Metabolic Stress Modulates Alzheimer's β-Secretase Gene Transcription via SIRT1-PPARγ-PGC-1 in Neurons

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SUMMARY

Classic cardio-metabolic risk factors such as hypertension, stroke, diabetes, and hypercholesterolemia all increase the risk of Alzheimer's disease. We found increased transcription of β-secretase/BACE1, the rate-limiting enzyme for Aβ generation, in eNOS-deficient mouse brains and after feeding mice a high-fat, high-cholesterol diet. Up- or downregulation of PGC-1α reciprocally regulated BACE1 in vitro and in vivo. Modest fasting in mice reduced BACE1 transcription in the brains, which was accompanied by elevated PGC-1 expression and activity. Moreover, the suppressive effect of PGC-1 was dependent on activated PPARγ, likely via SIRT1-mediated deacetylation in a ligand-independent manner. The BACE1 promoter contains multiple PPAR-RXR sites, and direct interactions among SIRT1-PPARγ-PGC-1 at these sites were enhanced with fasting. The interference on the BACE1 gene identified here represents a unique noncanonical mechanism of PPARγ-PGC-1 in transcriptional repression in neurons in response to metabolic signals that may involve recruitment of corepressor NCoR.

INTRODUCTION

Alzheimer's disease (AD) is one of the most devastating neurodegenerative disorders and is characterized by the two pathological hallmarks of amyloid plaques and neurofibrillary tangles. Amyloid peptides (Aβ), the major constituent of plaques, are generated by sequential proteolytic cleavage of the amyloid precursor protein (APP) via β-secretase (BACE1) and the γ-secretase complex (Hardy and Selkoe 2002). The expression and activity levels of BACE1 are elevated in AD brains and correlate with the specific regions affected by Aβ deposition. Taken together with the observation that BACE1-deficient mice display

diminished amyloid pathology (Vassar et al., 2009), current evidence strongly suggests that BACE1 elevation leads to enhanced Aβ production and deposition in AD. Given the central role of Aβ in AD pathogenesis and the fact that BACE1 is the rate-limiting enzyme in APP processing and Aβ generation, BACE1 remains one of the most important therapeutic targets for treating AD.

BACE1 expression is tightly regulated at multiple levels between transcription and posttranslation (Rossner et al., 2006). A number of transcription factors (TFs) have been identified that positively or negatively regulate BACE1 gene expression in both basal (Sp1, YY1, and HNF3β) (Ge et al., 2004; Sun et al., 2005) and cell stress conditions (e.g., HIF-1α during hypoxia and NF-κB and PPARγ with inflammation) (Guglielmotto et al., 2012). We recently reported differential regulation of BACE1 by oxidative and nitrosative signals (Kwak et al., 2011), both of which are common denominators in age-related diseases.

While the molecular mechanism underlying ischemia/hypoxia or reactive oxygen species (ROS)-induced BACE1 activation and APP processing has been extensively studied, relatively little is known about BACE1 regulation by metabolic stress. The vast majority of AD cases are late onset and sporadic (SAD) in origin, with age being the most profound risk factor. Multiple environmental factors, such as diet and lifestyle, along with genetic factors are all significant contributors. Epidemiological, clinical, and experimental evidence strongly link metabolic defects with functional alterations associated with aging of the brain and with AD pathogenesis. Thus, classic cardio-metabolic risk factors such as hypertension, cerebral hypoperfusion, diabetes mellitus, and hypercholesterolemia have been shown to increase the risk of SAD (Bhat 2010; Craft 2009; de la Torre 2009; Martins et al., 2006).

In this study, we sought to investigate the potential role of the sirtuin 1 (SIRT1), peroxisome proliferator-activated receptor gamma (PPARγ), and its coactivator (PGC-1) pathway in regulating BACE1 expression. Using in vitro and in vivo experimental systems mimicking metabolic stress (e.g., glucose depletion, hyperglycemia, high-fat/high-cholesterol (HFC) diet, and fasting), our work demonstrates the potent suppressive effects of these key regulators on BACE1 transcription in response to cellular metabolic status.
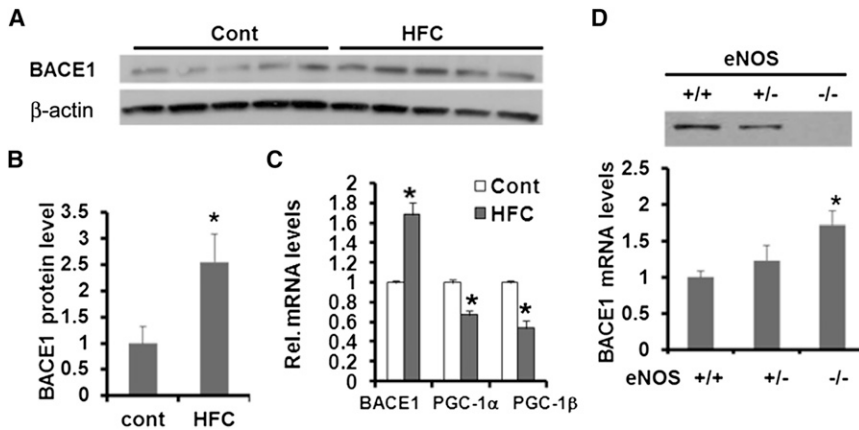
Figure 1. HFC Feeding or eNOS Deficiency Increases BACE1 Expression
(A) Western blot analysis of BACE1 protein expression from the forebrain extracts of mice fed HFC and control chow diets (n = 5/group; 9 months of age).
(B) Quantification of (A) by densitometry.
(C) Relative mRNA levels of BACE1 and PGC-1α determined by qRT-PCR from the same forebrain samples as in (A).
(D) Relative forebrain BACE mRNA levels of mice with partial and complete eNOS deficiency (n = 5/group). Western blot of eNOS protein expression is shown on the top panel. *p < 0.05.

