Research Article

Transcriptional Activity of PGC-1α and NT-PGC-1α Is Differentially Regulated by Twist-1 in Brown Fat Metabolism

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1. Introduction

The transcriptional coactivator PGC-1α was first identified as a coactivator of PPARγ in brown adipose tissue and is now known to interact with a broad range of nuclear receptors and transcription factors to regulate mitochondrial biogenesis in most tissues but also control adaptive thermogenesis, fatty acid/glucose metabolism, ROS metabolism, and muscle fiber type switching in a tissue-specific manner [1–7]. The function of PGC-1α among tissues is regulated by signaling inputs that increase transcription of PGC-1α and modulate the transcribed protein through tissue-specific posttranslational modifications [8–14]. This allows PGC-1α to function as a key regulator to link nutritional and environmental stimuli to the tissue-specific transcriptional programs.

Alternative splicing of PGC-1α produces an additional transcript that encodes a shorter isoform called NT-PGC-1α (aa 1–270) [15]. NT-PGC-1α is coexpressed with PGC-1α in metabolically active tissues and its expression is coregulated by the nutritional and environmental cues which activate the gene [15–17]. PGC-1α is a short-lived nuclear protein containing 797 amino acids. A variety of post-translational modifications enhance the stability and activity of PGC-1α by decreasing its targeting to the proteosome. In contrast, NT-PGC-1α is relatively stable since it is less effectively targeted to the proteosome due to lack of the C-terminal domain involved in mitochondrial fatty acid oxidation and uncoupling (CPT1β, UCP1, and ERRα). In contrast, NT-PGC-1α-mediated induction of these genes is unaffected by Twist-1. These findings show that differences in inhibitory protein-protein interactions of PGC-1α and NT-PGC-1α with Twist-1 lead to differential regulation of their function by Twist-1.
of PGC-1α (aa 200–400) in C2C12 myoblasts [18]. The docking of p160MOB on PGC-1α inhibits transcription of PGC-1α target genes [9, 18]. RIP140 is a transcriptional corepressor for a number of nuclear receptors in adipose tissue and skeletal muscle where it represses many PGC-1α target genes [19]. Mechanistically, RIP140 directly interacts with PGC-1α (aa 184–797) and suppresses its activity [20]. Recently, the transcription factor Twist-1 was also identified as a negative regulator of PGC-1α in brown adipose tissue. Twist-1 is a helix-loop-helix (HLH)-containing transcription factor involved in early development, apoptosis, cancer, and osteoblast differentiation [21–23]. A recent study reported that Twist-1 is recruited to the PGC-1α target genes by docking to the C-terminal domain of PGC-1α (aa 350–797) to negatively modulate oxidative metabolism and UCP1-dependent uncoupling in brown adipose tissue [24]. Interestingly, all of these negative regulators bind to the central to C-terminal region of PGC-1α, suggesting that these regulators would have little or no inhibitory effect on NT-PGC-1α function in the nucleus.

The present study was designed to investigate the effect of known PGC-1α repressors on NT-PGC-1α function and found that Twist-1 plays a differential role in the regulation of PGC-1α and NT-PGC-1α activity in brown adipocytes. Twist-1 significantly suppressed PGC-1α-mediated activation of CPT1β, UCP1, and ERRα by docking to the C-terminal region of PGC-1α. In contrast, NT-PGC-1α-dependent induction of these genes was not affected by Twist-1 due to lack of interaction with Twist-1.

2. Materials and Methods

2.1. Cell Cultures and Brown Adipocyte Differentiation. COS-1 cells were grown in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (Invitrogen). Immortalized PGC-1α-deficient mouse brown preadipocyte cell lines expressing empty vector, PGC-1α, or NT-PGC-1α [16] were maintained in DMEM supplemented with 10% FBS, 1% glutamine, and 1% penicillin/streptomycin. Preadipocytes were grown to confluence in culture medium supplemented with 20 nM insulin and 1 mM T3 (differentiation medium). Differentiation of brown adipocytes was induced (day 1) by incubating the cells in differentiation medium supplemented with 0.5 mM isobutylmethylxanthine (IBMX), 0.5 μM Dexamethasone, and 0.125 mM indomethacin for 48 hours. Thereafter, the cells were maintained in differentiation medium until day 7, followed by treatment with dibutyryl cAMP for 4 h.

