Brief Communication

MOLECULAR METABOLISM

Cartilage oligomeric matrix protein is differentially expressed in human subcutaneous adipose tissue and regulates adipogenesis

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ABSTRACT

Background: The composition of the extracellular matrix (ECM) impacts adipocyte function and might determine adipose tissue (AT) function and distribution. Cartilage oligomeric matrix protein (COMP), a matricellular protein usually studied in bone and cartilage, is highly differentially expressed between subcutaneous abdominal and gluteal AT.

Objective: This study aimed to explore COMP's role in human subcutaneous abdominal and gluteal AT and preadipocyte biology.

Methods: *COMP* mRNA levels were measured in whole AT and immortalised preadipocytes via quantitative (q)-PCR. Tissue and cellular COMP protein were measured via western blot and immunohistochemistry; plasma COMP was measured by ELISA. The effect of COMP on adipogenesis in immortalised preadipocytes was evaluated by qPCR of adipogenic markers and cellular triacylglycerol (TAG) accumulation.

Results: qPCR analysis of paired subcutaneous abdominal and gluteal AT biopsies ($n = 190$) across a range of BMI (20.7−45.5 kg/m^2) indicated ~3-fold higher *COMP* expression in gluteal AT ($P = 1.7 \times 10^{-31}$); protein levels mirrored this. Immunohistochemistry indicated COMP was abundant in gluteal AT ECM and co-localised with collagen-1. AT *COMP* mRNA levels and circulating COMP protein levels were positively associated with BMI/adiposity but unrelated to AT distribution. *COMP* expression changed dynamically during adipogenesis (time × depot, $P = 0.01$). Supplementation of adipogenic medium with exogenous COMP protein (500 ng/ml) increased *PPARG2* expression ~1.5-fold ($P = 0.0003$) and TAG accumulation ~1.25-fold in abdominal and gluteal preadipocytes ($P = 0.02$).

Conclusions: We confirmed that COMP is an ECM protein which is differentially expressed between subcutaneous abdominal and gluteal AT. Despite its depot-specific expression pattern, however, AT *COMP* mRNA levels and plasma COMP concentration correlated positively with overall obesity but not body fat distribution. Exogenous COMP enhanced adipogenesis. These data identify COMP as a novel regulator of AT and highlight the importance of the ECM to AT biology.

Keywords Adipose; Adipogenesis; Preadipocyte; Extracellular matrix

1. INTRODUCTION

Remodelling of the extracellular matrix (ECM) is crucial for adipogenesis [1,2]. Mounting data indicate that altered expression of ECM components such as collagen [3] and hyaluranon [4] can modify adipose tissue (AT) function and thereby influence overall metabolic health. Adipocytes within AT are embedded in a three-dimensional ECM scaffold whose spatial arrangement is co-ordinated by matricellular proteins; these proteins physically interact with ECM components but do not directly contribute to the ECM's structural integrity [5].

Matricellular proteins are secreted modular molecules implicated in numerous processes ranging from proliferation, migration, and differentiation to angiogenesis and wound healing [6]. The thrombospondins comprise five calcium-binding matricellular proteins that regulate AT biology.

Thrombospondin-1 (THBS1) is an adipokine implicated in the development of obesity and metabolic disease [7]. The *THBS1* gene is more highly expressed in visceral compared to subcutaneous abdominal AT [8] with mRNA levels in the latter reportedly correlating positively with BMI but correlating negatively with insulin sensitivity [9]. Further,

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Abbreviations: Abd, abdominal; And/Gyn, android/gynoid fat mass ratio; ANOVA, analysis of variance; AT, adipose tissue; BMI, body mass index; CD36, cluster of differentiation 36; COMP, cartilage oligomeric matrix protein; ECM, extracellular matrix; ERK1/2, extracellular signal-regulated kinase-1/2; Glut, gluteal; HRP, horseradish peroxidase; OBB, Oxford Biobank; PPIA, peptidylprolyl isomerase A; PSACH, pseudoachondroplasia; PVDF, polyvinylidene fluoride; qPCR, quantitative polymerase chain reaction; TAG, triacylglycerol; THBS, thrombospondin

Received July 3, 2018 • Revision received July 17, 2018 • Accepted July 23, 2018 • Available online xxx

https://doi.org/10.1016/j.molmet.2018.07.005

Brief Communication

circulating THBS1 protein levels were found to positively correlate with hypertension, hyperglycaemia, and central obesity in a Japanese cohort [10]. *Thbs1* knock-out mice are protected from the deleterious effects of high-fat diet feeding [11–13]. Thbs2 has also been reported to exert anti-adipogenic effects in mice [14] although this finding has not been replicated [15].

THBS5 (also called cartilage oligomeric matrix protein; COMP) exhibits a striking depot-specific expression pattern between subcutaneous abdominal and gluteal AT [16]. Given that most differentially-expressed genes between white AT depots tend to be development-related transcriptional regulators such as *HOX* genes [16–18], regional variation in the expression of ECM components may be important in the context of depot-specific AT biology.

COMP has primarily been studied in the context of bone as mutations in *THBS5* cause severe skeletal malformations, principally pseudoachondroplasia (PSACH) [19]. COMP coordinates collagen fibrillogenesis [20], but PSACH-causing mutations disrupt this process [21], typically by causing intracellular retention of mutant COMP protein which results in chondrocyte death [22]. It has been proposed that plasma COMP protein levels represent a biomarker of osteoarthritis progression [23]. With central obesity being associated with an increased risk of developing metabolic and cardiovascular disease as well as osteoarthritis [24], there is a growing appreciation of cross-talk between bone and AT via circulating factors in health and disease [25]. Based on its multi-functional properties and well-established role in cartilage/bone biology, COMP is an attractive candidate molecule to study in the context of AT. The aim of this study was to investigate *COMP* expression in human subcutaneous abdominal and gluteal AT and preadipocytes and relate this to AT distribution and function.

