Brief Communication

MOLECULAR METABOLISM

Distinct adipocyte progenitor cells are associated with regional phenotypes of perivascular aortic fat in mice

Khanh-Van Tran [1,2,*,3], Timothy Fitzgibbons [1,2,3], So Yun Min [1], Tiffany DeSouza [1], Silvia Corvera [1]

ABSTRACT

Objective: Perivascular adipose tissue depots around the aorta are regionally distinct and have specific functional properties. Thoracic aorta perivascular adipose tissue (tPVAT) expresses higher levels of thermogenic genes and lower levels of inflammatory genes than abdominal aorta perivascular adipose tissue (aPVAT). It is not known whether this distinction is due to the *in-vivo* functional environment or to cell-autonomous traits that persist outside the *in-vivo* setting. In this study, we asked whether the progenitor cells in tPVAT and aPVAT have cell-autonomous traits that lead to formation of regionally distinct PVAT.

Methods: We performed microarray analysis of thoracic and abdominal peri-aortic adipose tissues of C57Bl/6J mice to define gene expression profile of each depot. To derive adipocyte progenitor cells, C57Bl/6J mice were sacrificed and thoracic and abdominal aorta fragments were embedded in Matrigel and cultured under pro-angiogenic conditions. Adipogenesis was induced using the *Ppar-γ* agonist rosiglitazone, a thiazolidinedione (TZD). TZD-induced adipocyte populations were analyzed using immunofluorescence and qRT-PCR.

Results: Microarray analysis showed that tPVAT expressed higher levels of transcription factors related brown adipose tissue development compared to aPVAT. Classic brown adipose tissue (BAT) genes such as *Ucp-1*, *Prdm16*, *Dio2*, *Slc27a* displayed a concordant trend of higher level expression in tPVAT, while white adipose tissue (WAT) genes such as *Hoxc8*, *Nnat*, *Sncg*, and *Mest* were expressed at a higher level in aPVAT. The adipokines resistin and retinol binding protein 4 were also higher in aPVAT. Furthermore, adipocyte progenitors from abdominal and thoracic aortic rings responded to TZD with expression of canonical adipocyte genes *Acrp30*, *Plin1*, and *Glut4*. Adipocytes differentiated from thoracic aorta progenitors displayed markedly higher induction of *Ucp-1* and *Cidea*.

Conclusions: Thoracic aorta PVAT expresses higher levels of brown adipocyte transcription factors than aPVAT. Precursor cells from the thoracic aorta give rise to adipocytes that express significantly higher levels of *Ucp-1* and *Cidea ex vivo*, suggesting that progenitor cells in tPVAT and aPVAT have cell-autonomous properties that dictate adipocyte phenotype.

Keywords Perivascular adipose tissue; Adipocyte precursors; Progenitors; Ucp-1; Adipogenesis; Aorta

1. INTRODUCTION

Perivascular adipose tissue (PVAT) is a newly recognized adipose depot with highly active endocrine and paracrine functions. PVAT secretes a multitude of adipokines and other factors that have important physiological and pathophysiological implications for the adjacent blood vessels [1–6]. Recent studies show that murine thoracic PVAT produces numerous factors, including adiponectin, leptin, H_2S, and other unidentified factors, collectively referred to as adipocyte-derived relaxing factors, that influence vascular function [7–13]. These secreted factors can facilitate a vasodilatory effect through an endothelium-dependent mechanism via nitric oxide (NO) synthesis and release or an endothelium-independent mechanism via the generation of hydrogen peroxide [10,14]. On the other hand, in obese humans or mice, PVAT has been shown to release factors to promote inflammation, vasoconstriction, or vascular smooth muscle cell proliferation that are detrimental to vascular function [15,16]. Therefore, the physiological importance of PVAT in vascular homeostasis is undisputed and is an area of intense investigation.