RESULTS

Elevated BACE1 Transcription by Feeding a HFC Diet or eNOS Deficiency

Disrupted cholesterol homeostasis causes excessive Aβ generation (Puglielli et al., 2003). Previously, we observed that young C57BL/6 mice consuming a HFC diet containing 21% milk fat and 1.25% cholesterol for 8 weeks demonstrated learning/memory deficits and neuroinflammation (Thirumangalakudi et al., 2008). Since high cholesterol is the biggest risk factor for SAD in middle-aged individuals, we repeated the 8 week HFC feeding regimen on middle-aged mice. BACE1 gene and protein expression levels were found to be increased over 2-fold in the mouse forebrains after HFC feeding (Figures 1A and 1B). Furthermore, we found that both PGC-1α and PGC-1β messenger RNA (mRNA) levels were significantly reduced following the HFC diet (Figure 1C). BACE1 reduction appeared to be at the transcription level, since we did not detect significant changes in the protein level of GGA3, a key regulator of BACE1 trafficking and lysosomal degradation (Tesco et al., 2007), or p-eIF2α (O'Connor et al., 2008) (Figure S1).

Recently, we demonstrated that BACE1 is highly susceptible to nitric oxide (NO)-mediated regulation. NO generated by the endothelial NO synthase (eNOS) suppresses BACE1 transcription via cGMP-PKG signaling (Kwak et al., 2011). Consistent with this, BACE1 protein expression in the brains of eNOS-deficient mice was found to be elevated (Austin et al., 2010). We observed that the frontal brains of 4-month-old eNOS$^{-/-}$ mice displayed significantly increased levels of BACE1 mRNAs (Figure 1D) while heterozygous mice did not show significant alteration.

Fasting Reduces BACE1 Expression in Mice

Calorie restriction can activate the AMP-activated protein kinase (AMPK)-SIRT1-PGC-1 pathway (Qin et al., 2006). We therefore examined the effect of fasting on BACE1 levels in mouse brains and found that BACE1 expression was significantly reduced at the 24 hr time point (Figures 2A and 2B), accompanied by marked activation of AMPK and increased PGC-1α/PGC-1β (Figures 2C and 2D). This inverse correlation between BACE1 and PGC-1 was also detected at mRNA levels (Figure 2E). Fast-

ing for 24 hr resulted in >85 mg/dl blood glucose levels, which are still within the normal range (Figure S2).

PGC-1 Regulates BACE1 In Vitro and In Vivo

Since AMPK and PGC-1 can both be activated by cGMP-PKG signal transduction (Nisoli et al., 2003), we investigated whether these key metabolic regulators have a direct role in suppressing BACE1. In HEK293 cells, we found that overexpression of PGC-1α suppressed basal transcription of endogenous BACE1 mRNA, resulting in a 2-fold reduction of mRNA and protein levels (Figures 3A and 3B). On the other hand, downregulation of either PGC-1α (52%) or PGC-1β (38%) gene transcription in rat primary cortical neurons via specific small interfering RNA (siRNA) delivered from adenoviral transduction resulted in >2-fold upregulation of BACE1 protein expression (Figures 3C and 3D). A direct role for PGC-1 in suppressing BACE1 expression was also further confirmed by in vivo approaches. Overexpression of PGC-1α (>7-fold) via AAV2-mediated gene transfer severely diminished BACE1 protein expression in the hippocampi of Tg2576 (a familial AD [FAD] model overexpressing an APP$_{Swedish}$ mutant), while downregulation of PGC-1α (1.9-fold) augmented BACE1 expression by 2.6-fold in the hippocampi of wild-type (WT) C57BL/6 mice (Figures 3E and 3F). The effects were confirmed to be on modulation of BACE1 transcription, as evidenced by the altered mRNA levels (Figure 3G). We found comparable effects of PGC-1α and PGC-1β in suppressing BACE1 upon transfection, indicating that they are interchangeable.

PGC-1's Effect on BACE1 Requires Deacetylation by SIRT1

As with elevating PGC-1 levels, overexpression of SIRT1 suppressed BACE1 transcription, while deacetylase-inactive (DN) H355A SIRT1 had the opposite effect in HEK293 cells (Figures 4A and 4B). Treatment with the SIRT1 activating agent, resveratrol, repressed BACE1 transcription in a dose-dependent manner (Figure 4C); concentrations greater than 50 μM caused cytotoxicity in primary cultured neurons. On the contrary, the SIRT inhibitor, sirtinol, significantly augmented BACE1 levels at 10 μM (Figures 4D and 4E). BACE1 expression was upregulated 1.8-fold (Figure 4D) by 5 mM nicotinamide. Notably, coexpression of PGC-1 and the DN-SIRT1 plasmid constructs in HEK293 cells completely abolished the suppressive effect
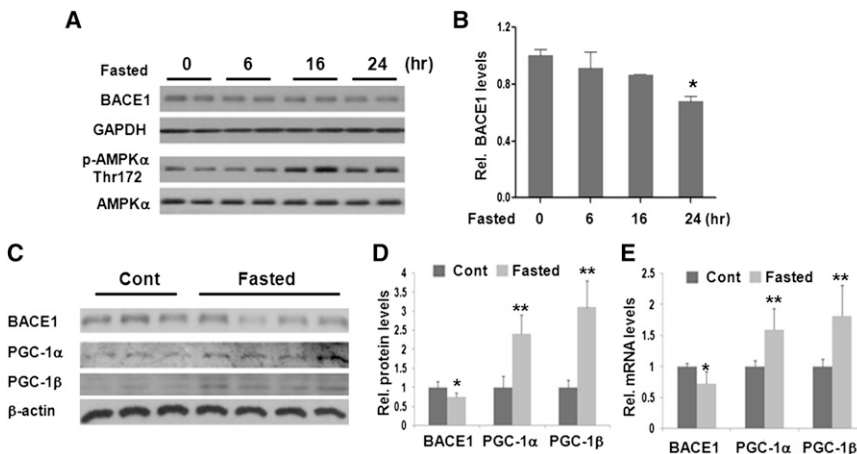
Figure 2. Fasting Reduces BACE1 Expression in Mouse Brains

(A) Fasting reduces BACE1 expression in mouse forebrains at 24 hr time point as determined by western blot (n = 2/time point).

