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Silencing Mediator of Retinoid and Thyroid Hormone Receptors (SMRT) regulates glucocorticoid action in adipocytes

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ABSTRACT

Local modulation of glucocorticoid action in adipocytes regulates adiposity and systemic insulin sensitivity. However, the specific cofactors that mediate glucocorticoid receptor (GR) action in adipocytes remain unclear. Here we show that the silencing mediator of retinoid and thyroid hormone receptors (SMRT) is recruited to GR in adipocytes and regulates ligand-dependent GR function. Decreased SMRT expression in adipocytes *in vivo* increases expression of glucocorticoid-responsive genes. Moreover, adipocytes with decreased SMRT expression exhibit altered glucocorticoid regulation of lipolysis. We conclude that SMRT regulates the metabolic functions of GR in adipocytes *in vivo*. Modulation of GR–SMRT interactions in adipocytes represents a novel approach to control the local degree of glucocorticoid action and thus influence adipocyte metabolic function.

1. Introduction

Glucocorticoids exhibit diverse metabolic and anti-inflammatory functions. While the anti-inflammatory effects of glucocorticoids can be beneficial clinically, therapeutic doses of glucocorticoids lead to severe metabolic dysfunction (Friedman et al., 1996). In particular, excess glucocorticoid action leads to obesity, insulin resistance, and type 2 diabetes mellitus. Recent work suggests that increased local production of glucocorticoids in adipocytes leads to metabolic dysfunction. For example, an increase in adipocyte 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1), which increases the conversion from inactive to active glucocorticoids, results in insulin resistance, obesity, hypertension, and dyslipidemia (Masuzaki et al., 2001, 2003), whereas adipocyte-specific knock-out of 11βHSD1 leads to resistance to both obesity and insulin resistance (Kershaw et al., 2005). Thus, identification of the pathways leading to local activation of glucocorticoid action presents a promising target for the treatment of obesity and the metabolic syndrome.

Glucocorticoids act by binding to the glucocorticoid receptor (GR). In the absence of ligand, the GR is predominantly located in the cytoplasm. Recent studies have suggested a model for GR action (Heitzer et al., 2007), in which upon glucocorticoid binding, the GR translocates to the nucleus and binds to regions of DNA known as glucocorticoid response elements (GREs). Glucocorticoid binding to GR also induces a conformational change that allows the GR dimer to recruit a class of nuclear proteins termed coactivators. The recruitment of coactivators to the GR, via their effects on histone acetyltransferase activity and other pathways, then leads to increased DNA transcription of target genes.

There exists another class of nuclear proteins, termed corepressors, whose role in GR action is much less clear. Corepressors, such as the silencing mediator of retinoid and thyroid hormone receptors (SMRT) and the nuclear corepressor protein (NCoR), classically repress the transcriptional activity of a variety of nuclear receptors, such as the thyroid hormone receptor (TR) and the retinoic acid receptor (RAR) (Chen and Evans, 1995; Horlein et al., 1995; Sande and Privalsky, 1996). More recent work suggests that SMRT and NCoR also modulate peroxisome proliferator-activated receptor gamma (PPARγ) action in adipocytes, leading to a derepression of the adipogenic program (Guan et al., 2005; Li et al., 2011; Nofsinger et al., 2008; Yu et al., 2005). However, the role of corepressors in GR action is much less clear, in part because the GR is predominantly located in the cytoplasm in the absence of ligand and thus does not exert basal (ligand-independent) repression.

A few studies have suggested that corepressors modulate GR transcriptional activity (Blackford et al., 2012; Bush et al., 2012; Hong et al., 2009; Ronacher et al., 2009; van der Laan et al., 2008; Wang et al., 2004), but these studies have been mainly limited to an

Abbreviations: SMRT, Silencing Mediator of Retinoid and Thyroid Hormone Receptors; NCoR, Nuclear Corepressor Protein; GR, Glucocorticoid Receptor; PPAR, Peroxisome Proliferator- Activated Receptor; GRE, Glucocorticoid Response Element.

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evaluation of the effects of SMRT on GR action in immortalized cell lines, and have not been able to determine how SMRT modulates GR action in metabolism. We have developed a SMRT knock-out (KO) mouse model that allows an evaluation of SMRT function on GR action in adipocytes. Although SMRT–/– mice die *in utero*, SMRT+/– mice survive, and exhibit increased adiposity when challenged with a high-fat diet (HFD) (Sutanto et al., 2010). The enhanced adiposity in the mice is due in part to greater caloric intake, however, there was also enhanced adipogenesis, likely due to the absence of SMRT repression of PPARγ. The increased activation of PPARγ also led to a modest increase in the insulin sensitivity of the adipocytes. The strong effects of the knockout on adipogenesis contrasted with the relatively mild effects on insulin sensitivity, and led us to suspect that SMRT modulates the activities of a variety of nuclear receptors, some of which may enhance insulin sensitivity and others that impair this process. Here we show that SMRT regulates GR action in adipocytes, and that altering the ability of SMRT to repress adipocytic GR action represents a novel mechanism to alter local adipocyte GR signaling and modulate adipocyte insulin sensitivity.

2. Materials and methods

2.1. Mouse treatment and care

Mice were housed in a specific pathogen-free barrier facility with a 12-h light/dark cycle, with free access to water and a standard chow diet at the University of Chicago. We used age-matched males for each experiment and littermates when possible. All animal husbandry and animal experiments were approved by the University of Chicago Institutional Animal Care and Use Committee. After weaning at 4 weeks of age, ear clippings were obtained for genotyping by PCR. At 12–16 weeks of age, male mice were sacrificed using isoflurane and cervical dislocation.

