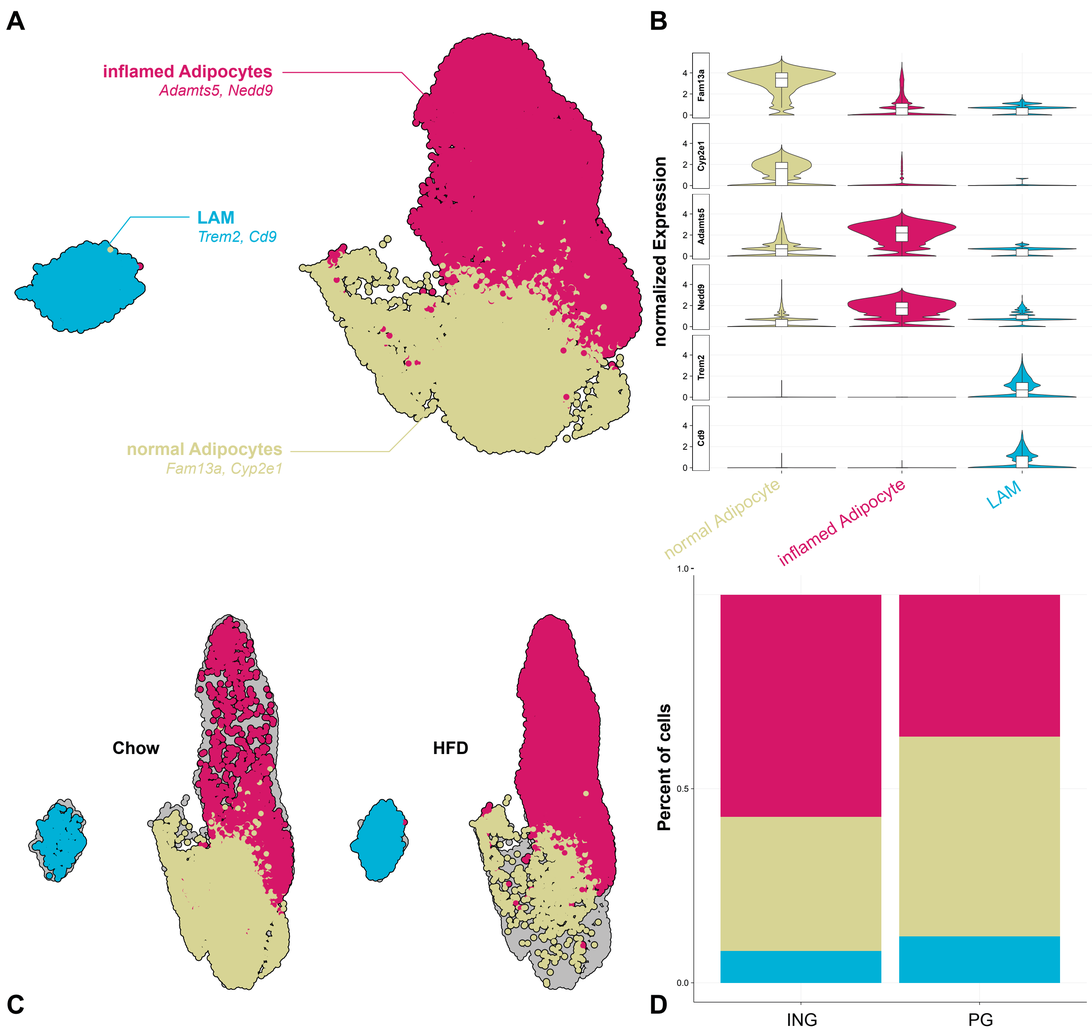

Fig. 1: (A) UMAP of 24,082 cells shows three separate clusters, one LAM cluster (light blue) and two adipocyte clusters (red and beige), (B) Marker genes for normal adipocytes (Fam13a, Cyp2e1), inflamed adipocytes (Adamts5, Nedd9) and LAM (Trem2, Cd9) show unique expression patterns in their clusters, (C) UMAP split by LFD vs. HFD shows significant transition of normal adipocytes into fibro-inflammatory adipocytes during weight gain, (D) Ratios of clusters split by depot show a higher amount of in- flamed adipocytes in perigonadal adipose tissue depot than in inguinal adipose tissue, indicating a time delay of inflammation between subcutaneous and visceral adipose tissue depots.

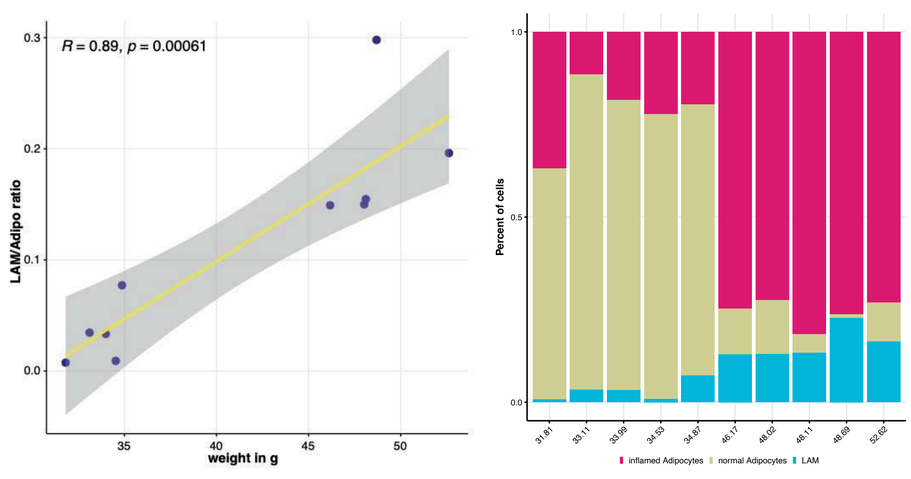

Fig. 2: Correlation of weight and LAM/Adipo ratio of mice (left). Distribution of cell subpopulations in different animals, ordered by LAM/Adipo ratio (right).

Discussion

Our study contributes to a quantitative understanding of adipocytes, LAM macrophages, and their interactions in different severity levels of obesity. It validates the phenotypic switch of adipocytes into fibro-inflammatory adipocytes during HFD. We provide further confirmation for LAM to be an important player in retaining inflammation. With the LAM/Adipo ratio, we suggest a novel measurement for aligning single-cell samples in a biologically meaningful order, that can replace weight or BMI to a certain extent, when applied to a cohort with low biological and non-biological batch effects. Certainly, a larger and well annotated scRNA-seq dataset is needed to further validate our findings. The condition of extreme obesity should be studied in depth to replace the linear with a higher-order model. Further research and in-vitro validation is needed to elaborate on the topic of adipocyte-LAM cell interactions. This includes validation on the protein level by flow cytometry or mass spectrometry.

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