(B) Quantification of (A) based on densitometry.

(C) Western blot analysis of BACE1 and PGC-1α/PGC-1β protein in mouse forebrain after 24 hr of fasting.

(D) Quantification of westerns in (C).

(E) mRNA abundance of BACE1 and PGC-1α/PGC-1β. Data are represented as means ± SD; n = 3–5 mice/group; *p < 0.05.

mediated by PGC-1 on BACE1 protein levels (Figure 4E). DN-SIRT1 coexpression was found to increase PGC-1 acetylation. Under an extreme no glucose (NG) condition, which is known to cause neuronal toxicity, BACE1 transcription and expression were found to be elevated, accompanied by decreased expression levels of SIRT1 and PGC-1 as well as PGC-1 activity (increased acetylation); overexpression of PGC-1 completely reversed the detrimental effect of NG on BACE1 (Figure S4).

Recently, SIRT1 was also reported to activate PPARγ via deacetylation on K268 and K293 in "browning" adipocytes (Qiang et al., 2012). Since PGC-1 was classically defined as the coactivator to PPARγ, we also investigated the role of PPARγ and its deacetylation in BACE1 suppression. In cultured neurons, treatment with rosiglitazone (PPARγ ligand) and GW9662 (irreversible antagonist) led to opposing effects on BACE1 expression in a dose-dependent manner (Figure 5A). Interestingly, PGC-1's suppressive effect was largely abolished by GW9662, but unaffected by rosiglitazone, indicating a ligand-independent mechanism (Figure 5B). In 24 hr fasted mouse brains, the acetylated forms of both PGC-1 and PPARγ were reduced (Figure 5C). Notably, overexpression of nonacetylated PPARγ (K268T and/or K293T), which represents the active form, repressed BACE1 transcription to a similar degree to that of PGC-1 or SIRT1 in a ligand-independent manner (Figure 5D). Surprisingly, overexpression of WT PPARγ did not exert a significant effect on BACE1, indicating that the molecule is not limiting in neurons. In addition, coexpression of SIRT1 with PPARγ resulted in an effect similar to that of PGC-1 or mutant PPARγ. However, coexpression of PGC-1 with WT SIRT1 did not exert a further additive effect. Similar results were observed not only on the endogenous BACE1 mRNAs, but also on the transfected rBACE1-luciferase (Luc) reporter assay (Figure S5). Taken together, these data strongly indicate that SIRT1 is the upstream regulator of PPARγ and PGC-1.

SIRT1, PPARγ, and PGC-1 Directly Interact with the Promoter

To investigate the mechanism by which PPARγ-PGC-1α represses BACE1 transcription, we first investigated if these molecules play a suppressive role on BACE1 via the PPARγ-responsive element (PPRE) identified previously under inflammatory conditions (Sastre et al., 2006). Analysis of the BACE1

promoter identified four potential PPAR-RXR-responsive elements (Figure S6, sites 1–4). We generated a series of deletion mutants for the BACE1 promoter and compared their promoter activities. All of the deletions resulted in significantly increased basal promoter activity with an exception on the F9 construct (Figures 6A and 6B). The findings that constructs F7 and F8 resulted in a 2-fold increase in BACE1 promoter activity suggested that the −1,541/−1,209 region contains elements that negatively regulate BACE1 (e.g., sites 1 and 2). The F9 construct, which lacks the HNF-3 binding site, displayed normal BACE1 promoter activity, as compared to WT F1, suggesting that HNF-3 is a strong positive TF. Further deletion (F2) up to the −753 region regained increased BACE1 promoter activity, indicating the presence of additional negative regulatory elements. Notably, the repressive effect of PGC-1 overexpression was lost from all of the deletion promoters, indicating that PGC-1 suppresses in part through the N-terminal region.

Between sites 1 and 2, only the first site displays a typical PPRE motif. Further disruption of the −1,357/−1,333 PPRE by site-directed mutagenesis led to a 36% increase in luciferase activity. In addition, mutation of the YY1 site-mutated promoter resulted in a 48% reduce in reporter activity (Figures 6C and 6D), consistent with the report that YY1 is a key positive TF for BACE1 (Nowak et al., 2006). Interestingly, when either the PPRE or YY1 site was mutated, PGC-1 overexpression lost its suppressive effect on BACE1 promoter transcription. Downregulation of PGC-1 had no effect on the BACE1-luc with a mutated PPRE. These results strongly indicated that PPRE was the major site at which PGC-1 exerts its regulatory effect on the BACE1 promoter. To seek more direct in vivo evidence of PGC-1's effect on this PPRE, we conducted a chromatin immunoprecipitation (ChIP) assay on the 24 hr fasted mouse brains to show that the SIRT1, PGC-1, and PPARγ proteins are associated with the first PPRE site (Figures 7A and S7A). Indeed, similarly enhanced protein-protein interactions between PGC-1 and SIRT1 and with PPARγ were detected by coimmunoprecipitation in the fasted brains (Figure 7D).