2. METHODS

2.1. Immunohistochemistry

AT biopsies collected via gun biopsy under local anaesthetic (1% lignocaine) were fixed in 10% formaldehyde, embedded in paraffin wax, and cut into 5 μm sections. Sections were dewaxed, rehydrated (ethanol), and antigen retrieval was performed (heating in 1 mM sodium citrate). Endogenous peroxidase activity was blocked (0.3% H_2O_2 in methanol), and auto-fluorescence was quenched (1.5% glycine). Sections were blocked in 1/20 swine serum (Dako) and incubated with goat anti-COMP (1/100; AF3134; R&D Systems) and rabbit anti-collagen 1 (1/300; ab34710; Abcam) overnight at 4 °C. Sections were washed and incubated with horseradish peroxidase (HRP)-conjugated donkey anti-goat antibody (1/50; sc2020; Santa Cruz Biotechnology) to label COMP staining; the signal was amplified via tyramide amplification (0.3% H_2O_2 with 5% tyramide 488). Sections were then blocked in 1/20 goat serum (Dako) and incubated in goat anti-rabbit 568 (1/250; A11036; Life Technologies) to co-label collagen 1 staining. Sections were mounted and visualised using a Radiance 2100 laser scanning system confocal microscope (Bio-Rad); images were captured using Laser sharp software (Bio-Rad).

2.2. AT sample collection

Paired AT samples were taken from 97 females and 93 men in the Oxford Biobank (OBB) [26] by needle biopsy from the periumbilical (subcutaneous abdominal AT) and upper buttock (gluteo-femoral AT) areas under local anaesthetic (1% lignocaine) and immediately stored in RNAlater (ThermoFisher Scientific). Donors had a median age of 45 years (range 33–53 years) and median body mass index (BMI) of 25.6 kg/m^2 (range 18.8–46.2 kg/m^2). The taking of human AT samples was approved by the Oxfordshire Clinical Research Ethics Committee; all participants gave written informed consent.

2.3. Preadipocyte culture and differentiation

Immortalised subcutaneous abdominal and gluteal preadipocyte cell lines were generated, maintained and differentiated as recently described [27]. COMP (ACRO Biosystems) reconstituted as previously described [28] was added to the growth medium or adipogenic cocktail where indicated.

2.4. Gene expression analysis

Total RNA was extracted from AT biopsies [29] and preadipocytes [30]. cDNA was synthesised from total RNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies). Quantitative (q)-PCR was performed on cDNA diluted 1/20 in triplicate with Kapa Probe Fast Mastermix (Kapa Biosystems) in an 8 μl reaction. The following TaqMan Assays-on-Demand (Applied Biosystems) were used: *COMP* (Hs00164359_m1); *PPARG2* (Hs01115510_m1); *FASN* (Hs00188012_m1); and *PPIA* (Hs99999904_m1). Data were captured on an ABI Prism 7900 HT. Relative transcript expression was calculated using the ΔΔCt relative quantification methods [31] where:

$$\Delta Ct = \text{Assay efficiency}^{(\text{minimum Ct} - \text{sample Ct})}$$

The ΔCt values of target genes were normalised to ΔCt of the reference transcript *peptidylprolyl isomerase A* (*PPIA*).

2.5. Western blotting

Whole AT biopsies lysates were prepared using an IKA homogeniser in ice-cold lysis buffer (8 M Urea; 1% SDS; 5% glycerol; 10 mM Tris–HCl; pH 6.8) and protease inhibitor cocktail (Complete EDTA-free; Roche). Whole cell lysates were processed as previously described [27]. Equal amounts of protein were loaded (50 μg/biopsy; 100 μg/cell lysate) and resolved by SDS-PAGE, transferred onto polyvinylidene (PVDF) membranes (Bio-Rad) and immunoblotted with: COMP (0.1 μg/ml; AF3134; R&D Systems); β-actin (1:2000; sc1616; Santa Cruz); and α-tubulin (1:2000; ab15246; Abcam) antibodies followed by an HRP-conjugated secondary antibody; goat anti-rabbit IgG (1:5000; 31460; ThermoFisher Scientific). Clarity enhanced chemiluminescence detection kit (Bio-Rad) was used for detection. Immunoblot images were captured on a Chemi-Doc XRS+ (Bio-Rad) and analysed using ImageJ (National Institute of Health, USA) software.

2.6. Measurement of cellular triacylglycerol content

Adipocyte triacylglycerol (TAG) content was measured in differentiated immortalised subcutaneous abdominal and gluteal preadipocytes on day 14 of adipogenesis using an ILAB 650 clinical analyser (Instrumentation Laboratory UK) according to the previously described method [27].

2.7. Quantification of plasma and cell media COMP concentrations

COMP concentration in preadipocyte-conditioned media and plasma was determined using ELISA (R&D Systems) in duplicate. Plasma samples were selected from participants in the OBB with distinctly different AT distribution phenotypes. Using dual X-ray absorptiometry-derived body composition data on 4900 participants, individuals from the top vs. lowest tertile of Android/Gynoid fat mass ratio were pair matched for total fat mass percentage, age, and sex (80 men; 72 women). Anthropometric characteristics for the study cohorts are presented in Supplementary Information Table 1. Depot-specific COMP release by AT into the bloodstream was measured using arterio-