2.2. Luciferase Reporter Assay. COS-1 cells were transiently transfected using FuGENe6 (Roche) with following plasmids. For a transcriptional repression assay with RIP140, GAL4-responsive luciferase reporter (pGK), GAL4-DBD-fused mouse ERRα-LBD, and plasmids expressing PGC-1α-HA, NT-PGC-1α-HA, and RIP140 were used. For a transcriptional repression assay with Twist-1, (PPRE)γ-TK-luc, pSV sport-PPARγ1, and plasmids expressing PGC-1α-HA, NT-PGC-1α-HA, and Twist-1 were used. pRL-SV40 control plasmid expressing Renilla luciferase was used for normalization. Cells were harvested for luciferase assay 48 h after transfection, and luciferase activity was determined using a Promega Dual Luciferase assay kit (Promega). The firefly luciferase activity was normalized with Renilla luciferase activity. Data represent mean ± SEM of at least four independent experiments.

2.3. Immunoprecipitation and Western Blot. COS-1 cells were transfected with pcDNA3.1-Flag-Twist-1 and pcDNA3.1-PGC-1α-HA or pcDNA3.1-NT-PGC-1α-HA. Cells were harvested 48 h after transfection, washed with ice-cold PBS, and lysed in IP buffer (20 mM HEPES-NaOH, pH 7.0, 150 mM NaCl, 0.2% NP-40) supplemented with a protease inhibitor cocktail (Roche). Lysates were precleared with protein G-agarose beads and immunoprecipitated with IgG and anti-Flag antibody (Sigma) overnight at 4°C. After washings, immunoprecipitates were subjected to Western blot analysis using anti-PGC-1α antibody directed against the N-terminus of PGC-1α (aa 1–200) and anti-Flag antibody.

2.4. Retroviral Infection. GP-293 cells were cotransfected with pVSV-G and pBABE-zeo or pBABE-zeo-Twist-1 using Profection transfection system (Promega). Following transfection, the cells were incubated at 32°C to increase viral titer. Virus-containing medium was collected, filtered through the 0.45 μm filter, and used to infect target cells. Immortalized PGC-1α-deficient mouse brown preadipocyte cells that ectopically express empty vector, PGC-1α, or NT-PGC-1α [16] were infected with the viral supernatant supplemented with 8 μg/ml polybrene. The medium was aspirated after 2 h and replaced with fresh viral supernatant, and the procedure was repeated. After 8 h of infection, the cells were replaced with fresh DMEM medium supplemented with 10% FBS, 1% glutamine, and 1% penicillin/streptomycin. Selection was initiated with zeocin (Invitrogen) 48 h after infection.

2.5. Real-Time PCR Analysis. Total RNA was isolated from brown adipocytes using Tri-Reagent (Molecular Research Center) and RNeasy kits (Qiagen). For quantitative RT-PCR analysis, 2 μg of RNA samples were reverse transcribed using oligo dT primers and M-MLV reverse transcriptase (Promega), and 4 ng of cDNA were used in quantitative PCR reactions in the presence of a fluorescent dye (Cybergreen, Takara) on Applied Biosystems 7900 (Applied Biosystems). Relative abundance of mRNA was normalized to that of cyclophilin mRNA. The primers for Twist-1, UCP1, CPT1β, Cox7a1, ERRα, VLCAD, PPARα, and cyclophilin were previously described [15, 16, 24].

3. Results

3.1. Twist-1 Negatively Regulates PGC-1α but Not NT-PGC-1α. Brown adipose tissue expresses two PGC-1α isoforms (PGC-1α and NT-PGC-1α), both of which regulate transcription of mitochondrial and thermogenic genes by promoting the activity of several nuclear receptors including PPARs [15–17]. RIP140 and Twist-1, which have been shown to negatively regulate PGC-1α activity in adipose tissue, were
3.2. Twist-1 Does Not Interact with NT-PGC-1α