By in vivo ChIP, we further detected increased binding of SIRT1, PPARγ, and PGC-1 on the other three elements (sites 2–4) in the fasted brains (Figures 7B and S7B), with the most prominent enhancement observed on sites 1 and 3. Interestingly, we also detected markedly enhanced binding of a nuclear
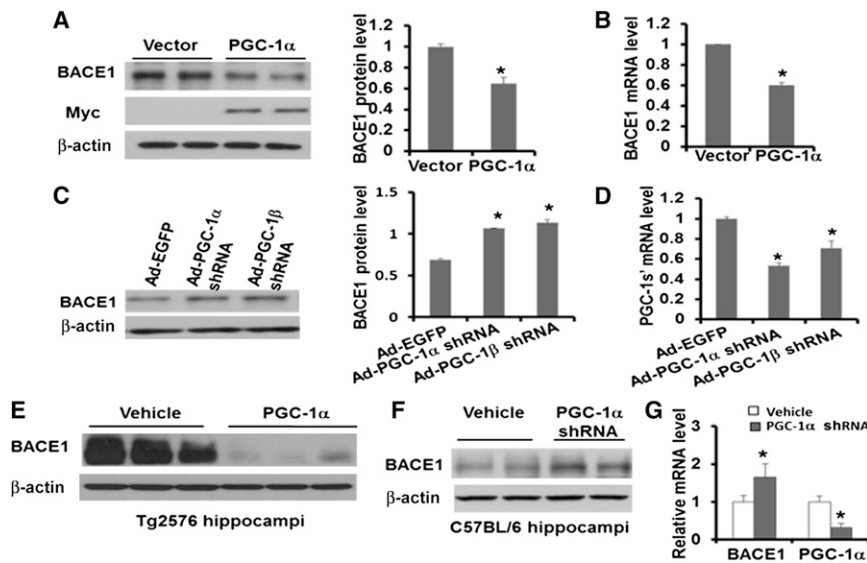
Figure 3. Suppression of BACE1 Expression In Vitro and In Vivo by PGC-1

(A) Effects of overexpressed myc-tagged-PGC-1α on BACE1 protein expression. Protein levels were measured in HEK293 cells 48 hr after transient transfection (2 μg) by western blot analysis. The BACE1 monoclonal antibody (3D5) used was raised in BACE1 null mice. PGC-1α protein was detected by an anti-Myc antibody. Protein levels in the left panel were quantified by densitometry analysis (right panel).

(B) Effect of overexpressed PGC-1α on the endogenous levels of BACE1 mRNA as determined by qRT-PCR 48 hr after transfection.

(C) Downregulation of PGC-1 (PGC-1α or PGC-1β) by shRNA upregulates BACE1 expression in primary neurons. Rat primary neurons (DIV10) were infected with PGC-1 shRNA adenovirus for 48 hr. BACE1 protein expression was detected by western blot and quantified based on data from four experiments. Infection efficiency was monitored by the EGFP signals under microscope and by qRT-PCR.

(D) PGC-1α and PGC-1β mRNA levels following knockdown are shown.

(E) Effects of PGC-1α overexpression and downregulation on BACE1 expression in vivo. AAV2-PGC-1α or AAV2-EGFP (vehicle, 10^9 virus particles in 1 μl) was injected into the CA1 region of Tg2576 mouse hippocampi (n = 5/group, 5-month-old mice), and BACE1 protein was determined by western blot analysis 4 weeks later.

(F) AAV2-PGC-1α-shRNA and AAV2-EGFP (10^{10} viruses particles in 1 μl) were injected into the CA1 of C57BL/6 mice (n = 5/group; 4-month-old mice), and BACE1 level was determined 4 weeks later.

(G) The mRNA levels of BACE1 and PGC-1α in hippocampi as determined from the same animals for the studies in (E) and (F). No cytotoxicity was detected at the time of the in vitro assays. Data are represented as means ± SD from five to seven independent experiments; *p < 0.05, **p < 0.01.

receptor corepressor (NcoR) on sites 1 and 3. Based on our preliminary results, we did not see binding of the other major ligand-independent corepressor SMRT/NCoR2 to the BACE1 promoter, suggesting a corepressor specificity of NCoR in terms of negative regulation of BACE1. Further ChIP analysis was conducted on the three positive sites (HNF-3, Sp1, and YY1). As predicted, none of the binding of these positive transcription factors was significantly altered upon fasting (Figures 7C and S7C), while enhanced PGC-1 was found at the HNF-3 and Sp1 sites. SIRT1 binding was either undetected or unaltered.

DISCUSSION

This work was designed to explore mechanisms through which the altered expression of the components of the SIRT1-PPARγ-PGC-1 pathway may regulate BACE1 expression. Herein, we present in vitro and in vivo evidence for the transcriptional regulation of BACE1 by this dominant metabolic signaling pathway under basic and metabolic stress conditions. Our findings provide a fresh molecular basis for metabolic factors regulating a crucial and rate-limiting enzyme in AD pathogenesis. Given the increasing recognition of several major cardio-metabolic risk factors (e.g., central nervous system [CNS] insulin resistance, high cholesterol, ApoE genotype, insufficient eNOS, etc.) in accelerating AD development and progression, the unique transcriptional regulation of BACE1 identified here may represent a unifying and central mechanism for aberrant amyloidogenesis induced by increased metabolic stress.

PGC-1α was classically defined as a transcription coactivator that interacts with a broad range of TFs that participate in many biological processes, including adaptive thermogenesis, mitochondrial biogenesis, glucose/fatty acid metabolism, muscle fiber-type switching, and heart development (Lin et al., 2005). Dysregulated PGC-1α has been implicated in pathogenic conditions such as obesity, type 2 diabetes, and cardiomyopathy. The evidence for PGC-1α transcriptional interference in neurodegeneration was initially supported by the discovery of Huntington-like striatal degeneration in PGC-1-deficient mice (Cui et al., 2006; Lin et al., 2004; Weydt et al., 2006). Its role was recently extended to Parkinson's disease (Clark et al., 2011; Zheng et al., 2010) and to non-neuronal lineages (Tsunemi and La Spada 2012; Xiang et al., 2011). However, little is known of its role in AD other than reduced expression in the brains of AD patients and Tg2576 mice with insulin resistance induced by a HFD (Ho et al., 2004; Katsouri et al., 2011; Qin et al., 2009). Both PGC-1 isoforms (PGC-1α and PGC-1β) are abundantly expressed and widely distributed in the brain and may be interchangeable for certain functions, including neuronal mitochondrial biogenesis (Wareski et al., 2009), as well as in BACE1 transcriptional regulation as reported here. Recent studies in CaMKII-specific PGC-1α conditional knockout (KO) mice (Ma et al., 2010) suggested a crucial role for PGC-1 in neuronal function with forebrain neurons as an important and integral part of the neural circuitry in governing energy balance.

