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Adipocyte-secreted chemerin is processed to a variety of isoforms and influences MMP3 and chemokine secretion through an NFkB-dependent mechanism

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Obesity is associated with white adipose tissue (WAT) remodelling characterized by changes in cellular composition, size, and adipokine secretion. Levels of the adipokine chemerin are positively associated with obesity; however, the biological function of chemerin in WAT is poorly understood. We identified factors involved in WAT remodelling, including matrix metalloproteinase (Mmp)3 and chemokines (Ccl2, 3, 5, 7), as novel targets of chemerin signalling in mature adipocytes. Inhibition of chemerin signalling increased MMP activity and the recruitment of macrophages towards adipocyte-conditioned media. These effects were mediated through increases in NFkB signalling, suggesting that chemerin exerts an anti-inflammatory influence. We also demonstrate that multiple chemerin isoforms are present in adipocyte-conditioned media and that adipocyte-secreted chemerin, but not synthetic chemerin, recapitulates the activity of endogenous chemerin. Considered altogether, this suggests that endogenously secreted chemerin plays an autocrine/paracrine role in WAT, identifying chemerin as a therapeutic target to modulate adipose remodelling.

1. Introduction

Obesity is characterized as the accumulation of abnormal or excessive amounts of white adipose tissue (WAT), which generally results from situations in which energy intake exceeds energy expenditure. Recent reports published by the World Health Organization indicate that more than 1.9 billion adults are currently overweight or obese (WHO, 2015). This is an alarming health concern as pathological changes in WAT structure and function with obesity are thought to promote a state of chronic low-grade inflammation, insulin resistance, and contribute to the increased risk for obesity-associated disorders (Sun et al., 2011; Suganami et al., 2012). WAT is comprised primarily of lipid-filled adipocytes, but also contains other cell types including preadipocytes, endothelial cells, fibroblasts, and leukocytes, which are supported by a rich extracellular matrix (ECM) (Mariman and Wang, 2010; Divoux and Clement, 2011; Martinez-Santibanez and Lumeng, 2014). With obesity, excessive lipid storage accelerates adipose tissue remodelling characterized by adipocyte hypertrophy and hyperplasia as well as increased angiogenesis, macrophage infiltration, and changes in ECM composition and structure (Sun et al., 2011; Martinez-Santibanez and Lumeng, 2014). These changes are associated with altered production of inflammatory mediators and signalling molecules from both infiltrating immune cells and adipocytes. Obesity also alters the secretion of several adipose-derived signalling molecules (adipokines) resulting in undesirable paracrine and endocrine effects on adiposity, energy metabolism, neuroendocrine function, and inflammation (Ouchi et al., 2011; Poulos et al., 2010). For example, clinical and experimental studies have demonstrated that circulating levels of the adipokine chemerin, originally identified as a potent chemoattractant for cells expressing chemokine-like receptor 1 (CMKLR1) (Wittamer et al., 2003), are positively correlated with adiposity and the development of metabolic disorders including metabolic insulin resistance

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and dyslipidemia that may contribute to an increased risk for type 2 diabetes and cardiovascular disease (for review, see (Rourke et al., 2013)). Chemerin is expressed at high levels in mature adipocytes and WAT is considered a primary modifiable source of both local and systemic chemerin. Chemerin is secreted from adipocytes as inactive prochemerin, which undergoes extracellular processing to a variety of bioactive isoforms through the removal of a number of amino acids from the C-terminus (Rourke et al., 2013; Zabel et al., 2014). Adipocytes express several proteases, including the serine proteases elastase and tryptase, which are capable of bioactivating chemerin (Parlee et al., 2012). However, to date, the distribution of adipocyte-derived chemerin isoforms remains unclear and the majority of past studies focused on the role of the chemerin-157 isoform. Bioactive chemerin binds and activates two receptors – CMKLR1 and G protein coupled receptor 1 (GPR1) – as well as the non-signalling receptor C–C chemokine receptor-like 2 (CCRL2) (Wittamer et al., 2003; Barnea et al., 2008; Meder et al., 2003; Zabel et al., 2008). Both CMKLR1 and GPR1 are highly expressed in WAT, with CMKLR1 expressed in adipocytes and the stromal vascular fraction (SVF), while GPR1 is enriched in the SVF. While CMKLR1 is expressed at various levels on leukocytes, preadipocytes, and endothelial cells, it is unknown which cell type(s) in the SVF expresses GPR1 (Goralski et al., 2007; Rourke et al., 2014). Previous studies have demonstrated that chemerin expression is induced by adipocyte hypertrophy (Bauer et al., 2011) and plays a role in adipogenic processes including adipocyte differentiation (Goralski et al., 2007; Muruganandan et al, 2010, 2011), glucose uptake (Kralisch et al., 2009; Takahashi et al., 2008), and lipolysis (Goralski et al., 2007; Roh et al., 2007; Shimamura et al., 2009). However, the mechanisms of chemerin signalling in adipocytes, including chemerin isoform distribution and the activation of intracellular signalling pathways, remain unclear.