Tested for their effect on regulation of NT-PGC-1α function, NT-PGC-1α (aa 1–270) partially contains a docking region for RIP140 that binds to the wide region of PGC-1α (aa 186–797), whereas the C-terminal Twist-1-binding domain (aa 353–797) is missing in NT-PGC-1α (Figure 1(a)). To assess the ability of these transcriptional regulators to modulate NT-PGC-1α activity, we carried out transient cotransfection and luciferase reporter assays as described in Materials and Methods. For a transcriptional repression assay, Flag-Twist-1 was coexpressed with NT-PGC-1α(1–270) partially contains a docking region for RIP140 (data not shown). Coexpression of RIP140 with PGC-1α significantly inhibited the ability of PGC-1α to increase Gal4-ERRα-LBD-mediated transcription of the reporter gene (Figure 1(b)). Similarly, RIP140 suppressed NT-PGC-1α-mediated induction of the reporter gene (Figure 1(b)), indicating that amino acids 186–270 in NT-PGC-1α are sufficient for RIP140 binding and repression. A transcriptional repression assay with Twist-1 showed that Twist-1 largely suppressed the ability of PGC-1α to increase PPARγ-mediated transcription, whereas NT-PGC-1α-dependent increase of reporter gene expression was not affected by Twist-1 (Figure 1(c)).

3.3. Twist-1 Differentially Regulates a Subset of PGC-1α- and NT-PGC-1α-Target Genes in Brown Adipocytes.

Twist-1 is selectively expressed in adipose tissue and its overexpression in brown adipocytes specifically suppresses PGC-1α-mediated activation of fatty acid oxidation and uncoupling genes [24]. Since NT-PGC-1α is coexpressed with PGC-1α in brown adipocytes and regulates many PGC-1α target genes [15–17], we hypothesized that PGC-1α and NT-PGC-1α are differentially regulated by Twist-1 in brown adipocytes. To investigate a differential role of Twist-1 in PGC-1α- and NT-PGC-1α-target gene expression in brown adipocytes, the PGC-1α-deficient mouse brown preadipocyte cell lines expressing empty vector, PGC-1α, or NT-PGC-1α [16] were transduced with Twist-1 retrovirus to overexpress Twist-1. PPAR expression is not affected by PGC-1α or NT-PGC-1α [26]. In addition, Twist-1 does not change PPAR expression or activity [24]. After retroviral infection, the mRNA levels of Twist-1 were ~16- and ~12-fold increased in
the PGC-1α-deficient brown preadipocytes expressing PGC-1α and NT-PGC-1α, respectively (Figure 3).

These brown preadipocyte cell lines were then differentiated for 7 days and treated with dibutyryl cAMP to maximize protein stability/activity of PGC-1α and increase nuclear retention of NT-PGC-1α [15, 16]. In response to cAMP-induced signaling, p38 MAPK increases stabilization and activation of PGC-1α protein by preventing its proteosomal targeting [8], whereas cAMP-activated PKA phosphorylates NT-PGC-1α, leading to inhibition of CRM1-mediated nuclear export [16]. Expression of αP2, a marker of adipocyte differentiation, was relatively comparable among the cell lines (Figure 4(a)). In agreement with previous findings [24], overexpression of Twist-1 in differentiated brown adipocytes significantly suppressed PGC-1α-mediated induction of CPT1β, UCP1, and ERRα, which are implicated in mitochondrial fatty acid oxidation and uncoupling (Figure 4(b)). In contrast, Twist-1 had no suppressive effect on NT-PGC-1α-dependent induction of CPT1β, UCP1, and ERRα (Figure 4(b)), indicating that Twist-1 differentially regulates PGC-1α and NT-PGC-1α activity in brown adipocytes. With no addition of dibutyryl cAMP, PGC-1α and NT-PGC-1α are able to activate their target gene expression although their transcriptional activity is reduced. In the absence of cAMP signaling activation, cAMP-dependent increase of basal UCP1 levels was ∼80% reduced, leading to large fold changes in PGC-1α- and NT-PGC-1α-mediated induction of UCP1 (12.2-fold and 10.3-fold, respectively) (Figure S1, available online at doi: 10.1155/2012/320454).

Similarly, Twist-1 significantly suppressed PGC-1α-mediated induction of UCP1, whereas NT-PGC-1α-mediated increase of UCP1 gene expression was not affected by Twist-1 (Figure S1, available online at doi: 10.1155/2012/320454). Expression of many mitochondrial genes and nuclear receptors is also regulated by PGC-1α and NT-PGC-1α [15–17]. However, neither PGC-1α- nor NT-PGC-1α-dependent induction of Cox7a1, PPARα, and VLCAD (Figure 4(c)) and MCAD, Atp5b (not shown) was affected by Twist-1. This suggests that Twist-1 negatively regulates only a subset of PGC-1α target genes in brown adipocytes.