Caloric restriction has been shown to induce multiple changes in glucose metabolism and extend lifespan in a broad spectrum of species, possibly attributable to the activated AMPK-SIRT1-PGC-1 signaling pathway. In the liver, SIRT1, the NAD-dependent protein deacetylase, controls the gluconeogenic/glycolytic pathways in response to fasting through interaction with and
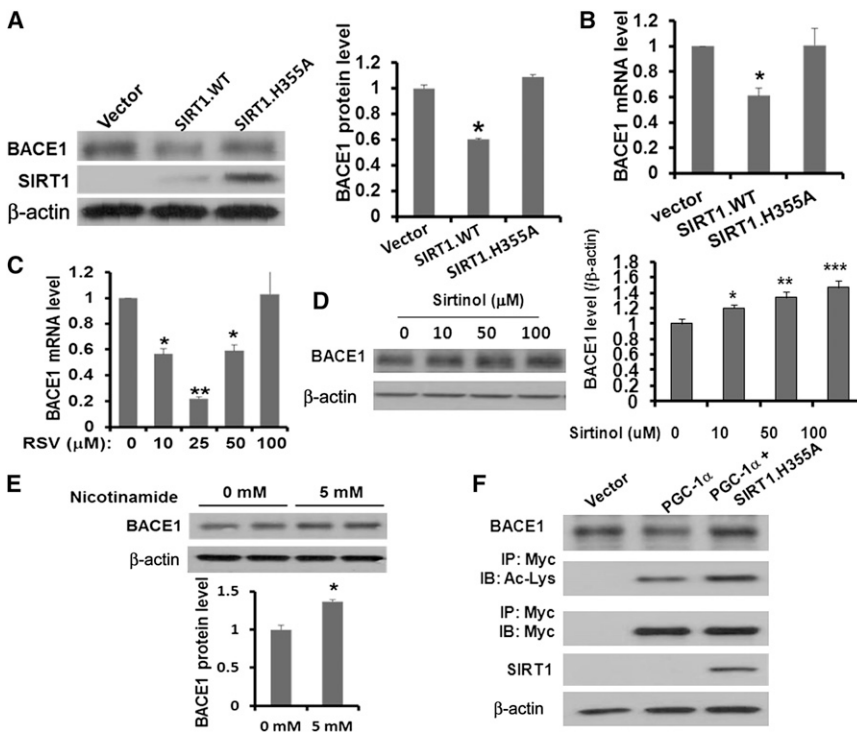
Figure 4. PGC-1 Suppression of BACE1 Requires SIRT1 Deacetylase Activity

(A) Effects of overexpressed WT and a dominant-negative mutant SIRT1 (SIRT.H355A) on BACE1 protein expression HEK293 cells. Protein levels were detected in HEK293 cells 48 hr after transient transfection.

(B) The endogenous BACE1 mRNA levels upon SIRT1 transfection were determined by qRT-PCR and quantified based on three independent experiments.

(C–E) Effects of resveratrol (C), sirtinol (D), and nicotinamide (E) at various concentrations on BACE1 mRNA or protein levels in primary neurons 6 hr after treatment. Data are represented as means ± SD from three independent experiments; *p < 0.05; **p < 0.01.

(F) Effect of SIRT1-mediated deacetylation of PGC-1β on BACE1 suppression. Myc-tagged PGC-1β was cotransfected with dominant-negative mutant SIRT1 in HEK293 cells, and protein levels were determined 48 hr later. PGC-1 acetylation was detected after immunoprecipitation with an anti-Myc antibody and then probed with anti-acetylated lysine antibody (Cell Signaling Technology).

deacetylation of PGC-1α at specific lysine residues in an NAD(+)-dependent manner (Rodgers et al., 2008). These findings regarding the basic pathways of energy homeostasis in the liver appear to be replicated in the brain during the regulation of BACE1. Under nonfasting conditions, PGC-1α is expressed at very low levels in the brain but PGC-1α is induced by fasting. The expression and activity of PGC-1α in the brain are modulated by transcriptional activation as well as by SIRT1-mediated deacetylation, which increases PGC-1 activity. Like PGC-1, SIRT1 has emerged as a major regulator of mammalian transcription in response to cellular metabolic status and stress. It has been shown to positively regulate α-secretase promoter transcription, leading to reduced amyloid deposition (Donmez et al., 2010). As with exercise (Lazarov et al., 2005), calorie restriction mitigated excessive amyloidogenesis in Tg2576 brains via activation of α-secretase through SIRT1-mediated transcriptional activation of FoxO3a (Qin et al., 2008).

Is Transcriptional Dysregulation of BACE1 a Crucial Mechanism in SAD?