Among the most studied murine PVAT is adipose tissue surrounding the aorta. Along the aorta, the phenotype of the PVAT is unique depending on anatomical location [17]. The levels of inflammatory genes and markers of immune cell infiltration are greater in aPVAT than in tPVAT [18]. The aPVAT phenotype is more pro-inflammatory

[1]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, 01655, USA [2]Department of Medicine, University of Massachusetts Medical School, Worcester, MA, 01655, USA

[3] Khanh-Van Tran and Timothy Fitzgibbons contributed equally to this work.

*Corresponding author. University of Massachusetts Medical School, 368 Plantation Street, AS7-1018, Worcester, MA, 01605, USA.

E-mails: Khanh-van.tran@umassmemorial.org (K.-V. Tran), Timothy.Fitzgibbons@umassmemorial.org (T. Fitzgibbons), ssso1019@hanmail.net (S.Y. Min), Tiffany.DeSouza@umassmed.edu (T. DeSouza), Silvia.Corvera@umassmed.edu (S. Corvera).

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and atherogenic than the tPVAT phenotype and may explain why aneurysms more frequently occur in the abdominal aorta [17–19].

PVAT surrounding the murine thoracic aorta (tPVAT) exhibits phenotypic features of brown adipose tissue (BAT), and that surrounding the abdominal aorta PVAT (aPVAT) is more similar to white adipose tissue (WAT) [17–19]. White adipose tissue (WAT) classically functions to store excess energy in the form of triglyceride. In contrast, brown adipose tissue (BAT) oxidizes fatty acids to generate heat. "Beige" adipocyte exists in WAT and can be induced by cyclic adenosine monophosphate (cAMP) to express thermogenic genes to resemble BAT [20]. Although it is clear that there are inherent phenotypic differences between tPVAT and aPVAT, it is unclear whether these differences are the result of extrinsic anatomical factors or intrinsic cell autonomic properties. For example, higher beta-adrenergic signaling could induce expression of brown adipocyte genes even if precursors cells are inherently the same existing in different environments [21]. In contrast, the cells could be intrinsically different due to transcriptional programing and would develop into distinct adipocyte in an environment-independent manner.

In this study, we sought to answer this question by analyzing transcriptomic data from peri-aortic adipose tissue and studying progenitor cells that give rise to new adipocytes from different regions of mouse aorta. For this purpose, we used the traditional *ex vivo* aortic ring assay to study progenitor cells arising from thoracic and abdominal aortic regions. Originally, Nicosia and Ottinetti designed the *ex vivo* aortic ring assay to study angiogenesis [22]. Cells arising from aortic rings form tubular structures and lumenize. Since its first introduction, this method has been widely used to study angiogenesis. Adipocyte progenitor cells are known to reside in the vasculature of the adipose tissue [23–26]. The vasculature, such as the aorta, harbors PVAT progenitor cells. As such, we used the peroxisome proliferator-activated receptor gamma (*Ppar-γ*) agonist Rosiglitazone, a thiazolidinedione (TZD), to stimulate adipogenesis in precursor cells emerging from aortic explants from thoracic and abdominal aortic rings. We observed that cells emerging from the aortic ring take on adipocyte morphology, express adipocyte markers, and secrete adiponectin. Furthermore, we found that TZD-induced tPVAT expresses higher levels of hallmark brown adipocyte genes, uncoupling protein 1 (*Ucp-1)* and cell death activator CIDE-A (*Cidea*), than aPVAT. Our microarray analysis showed that transcription factors relating to brown adipocyte development are elevated in tPVAT compared to aPVAT. Taken together, our results demonstrate that preadipocytes residing in tPVAT and aPVAT have cell-autonomous characteristics that dictate phenotypic development.