4. Discussion

We previously reported that PGC-1α and NT-PGC-1α regulate a number of mitochondrial and thermogenic genes in brown adipose tissue [15–17]. Sympathetic stimulation of BAT by cold increases transcription of the PGC-1α gene by activating and recruiting cAMP-dependent transcription factors, ATF2 and CREB, to the PGC-1α promoter [1, 27]. Subsequent normal and alternative splicing of the transcribed RNA produce comparable mRNA levels of PGC-1α and NT-PGC-1α, respectively [15]. However, two transcripts produce structurally different proteins that possess fundamental differences in their protein size, stability, and localization. These different natures of two PGC-1α isoforms require different regulatory mechanisms to increase their transcriptional activity in response to the same signaling inputs. For example, cold/cAMP-activated p38 MAPK phosphorylation leads to stabilization and activation of PGC-1α protein by preventing its proteosomal targeting [8]. In contrast, cAMP-activated PKA phosphorylation increases nuclear
Figure 4: Differential effect of Twist-1 on PGC-1α and NT-PGC-1α target gene expression in brown adipocytes. (a), (b), (c) Quantitative real-time PCR analysis of gene expression in differentiated PGC-1α-null brown adipocytes expressing empty vector (pBABE), PGC-1α, and NT-PGC-1α with empty vector (white bars) or Twist-1 (black bars). Relative abundance of mRNA levels was normalized to that of cyclophilin mRNA. Data represent mean ± SEM of at least four independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; ns: not significant.
accumulation of NT-PGC-1α and subsequent recruitment to the transcriptional complexes [16].

Here we show an additional regulatory mechanism that differentially modulates transcriptional activity of PGC-1α and NT-PGC-1α in the nucleus of brown adipocytes. The mode of action is mediated by direct interaction of PGC-1α with a negative regulator Twist-1, which is abundantly expressed in brown adipocytes. Twist-1 is recruited to PGC-1α target genes by docking to the C-terminal region of PGC-1α and inhibits their expression by subsequently recruiting the histone deacetylase HDAC5 to the PGC-1α target gene promoters (e.g., UCP1 and CPT1) [24]. In contrast, Twist-1 has no inhibitory effect on NT-PGC-1α-mediated induction of NT-PGC-1α target genes since NT-PGC-1α does not recruit Twist-1 to its target gene promoters. Despite potential inhibition of all PGC-1α target genes by Twist-1, Twist-1 suppresses only a subset of PGC-1α target genes, including UCP1, CPT1β, and ERRα. Twist-1 is a basic helix-loop-helix (bHLH)-containing transcription factor that binds to the canonical E-box and the related sequences in the regulatory regions of target genes [28], thus raising a possibility that the presence of potential E-boxes on the PGC-1α target gene promoters further specifies a subset of PGC-1α/Twist-1 target genes. However, it seems unlikely that subsequent docking of Twist-1 to the potential E-boxes on the PGC-1α target gene promoters is required for its inhibitory effect since Twist-1-mediated suppression does not depend on its DNA-binding activity [24]. Instead, Twist-1 exerts its transcriptional repression on PGC-1α target genes by altering chromatin conformational states by recruitment of histone deacetylases (HDAC) [24]. Thus, it is likely that subsequent recruitment of additional regulators (e.g., histone deacetylases) to the PGC-1α target gene promoters further specifies a subset of PGC-1α/Twist-1 target genes.

5. Conclusion

Our findings demonstrate a differential regulation of PGC-1α and NT-PGC-1α activity by Twist-1 in brown adipocytes. Twist-1 suppresses PGC-1α-mediated transcriptional activation of a subset of PGC-1α target genes, including UCP1, CPT1β, and ERRα. In contrast, NT-PGC-1α-mediated induction of these genes is not affected by Twist-1.

Abbreviations

PGC-1α: Peroxisome proliferator-activated receptor γ coactivator 1α
ERRα: Estrogen-related receptor α
NRF1: Nuclear respiratory factor 1
CPT1β: Carnitine palmitoyltransferase 1β
UCP1: Uncoupling proteins 1
ROS: Reactive oxygen species
PKA: Protein kinase A
p160MBF: p160 Myb binding protein
RIP140: Receptor-interacting protein 140
MAPK: Mitogen-activated protein kinase

VLCAD: Very long-chain acyl-CoA dehydrogenase
MCAD: Medium-chain acyl-CoA dehydrogenase
ATF2: Activating transcription factor 2
CREB: cAMP response element-binding.

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