Despite overwhelming evidence of robust transcriptional activation of BACE1 in response to various stress conditions in numerous experimental models, including both cellular and rodent AD, the majority of earlier studies failed to detect significant elevation of the mRNA level of BACE in AD patient brains (Gatta et al., 2002; Preece et al., 2003; Yasojima et al., 2001), with one exception (Li et al., 2004). In addition, a recent large-scale, candidate-gene SNP-expression screen showed comparable *BACE1* expression in the cortex of late-onset AD/LOAD cases and age-matched controls (10.815 ± 0.038 in 187 cases of control versus 10.763 ± 0.039 in 176 cases of LOAD) (Webster et al., 2009). Since neuron density is much lower in AD cases, a

stable expression value actually indicates higher expression per neuron. The discrepancy between experimental models (Tg mice and cultured neurons) versus primary AD brain specimens may also arise in part from the sporadic nature of the majority of AD cases, with *BACE1* being tightly regulated by multiple mechanisms that are not necessarily mutually exclusive. It should be noted that one recent report found significantly increased BACE1 mRNAs in freshly isolated peripheral blood mononuclear cells (PBMCs) from a large cohort of AD patients, as compared to normal controls (Marques et al., 2012).

Although our data clearly indicate transcriptional regulation of BACE1 as the dominant event in response to metabolic stress, we cannot rule out the possibility of translational regulation. In a previous report, the treatment of mice with an acute energy inhibitor only increased BACE1 protein levels, but not mRNA levels, primarily due to elevated eIF2α phosphorylation via a translational control mechanism. Elevated p-eIF2α and BACE1 levels were also observed in 5XFAD transgenic mice and in human AD brains (O'Connor et al., 2008). It should be pointed out that the translational upregulation of BACE1 upon energy depletion using toxins to mitochondrial respiratory chains may be mechanistically different from fasting or calorie restriction used in our studies in terms of elicited changes in signaling pathways though PGC-1. We did not detect a significant increase in p-eIF2α in the mouse brains after HFC feeding. Nevertheless, the two major transcriptional and translational mechanisms need not be mutually exclusive, and it is unclear which mechanism may play a more important role in sporadic AD pathogenesis. Moreover, metabolic stress may elicit additional regulatory mechanisms posttranscriptionally (microRNA) (Wang et al., 2008) and posttranslationally (ubiquitination, S-nitrosylation, and oxidation) (Kwak et al., 2011). Together with
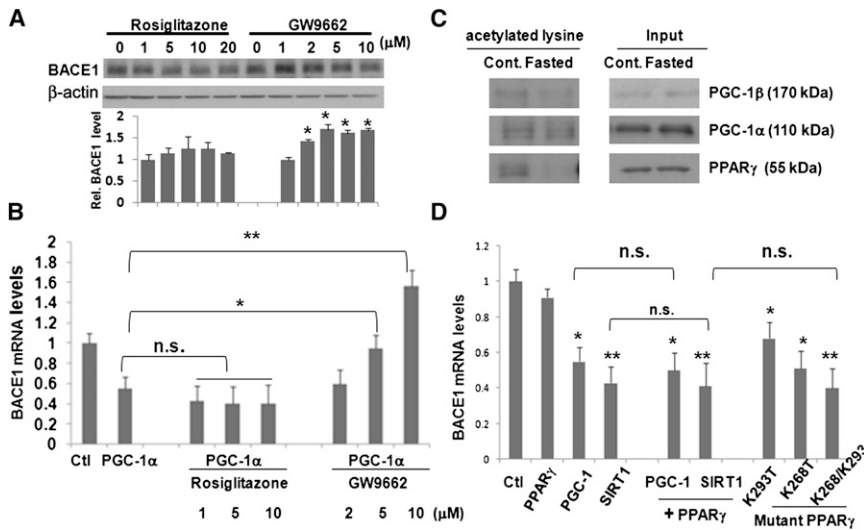
Figure 5. PGC-1's Suppressive Effect Requires Active PPARγ and Involves SIRT1-Mediated Deacetylation

(A) Opposing effects of rosiglitazone and GW9662 on BACE1 expression levels. Western blot analysis was conducted at 6 hr after treatment in neurons.

(B) PGC-1α effect is dependent on active PPARγ but not on its ligand. Rosiglitazone or GW9662 compounds were added to the HEK293 cells 24 hr after transfection with PGC-1α plasmid, and BACE1 gene transcription was determined 6 hr later by qRT-PCR. Data are represented as means ± SD from three independent experiments; *p < 0.05.

(C) Acetylation status of PPARγ, PGC-1α, and PGC-1β was determined by western blotting.

(D) PPARγ represses BACE1 in its active, non-acetylated form. Various plasmids were transfected, alone or in combination, into HEK293 cells, and BACE1 qRT-PCR was conducted 24 hr later. For (C) and (D), data are represented as means ± SD from three independent experiments; *p < 0.05, **p < 0.01. "n.s." indicates statistically nonsignificant.

recently disclosed epigenetic DNA methylation (Marques et al., 2012), BACE1 has proven to be tightly regulated by multiple mechanisms. Lastly, BACE1 protein degradation is emerging as a potentially important regulatory mechanism, and PGC-1α was reported to facilitate BACE1 protein degradation via the ubiquitin proteasome system (UPS) (Gong et al., 2010), which may also reflect one of the multiple effects of this key regulatory molecule. Interestingly, we also did not detect changes in the GGA3 protein upon metabolic stress, a mechanism discovered in response to cerebral ischemia and brain injury (Walker et al., 2012).

Perhaps due to technical limitations, several earlier studies did not see evidence of BACE1 transcriptional regulation under HFD-induced insulin resistance or by resveratrol (Ho et al., 2004; Vingtdeux et al., 2010). Of particular interest, the anti-amyloidogenic effect of resveratrol was attributed to the AMPK-mediated signal modulation of mTOR-autophagy, thereby facilitating BACE1 protein degradation and clearance (Dasgupta and Milbrandt 2007). Our study, based on the different experimental designs (NG, fasting, and HFC), has unequivocally demonstrated BACE1 transcriptional alteration as a key response to metabolic stress. Among the major components of the identified transcription circuitry and network are the PGC-1α-assisted PPARγ transcription complexes, which act via direct interaction with the PPRE located in the BACE1 promoter. We have also identified a molecular mechanism whereby SIRT1 functions in glucose homeostasis as a modulator of both PPARγ and PGC-1α. Enhanced functional interaction between SIRT1-PGC-1α, along with PPARγ-PGC-1α, has been found in fasting brains as detected by immunoprecipitation of molecular complexes (Figure 7D). These functional interacting partners have been identified as required to mediate nutrient control of glucose homeostasis in peripheral tissues (Rodgers et al., 2008). Hence, our findings demonstrate that the basic mechanisms of energy homeostasis can regulate BACE1.

