Hypoxic-SUGEN Rat Model of Pulmonary Hypertension Induces Proteome Changes to Visceral Adipose

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Introduction

- PAH demographics are shifting to an older, obese population garnering increased interest in the role of metabolic and endocrine disorders in PAH [1].
- Adipose is now considered an endocrine organ [2].
- Visceral adipose can be both anti-inflammatory and is more strongly related with cardiovascular disease (CVD) than is subcutaneous adipose [3,4].
- Localized, excessive perivascular adipose tissue (PVAT) surrounding the vasculature has been associated with vascular function and incident CVD [5-7].
- Identification of significantly up-down-regulated proteins in small visceral adipose depots, particularly PVAT, will aid in determining the mechanistic role of adipose tissue in the development of PAH and provide novel therapeutic targets.

Aim

- We investigated differential proteome profiles of abdominal and aortic perivascular adipose tissue (ePVAT) in a hypoxic-SUGEN PAH animal model [8].

Methods

Animals
- Adult male Sprague-Dawley rats
- SUGEN-Hypoxic (Sug/Hyp) exposure (n=12) [8]
  - Subcutaneous injection of SUG5416 (VEGF-inhibitor, 20mg/kg)
  - Hypoxic exposure (10% O2) for 3 weeks and returned to normoxic conditions (21% O2) for 12 weeks
- Control (CTL, n=12)
  - Maintained under normoxic conditions for the same duration
  - RV free wall thickness: Sug/Hyp > CTL, p<0.01
(Vevo 770 – VisualSonics, Toronto, Canada)

Visceral Adipose Tissue
- Abdominal adipose tissue, surrounding the abdominal organs
  - Removed and snap frozen
  - Sug/Hyp (n=4) and CTL (n=4)
- Aortic perivascular adipose tissue (ePVAT), descending thoracic aorta
  - Removed and snap frozen
  - Sug/Hyp (n=12) and CTL (n=12)

Proteomics
- Tissue preparation
  - Homogenized with mortar/pestle on dry ice
  - Tissue placed into an adipose-specific buffer (7 M Urea, 2 M Thiouria and 4% ASB-14)
- Difference Gel Electrophoresis (DGE)
  - Gradient gels prepared
  - Adipose tissue specific buffer (7 M Urea, 2 M Thiouria and 4% ASB-14)
  - 1st separation by isoelectric point (ETTAN IPGPhor)
  - 2nd separation by molecular weight (ETTAN DALT)
  - Multiple gels analyzed to incorporate eye-sawapping and internal standards
  - Gels scanned with Typhoon Trio
  - Gel images processed with DeCyder Software (GE Healthcare, Piscataway, NJ)
- Statistical threshold 2.0

Results

![Figure 1: A) Representative differential proteome of abdominal visceral adipose tissue. CTL (n=4) and Sug/Hyp (n=4). B) Heat map of CTL vs Sug/Hyp, Threshold 2.0. Total number of spots: 2718. Matched spots: 883. Significantly up-regulated: 11.5% (Red = Up-regulated, Green = Down-regulated, Black = No significant change).](image1)

![Figure 2: A) Representative differential proteome of aortic perivascular adipose tissue (ePVAT). CTL (n=12) and Sug/Hyp (n=12). B) Heat map of CTL vs Sug/Hyp, Threshold 2.0. Total number of spots: 1553. Matched spots: 998. Significantly up-regulated: 6.01% (Red = Up-regulated, Green = Down-regulated, Black = No significant change).](image2)

Discussion

Proteomics is an invaluable technique for providing a differential comparison of protein expression in biological tissue. In humans, differences in protein expression have been detected between various adipose depots (subcutaneous versus abdominal, visceral [9] and epicardial [10]), and visceral adipose between obesity-prone and obesity-resistant C57 mice [11].

A new PAH population is developing consisting of older and obese patients. It is well known that adipose tissue contributes to systemic inflammation and perivascular adipose tissue has a localized effect in CVD. The obesity epidemic and, in particular, increased volumes of adipose in close proximity to the vasculature may contribute to vascular remodeling and dysfunction in PAH.

Differential proteome analyses of various small vascular depots in close proximity to the heart and vasculature may provide insight into the mechanistic role of adipose in the development of PAH.

Summary

- First differential proteome analysis performed on the SUGEN-Hypoxic rat model of PAH [8].
- We were able to detect significant up- and down-regulated proteins between the CTL and Sug/Hyp groups in both visceral abdominal adipose and ePVAT.
- Our next steps include
  - A differential proteome analysis of the pericardial adipose
  - Identifying the significantly different proteins using a spot-picking technique
- Increased volumes of small visceral adipose depots, in particular the perivascular adipose surrounding the vasculature, may have a negative, local effect on vascular mechanics and remodeling.
- The localized effect due to perivascular adipose may influence vascular wall remodeling ranging from the pulmonary artery to the lung arterioles.

References


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