Elucidating the role of hyaluronic acid in adipocyte progenitor cell and macrophage behavior

Poster by: **Oscar Wang**, CAS 2026

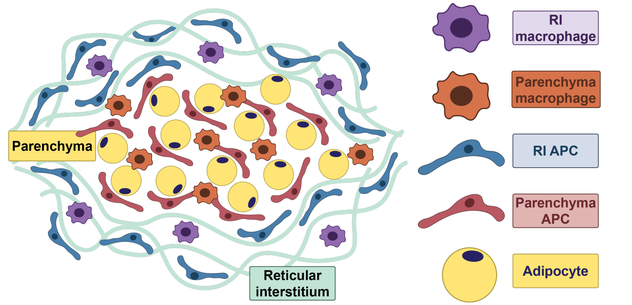
PI Mentor: **David Merrick, M.D. Ph.D.,** Perelman School of Medicine, Department of Medicine

Introduction

The **obesity epidemic** is a truly devastating crisis, given its ubiquity and the severity of its consequences for health and wellbeing. The prevalence of obesity in the U.S. and globally has approximately *tripled* since 1975, currently afflicting over 40% of the population.

The main culprit in this crisis is *dysfunctional* adipose tissue. **Adipose tissue** - commonly known as body fat - is an aggregation of heterogeneous cells including adipocytes, **adipocyte progenitor cells (APCs)**, **macrophages,** and endothelial cells.

Previous work has described several subpopulations of APCs with hierarchical adipogenic capacity and distinct anatomical niches within the adipose tissue. One type of APC resides predominantly in the **reticular interstitium (RI)** — a distinctive connective tissue niche surrounding the adipose tissue — and another type of APC resides primarily in the parenchyma of the tissue. Preliminary data from the lab show that a particular macrophage subpopulation is also concentrated in the RI.



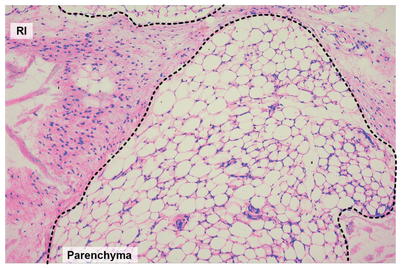
Our single cell RNA-sequencing (scRNA-seq) revealed differential **extracellular matrix (ECM)**-related gene expression between subpopulations of APCs and macrophages and in obese versus lean mice. Prior studies have shown increased ECM deposition and altered ECM composition in obesity. In addition, our prior data suggests structural changes to the ECM that heighten mechanical sensitivity in obesity. Together, this underlines the importance of studying the role of the ECM in adipose tissue dysfunction and points to **differential roles of APCs and macrophages in ECM maintenance that becomes maladaptive in obesity.**

Hyaluronic acid (HA) is a major structural component of the ECM that has important roles in cell communication and immune cell localization. HA plays important roles in several diseases, including cancer, and has been **implicated in obesity and type 2 diabetes**. The functions of HA depend on several factors including to which receptors it binds and its molecular weight. For example, **high molecular weight (HMW)** HA is associated with anti-inflammatory effects while **low molecular weight (LMW)** HA is associated with a pro-inflammatory effect.

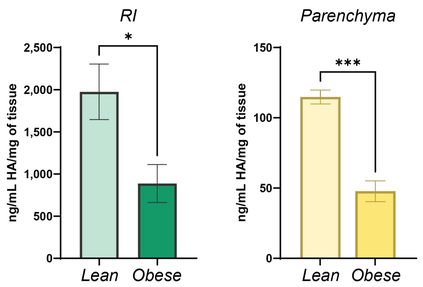
HA is concentrated in the RI and altered in obesity

Figure 1

a) Histology



b) HA ELISA



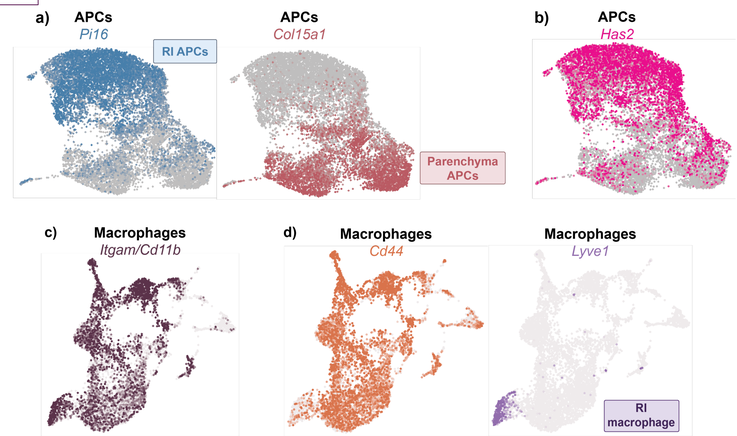
a) Histology of adipose tissue surrounded by RI. Pink is HA stained by Alcian Blue and purple are nuclei. HA is concentrated in the RI and present largely around adipocytes