Unique Molecular Mechanism Repressing BACE1 Transcription

This is a unique report of transcriptional inhibition by PPARγ-PGC-1α. The standard view of PPARγ-PGC-1-mediated transcription involves transcriptional upregulation of genes in a ligand-dependent manner. However, there are several reports suggesting that increases in PGC-1α levels can negatively impact the expression of certain genes via indirect and poorly understood mechanisms (Estall et al., 2009; Jeong et al., 2009; Zhang et al., 2004). One unresolved issue is how PPARγ-PGC-1 is able to repress transcription. There are several possible mechanisms: (1) The peroxisome proliferator responsive elements (PPREs) in the BACE1 promoter serve as negative PPREs and convert PPARγ into a transcriptional repressor. (2) PPARγ-PGC-1 act as scaffolding proteins rather than TF and coactivator, thereby allowing reverse recruitment of corepressors. Based on literature, PPARγ can either functionally antagonize certain positive TFs to repress transcription or recruit corepressors (Cohen 2006, Perissi et al., 2010). (3) SIRT1 represses PPARγ by docking with corepressors (NCoR and SMRT) as in adipocytes (Picard et al., 2004). (4) There are other critical proteins bound on the BACE1 promoter, such as HNF-3, YY1, or SP1, that convert PGC-1α into a transcriptional repressor. Our preliminary work has explored these possibilities and ruled out the PPRE. When two copies of each of the PPAR-RXR elements (sites 1–4) in the BACE1 promoter were ligated 5′ to the luciferase gene, none of these elements were able to repress expression of the luciferase reporter (F.-F.L., unpublished data). With respect to the involvement of corepressors, we found that NCoR was recruited into the promoter complexes with PPARγ, PGC-1, and SIRT1, and this recruitment was independent of rosiglitazone addition. Upon fasting, the association of both PGC-1 and NCoR was enhanced on the BACE1 promoter (Figure 7B). This finding supports the second and third possibilities, which are not mutually exclusive. The strongest finding of this work is on the coexistence of both corepressor NCoR and
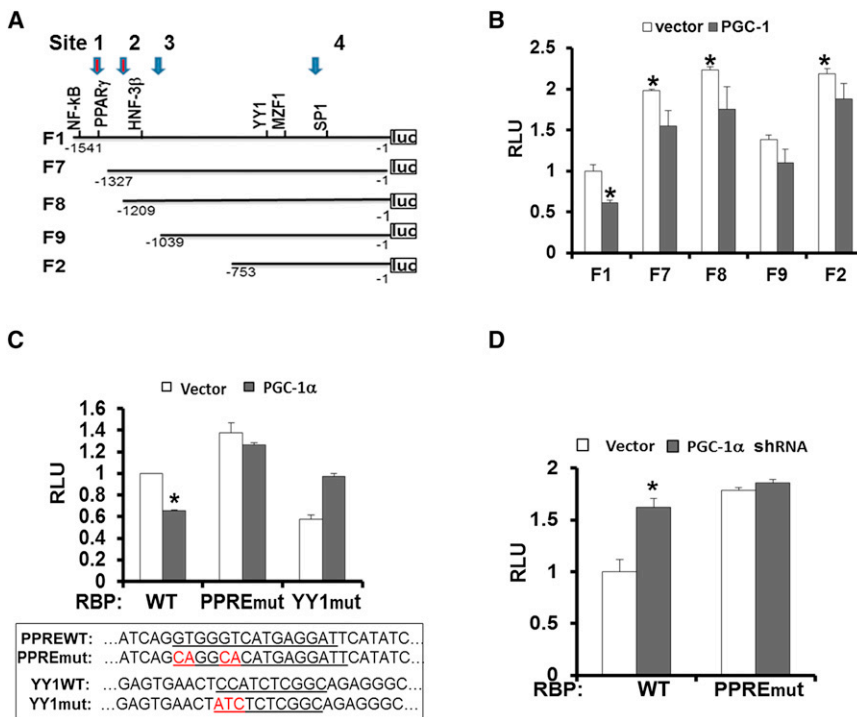
Figure 6. PGC-1's Suppressive Effect Is Largely Dependent on the First PPRE Site

(A) Schematic diagram of BACE1 promoter (rat, 1.54 kb) in pGL3-basic luciferase construct. Constructs F2–F9 represent four BACE1 promoter deletions.

(B) Effects of transfecting PGC-1α on BACE1 promoter activity. Relative BACE1 promoter activity from the various deletion constructs is presented as compared to full-length BACE1-luc (F1). Data are represented as means ± SD from five independent experiments; *p < 0.05.

(C) Effects of PPRE and YY1 mutants on BACE1 promoter activity. PGC-1α was cotransfected with rat BACE1 promoter (WT) or its mutants that are at PPRE or YY1 sites into HEK293 cells. Luciferase assay was performed 24 hr after transfection. Relative BACE1 promoter activity from the mutants and the effects of PGC-1α are presented as compared to pcDNA3.1 vector-cotransfected BACE1-luc (WT) activity.