2.2. RNA analyses

Total RNA from epididymal fat pads was extracted using e.Z.N.A Total RNA Kit II according to manufacturer's directions (Omega) before cDNA synthesis was performed using the QScript cDNA Super Mix (Quanta). Experiments were performed as described in the manual for SybrGreen (Quanta) with minor modifications. Each cDNA sample in triplicate was subjected to at least three individual PCR analyses using primers specific to Gilz, Lipin, or 18s RNA. RNA expression was normalized using 18s RNA, and 1.0 is defined as relative gene expression using WT adipose tissue. The primers used were Lipin: 5′-CCCTCGATTTCAACGTACCC and 5′-GCAGCCTGTGGCAATTCA (Donkor et al., 2007); Gilz: 5′-CAGCAGCCACTCAAACCAGC and 5′-ACCACATCCCCTCCAAGCAG; 18s RNA: 5′-GTAACCCGTTGAACCCCATT and 5′-CCATCCAATCGGTAGTAGCG (Wang et al., 2008).

2.3. MEF isolation

Mouse embryonic fibroblasts were isolated from embryonic day 13.5 embryos as previously described (Sutanto et al., 2010). MEF culture media included high-glucose DMEM, 8 mg/L biotin, 4 mg/L pantothenate, L-glutamine, 10% FBS, and Pen/Strep antibiotics.

2.4. Immunoprecipitation

Protein isolated from epididymal fat pads was incubated overnight with protein A/G magnetic beads (Millipore) and an Anti-SMRT (Millipore #17–10057) or Anti-GR (Santa Cruz #sc-1004) antibody. In the morning the beads were washed with RIPA buffer three times, then denatured in Laemmle buffer at 95 °C. The resulting proteins were run on a western blot and probed with Anti-GR (Santa Cruz #sc-1004), Anti-CBP (#sc-369), Anti-Med1 (#sc-5334), Anti-PPARγ (#sc-7273), or Anti-SMRT (Millipore #17–10057) antibodies. Blots were performed at least three times.

2.5. Chromatin immunoprecipitation (ChIP)

Epididymal fat pads were isolated from WT animals, minced, and incubated in DMEM with or without 100 nM dexamethasone for 2 hours. Cells were crosslinked with formaldehyde for 10 minutes and the reaction was quenched with glycine. The fat was then washed with PBS, homogenized with a polytron homogenizer, and sonicated. The resulting chromatin was incubated overnight with protein A/G magnetic beads and mouse IgG, Anti-SMRT, or Anti-GR antibodies. Beads were washed and eluted by shaking in buffer with proteinase K at 60 °C for 2 hours. DNA was purified from eluent using DNA spin columns and was analyzed by running PCR with primers specific to the glucocorticoid response element (GRE) of Gilz or Lipin. The primers used were Lipin (Zhang et al., 2008): 5′-TTCCCGGCTCGCATAAAGTA and 5′-AGGGAGTAGGCAGCCAAAGTC; Gilz (Neel et al., 2013): 5′-GAGCCCTTGAGAAACCAGTG and 5′-AGCTCTGGCAGAAAACGAAG. Representative blots from three ChIP assays are shown.

2.6. Luciferase assay

MEFs were isolated from WT and KO embryos as described previously (Sutanto et al., 2010). Cells were grown to 60% confluence in 6 well plates and transfected with plasmids containing a luciferase tagged GRE (a generous gift of Ella Atlas), the glucocorticoid receptor (GR), and a β-galactosidase-expressing vector. The GRE sequence was GGTACATTTTGTTCTAGCCAG, and 2 copies were cloned into the pGL2 luciferase vector (Atlas et al., 2014). The transfection was carried out using Transit-2020 (Mirus). Five hours post transfection cells were treated with 0, 1, 10, or 100 nM of dexamethasone (or ethanol) in DMEM + 0.5% FBS and allowed to incubate overnight. In the morning cells were washed and scraped. Luciferase activity was measured in 200 μL of cell slurry using a Lumat LB 9507 luminomator. Transfection efficiency was controlled by β-galactosidase activity, which was measured using Galacto-Light Plus (Applied Biosystems). The data presented are an average of three experiments, and each experiment was performed in triplicate.

2.7. Primary adipocyte isolation

Epididymal and subcutaneous WAT pads were minced in Krebs–Ringer bicarbonate HEPES buffer (KRBH) with 3% fatty acid free bovine serum albumin, 5 mM glucose, and 2 mg/mL type I collagenase (Worthington 49J11380). Samples were incubated at 37 °C, with agitation at 80 rpm for 1 hour. Tubes were then centrifuged at 1200 rpm for 1 min to separate the stromal vascular fraction and isolated primary adipocytes were transferred to 15-mL conical tubes and washed three times with KRBH + 500 nM adenosine.

2.8. Lipolysis assay

Glycerol release from primary adipocytes was monitored as a measure of lipolysis. Primary adipocytes isolated from 12- to 16-week-old animals as described earlier were used in lipolysis experiments. Freshly isolated primary adipocytes were incubated in KRBH buffer supplemented with 3% bovine serum albumin, 5 mM glucose, and 1 U/mL adenosine deaminase were treated overnight in triplicate in four treatment groups, basal with 100 nM PIA, 100 nM Dex, 100 ng/mL TNFα, or 100 nM Dex + 100 ng/mL TNFα. The infranatant medium was aspirated for measurement of glycerol, which was measured using Free Glycerol Reagent (Sigma). For each treatment condition, there were 4 biological replicates, and when the glycerol assay was performed it was done with technical triplicates.