2. MATERIAL AND METHODS

2.1. Microarray analysis

All animal experiments in this work has been approved by the Institutional Animal Care and Use Committee of University of Massachusetts Medical School. Briefly, C57Bl/6J mice were sacrificed using CO2 and cervical dislocation. The heart and vasculature were flushed with PBS. Thoracic PVAT directly adjacent to the lesser curvature of the aortic arch and aPVAT from above the renal arteries to the diaphragm were harvested and snap frozen in liquid nitrogen. RNA was isolated from thoracic and abdominal PVAT as previously described [19]. RNA concentrations were determined using a Nanodrop 2000 Spectrophotometer (Thermo Fisher, Wilmington, DE). The RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Only samples with a RNA integrity number 7.5 and normal 18- and 28-s fractions on microfluidic electrophoresis were used. RNA from two mice per tissue and diet was pooled for a total of 250 ng total RNA template for cDNA synthesis and in vitro transcription using the Ambion WT expression kit (Ambion, Carlsbad, CA). Second-strand cDNA was then labeled with the Affymetrix WT terminal labeling kit, and samples were hybridized to Affymetrix Mouse Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA). Gene chip expression array analysis for individual genes was performed as previously described, filtering for $p < 0.05$. Three biological replicate hybridizations per tissue were performed, for a total of 6 hybridizations. We analyzed microarray data using Affymetrix Transcriptome Analysis Console (TAC) Software, which integrates *limma*, a core component of Bioconductor project using the statistical computing language R [27]. Pathway analysis of transcriptomic data was performed using Broad Institute Molecular Signature Database: Hallmark Gene Set and well as Qiagen's Ingenuity® Pathway Analysis, with genes with $p < 0.05$ and a fold change of 2.

2.2. Aortic ring assay

C57Bl/6J mice were sacrificed as above. After cutting side branches throughout, the aorta was excised from the root to the femoral bifurcation. PVAT was removed under a dissecting microscope leaving the tunica externa intact. The thoracic aorta was determined to be segments from the aortic arch to the diaphragm and the abdominal aorta was determined to be segments from below the diaphragm to femoral bifurcation. The aortic fragments were washed with EGM-2 media and cut into 1 mm ring segments. Each aortic ring segment was embedded in a singular well of a 96-well plate containing 40 μL of Matrigel (BD Discovery Labware). After aorta was embedded in the 96-well plate, the plate was allowed to stand for 30 min in the 37 °C so that the Matrigel would solidify. The cells were fed with 200 μL of EGM-2 media, which was changed every other day. The mouse aorta fragments were mechanically excised, and the endothelial sprouts remaining in the Matrigel were isolated using Dispase II (Roche, 2.4 U/mL), centrifuged, and RNA extracted from the pellet using an Ambion RNA extraction kit. Probes used are specified in Table 5 of Appendix.

2.3. Staining and analysis

Pieces of aortic rings were embedded in Growth Factor Reduced Matrigel (BD Biosciences) in 35 mm glass-bottom culture dishes (MatTek Corporation), and cultured in EGM-2 media for 14 days. After 14 days in culture with and without rosiglitazone, explants were fixed in 4% Formaldehyde (Ted Pella, Inc.) in PBS for 15 min and permeabilized in 0.5% TX-100 in PBS for 30 min. The primary antibodies used to characterize the origin of cells growing from the explants were mouse Cd31 (BD Pharmingen, clone MEC 13.3, 1:50), mouse Cd34 (BD Pharmingen, clone RAM34, 1:50), monoclonal mouse Adiponectin (Peirce, PA1-054, 1:200), and polyclonal Guinea Pig Perilipin (Fitzgerald Industries International, 20R-PP004, 1:200). Secondary antibodies were species matched Alexa Fluor 594, and Alexa Fluor 488 (Millipore; Molecular probes, 1:500). Negative controls treated with irrelevant mouse IgG instead of primary antibody were processed simultaneously. All sections were counterstained with DAPI (Millipore, Molecular Probes.)

2.4. Western blotting

The mouse aorta fragments were mechanically excised and the sprouts remaining in the Matrigel were isolated using Dispase II (Roche, 2.4 U/mL). Cell lysates were prepared using the Complete