b) ELISAs of HA content in the RI or parenchyma from lean or obese C57BL/6 mice. HA is approximately 15 to 17– fold higher in the RI of mice. In both the RI and parenchyma, HA content is decreased. This ELISA measures general HA content with potential increased response to high molecular weight HA.

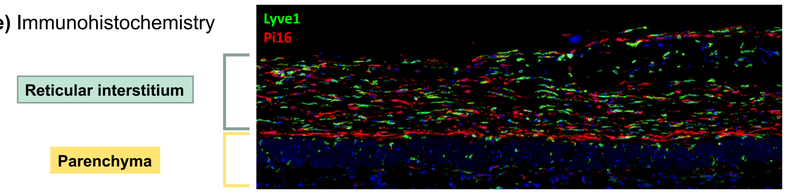
HA is a potential mediator of APC-macrophage crosstalk in adipose tissue

Figure 2

a-d) Single cell RNA-sequencing of adipose tissue



e) Immunohistochemistry



a) scRNA-seq expression of Pi16 (left) and Col15a1 (right). There are two major subtypes of APCs marked by expression of *Pi16* or *Col15a1*. *Pi16*+ APCs are found primarily in the RI while *Col15a1*+ APCs are found primarily in the parenchyma. *Pi16*+ APCs give rise to *Col15a1*+ APCs and *Col15a1*+ APCs give rise to adipocytes, outlining an adipogenic hierarchy along with anatomical niche.

b) scRNA-seq expression of Has2, an HA synthesizing enzyme. *Has2* is expressed by both RI and parenchyma APCs, with increased expression by RI APCs.

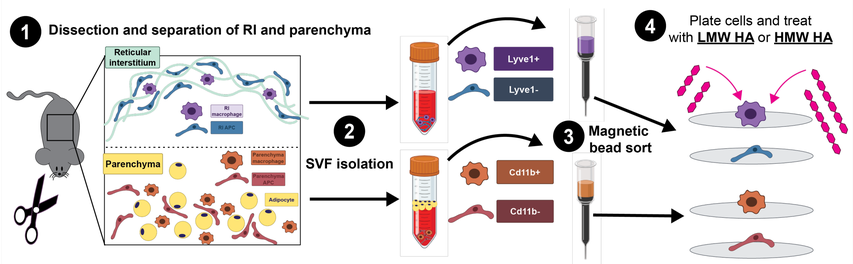
c) scRNA-seq expression of Itgam/Cd11b, a pan-macrophage marker

d) scRNA-seq expression of Cd44 (left) and Lyve1 (right). *Cd44* and *Lyve1* are receptors for HA. *Cd44* is expressed by most macrophages while *Lyve1* is expressed by a specific subtype of macrophages.

e) Immunohistochemistry of an adipose tissue section*. Lyve1+ macrophages are colored green and Pi16+ APCs are colored red, nuclei are blue. Lyve1+* macrophages are concentrated in the RI alongside *Pi16*+ APCs. This suggests crosstalk between RI macrophages and APCs, with HA as a potential mediator.

Does LMW HA and HMW HA differentially influence APC and macrophage gene expression profile?

Methods

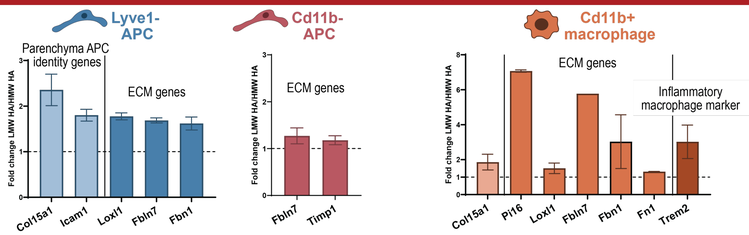


To further elucidate how the microenvironment - specifically LMW and HMW HA - around APCs and macrophages change their genetic expression profile C57BL/6 mice were dissected, and RI and parenchyma tissue was separated for downstream cell isolation and sorting. **The stromal vascular fraction (SVF)** was isolated, and cells from the RI were stained with an ***Lyve1+*** antibody and cells from the parenchyma were stained with a ***Cd11b+*** antibody. Magnetic beads were used to capture the positive cells, negative flowthrough cells were collected as well. Cells were plated (*Lyve1+, Lyve1-, Cd11b+, Cd11b-*) on collagen coated plates or cell bind plates. Cells were treated for **4 days** with LMW or HMW HA in the culture media. Cells were collected for RNA extraction and qPCR.