A primary interest in the prevention and treatment of obesity and associated disorders is to understand the mechanisms that underlie adipose tissue remodelling and changes in WAT function with obesity. We hypothesized that chemerin signalling plays a fundamental role in processes associated with adipose tissue remodelling. The goals of this study were to identify novel molecular targets of chemerin signalling in mature adipocytes and elucidate the signal transduction pathways downstream of CMKLR1 and/or GPR1 activation. We report that neutralization of endogenous chemerin signalling using an antibody-mediated approach increased the expression and activity of factors involved in adipose tissue remodelling through an NFkB-dependent pathway. Furthermore, we provide evidence that these effects are mediated through a unique adipocyte-derived chemerin isoform.

2. Methods

Reagents: Neutralizing mouse chemerin antibody, IgG control, recombinant mouse tumor necrosis factor (TNF)α, mouse chemerin (aa 17–156), and human chemerin (aa 21–157) were purchased from R&D (Minneapolis, MN, USA). Lipopolysaccharide (LPS) and Bay11-7082 were obtained from Sigma Aldrich (Oakville, ON). Dys10 was purchased from Genscript (Piscataway, NJ, USA).

Animals: C57Bl/6 mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Bone marrow-derived mesenchymal stem cells (MSCs) were isolated from 8 to 10 week old male mice and stromal vascular fraction cells were isolated from the subcutaneous white adipose depots of 10–14 week old female mice. Mice were maintained under specific pathogen free conditions, at 21 °C in a 12 h light:dark cycle with free access to food and water. Animals were sacrificed using an overdose (90 mg/kg) of pentobarbital sodium injected intraperitoneally followed by exsanguination via cardiac puncture. All experimental protocols were approved by the Dalhousie University Committee on Laboratory Animals and in accordance with the Canadian Council on Animal Care guidelines.

Primary cell isolation, culture, and treatment: MSCs were isolated from the bone marrow of wildtype mice as previously described (Muruganandan et al., 2010). Single colonies were isolated from this crude population of MSCs and tested for their ability to differentiate into adipocytes. Briefly, MSCs (~15,000 cells/48 well-plate, or 50,000 MSCs/24-well plate for microarray analysis) were plated and grown to confluence. To induce adipocyte differentiation, cells were grown in adipocyte induction media containing 50 μM ascorbic acid (Sigma), 5 μg/mL insulin (Roche, Mississauga, ON), 50 μM indomethacin (Sigma), and 0.1 μM dexamethasone (Sigma). This was considered day 0 of adipocyte differentiation. After 48 h, fresh maintenance media (250 μL) containing 5 μg/mL insulin was replaced every 48 h thereafter. MSCs with high adipogenic potential were expanded and stored in liquid nitrogen. Oil red O staining was performed as previously described (Muruganandan et al., 2010). For experiments, MSCs were used until approximately passage 20.

The stromal vascular fraction (SVF) was isolated from subcutaneous adipose tissue depots from mice as previously described (Muruganandan et al., 2011). All experiments were performed on freshly isolated cells. Experiments were performed on days 8–10 (MSC) or days 10–17 (SVF) following induction of differentiation and all treatments were performed in 100 μL of serum-free media. Lysate and media samples were stored at −80 °C until further analysis.

RNA isolation: Cells were lysed for RNA isolation in 350 μL of buffer RLT+ with 143 mM 2-mercaptoethanol with scraping and gentle mixing by pipette. RNA for downstream microarray experiments was isolated using the RNeasy RNA isolation kit with on-column DNAse digestion (Qiagen, Valencia, CA, USA). For all other experiments, RNA was isolated using the RNeasy Plus RNA isolation kit (Qiagen) according to manufacturer's recommendations.

Microarray analysis: Samples for microarray analysis were generated in triplicate and isolated independently. RNA was concentrated to a final volume of ~10 μL using a Speed Vac Plus SC100A with a universal vacuum system UVS400 (Savant, Fisher, Toronto, ON) for approximately 20 min with no heating. Microarray analysis was performed at The Centre for Applied Genomics at the Hospital for Sick Children (Toronto, ON). RNA BioAnalyzer analysis (Agilent, Santa Clara, CA, USA) was performed to verify quantity and quality of RNA samples, and microarray analysis was performed using a Mouse Gene 1.0 ST chip (Affymetrix, Santa Clara, CA, USA). The publicly available DNA-Chip Analyzer (dChip) software was used to analyze changes in gene expression between samples (Li and Wong, 2001).

qPCR analysis: cDNA was generated using EcoDry Premix Double-primed (Clontech, Mountain View, CA, USA) with 2 μg of total RNA and diluted to 10 ng/μl qPCR was performed using 1 μL of cDNA per reaction, with 0.5 μM primers, and FastStart SYBR green master mix (Roche). qPCR was performed on a LightCycler96 according to manufacturer's recommendations and analyzed using LightCycler96 software (Roche). Exon-spanning primers (Supplementary Table 1) were designed using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast). Relative gene expression was determined using the ΔΔCt method (Livak and Schmittgen, 2001) where each sample was normalized to cyclophilin A as the reference gene.

Western blotting: 6X SDS-PAGE sample buffer was added to 30–40 μL of conditioned adipocyte media, boiled for 5 min, and proteins separated on a 10% (MMP3) or 15% polyacrylamide gel (chemerin, CCL2). For analysis of non-denatured chemerin, 6X SDS-PAGE sample buffer without 2-mercaptoethanol was added to adipocyte media and samples were not boiled. Proteins were