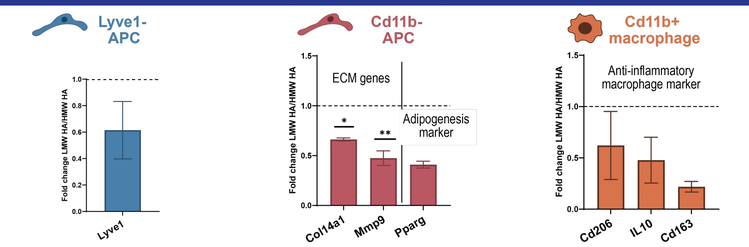
LMW HA induces differential gene expression in APC and macrophage subtypes relative to HMW HA

qPCR of sorted APCs and macrophages

Genes upregulated by LMW HA
(relative to HMW HA)



Genes downregulated by LMW HA
(relative to HMW HA)



qPCR of sorted APCs and macrophages after 4 days of treatment with either LMW or HMW HA. Gene expression was normalized to the housekeeping gene, TBP. Fold change of LMW HA over HMW HA was calculated by dividing gene expression of samples treated with LMW HA by average gene expression of HMW HA-treated samples. A value >1 means LMW HA upregulated gene expression relative to HMW HA treatment and vice versa. Lyve1- n =2, Cd11b- n=4, Cd11b+ n= 2. There were not enough Lyve1+ macrophages to treat and perform qPCR.

Conclusions and Future Directions

- LMW HA treatment on Lyve1- APC led to an increase in expression of ECM-related genes, which is a genetic marker for obesity, and a decrease in mmp9 and Pparg expression
- LMW HA treatment on Cd11b+ led to the increase in expression of pro-inflammatory macrophage marker and decrease in expression of anti-inflammatory macrophage markers
- LMW HA can contribute to the development of obesity through inducing pro-inflammatory macrophage polarization, increasing ECM-related genes and collagen deposition, and decreasing adipogenesis
- Use fluorescence activated cell sorting (FACS) to separate Lyve1+ and Lyve1- cells
- Incorporate monocyte into our experiment and figure out how to induce monocyte into intended polarization state
- Adjust HA treatment time scale and experiment with longer HA treatment

Sources

Lee, C.-H et al, *Int. J. Mol. Sci.* **(2021)**
Lim, H. Y. et al, *Immunity* **(2018)**
Alemzadeh, E et al, *Journal of biomedical materials research part B: applied biomaterials*, *108*(2), 555-567.
Caon I, Parnigoni A, Viola M, Karousou E, Passi A & Vigetti D. Cell Energy Metabolism and Hyaluronan Synthesis. Journal of Histochemistry and Cytochemistry 69, 35-47 (2021). https://doi.org:10.1369/0022155420929772
Rivas F, DeAngelis PL, Rahbar E & Hall AR. Optimizing the sensitivity and resolution of hyaluronan analysis with solid-state nanopores. Scientific Reports 12, 4469 (2022). https://doi.org:10.1038/s41598-022-08533-1
Park, M. J., Kim, M. S., Park, I. C., Kang, H. S., Yoo, H., Park, S. H., ... & Lee, S. H. (2002). PTEN suppresses hyaluronic acid-induced matrix metalloproteinase-9 expression in U87MG glioblastoma cells through focal adhesion kinase dephosphorylation. *Cancer research*, *62*(21), 6318-6322.
Zhu Y, Li N, Huang M, Bartels M, Dogné S, Zhao S, Chen X, Crewe C, Straub L, Vishvanath L, Zhang Z, Shao M, Yang Y, Gliniak CM, Gordillo R, Smith GI, Holland WL, Gupta RK, Dong B, Caron N, Xu Y, Akgul Y, Klein S & Scherer PE. Adipose tissue hyaluronan production improves systemic glucose homeostasis and primes adipocytes for CL 316,243-stimulated lipolysis. Nature Communications 12, 4829 (2021). https://doi.org:10.1038/s41467-021-25025-4
Tara E. Sutherland et al. ,The extracellular matrix and the immune system: A mutually dependent relationship.

Research project funded by PURM