(D) Effect of the PPRE-mutated promoter on PGC-1 in BACE1 suppression. AAV-PGC-1α shRNA was cotransfected with rat BACE1 promoter and its PPRE mutant into HEK293 cells for 24 hr. Data are presented as means ± SD from at least three independent experiments; *p < 0.05.

coactivator PGC-1 in a single complex. Moreover, when activated by deacetylation as demonstrated by the K mutants, PPARγ negatively regulated BACE1 transcription. Since we observed specific effects from overexpressing the mutant PPARγ at K268T and K293T in suppressing BACE1 transcription, and SIRT1 is known to deacetylate from these two residues, SIRT1 is likely responsible for the PPARγ activation in our case. However, it is not clear whether such deacetylation is ligand dependent as reported in adipocytes (Qiang et al., 2012); our data argue against a ligand-independent mechanism, likely reflecting a cell-type-specific mechanism in neurons. It is also unclear whether PPARγ activation by SIRT1 is required to recruit the corepressor NCoR. Although we detected in vivo binding of SIRT1, PPARγ, and PGC-1 on the multiple nuclear receptor response elements, with most prominent binding found on sites 1 and 3, PGC-1 was also found at the HNF-3 and Sp1 sites. There has been one previous report that resveratrol inhibited expression of the angiotensin II type 1 receptor gene through Sp1, raising the possibility that SIRT1 may inhibit BACE1 in part through Sp1 (Miyazaki et al., 2008). Based on the data we collected, we speculate a loop topology as illustrated in the Graphical Abstract in a simplified format: binding of the common factors to sites 1 and 3 and to HNF-3 may present the recruited corepressor to the positive TF. It is not yet known whether the corepressor NCoR is also recruited to these positive TFs and if its recruitment requires, or is assisted by, PGC-1.

Although the complex regulatory mechanism, in terms of the interplay between these molecules, warrants further investigation, our work strongly suggests that targeting BACE1 at the transcriptional level may be a viable approach for SAD, as demonstrated in a FAD mouse model using lenti-siRNA, leading to a complete reversal of excessive amyloidogenesis and neurodegeneration (Singer et al., 2005). Although our results based on

administration of the AAV2-PGC-1α viruses are preliminary due to a small number of Tg2576 mice (Figure 3), they show promise of the therapeutic value of overexpressing or activating PGC-1 in blunting amyloidogenesis in a FAD model. Furthermore, virus-mediated gene delivery of SIRT1 and PGC-1 have demonstrated proof of concepts in halting neurodegeneration in Huntington disease (HD) and AD mouse models (Jeong et al., 2012; Kim et al., 2007; Tsunemi and La Spada, 2012; Tsunemi et al., 2012). Our major findings on the molecular pathway under various metabolic stress conditions further suggest a promise of generalizing a PGC-1-based approach in SAD. Therefore, further characterization of the transcription network involving PGC-1α, SIRT1, and the BACE1 promoter will further validate the metabolic factors regulating this important gene in Alzheimer's etiology. More importantly, the study outcomes may be instrumental in future therapeutic design regarding targeting SAD.

EXPERIMENTAL PROCEDURES

Cell Culture, Drug Treatments, Plasmid Transfection, and Virus Infection

Rat primary cortical neurons (PRCN) were prepared as described (Chen et al., 2009). Glucose deprivation was performed with Dulbecco's modified Eagle's medium (DMEM; GIBCO) without glucose or Neurobasal medium without glucose overnight. Treatments with 10–100 μM resveratrol or 5 mM nicotinamide (Sigma-Aldrich) were performed overnight at 37°C. Transient transfections were performed using Lipofectamine 2000 (Invitrogen) with plasmids: pcDNA-PGC-1α and pcDNA-PGC-1β (Addgene); pcDNA4 TO-Myc.His-PGC-1α or β; pcDNA-SIRT1.WT and its mutant SIRT1.H355A. pGL3-basic luciferase reporter constructs and the site-directed mutagenesis was performed as described in our previous work (Chen et al., 2009; Kwak et al., 2011).

In Vivo Experiments on Mice

All animal care protocols and procedures were performed in accordance with the Animal Scientific Procedures Act and with the approval of the University
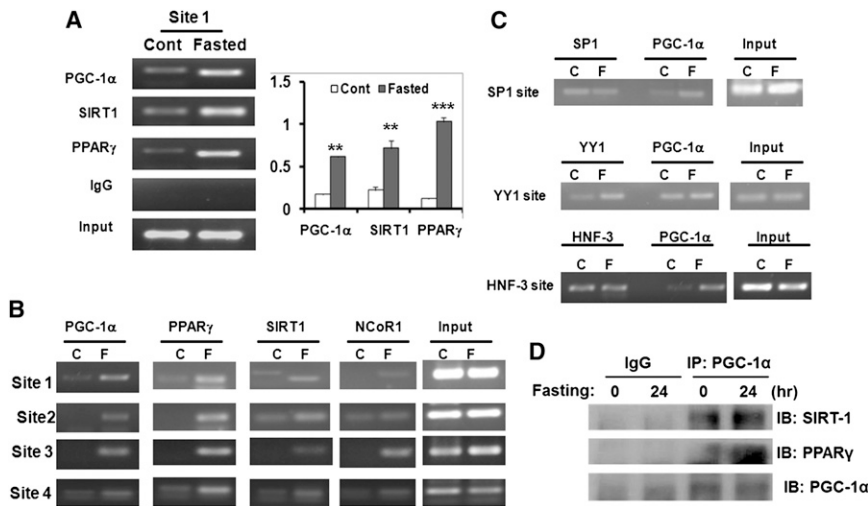
Figure 7. Enhanced In Vivo Binding of SIRT1, PPARγ, PGC-1α, and Corepressor NCoR to Multiple Sites of the BACE1 Promoter Region

(A) In vivo ChIP assay detected increased binding of endogenous PGC-1α, SIRT1, and PPARγ in the protein complex to the first PPRE site in mouse brains upon 24 hr fasting as compared to nonfasted samples. Frontal cortical tissues were pooled from four mice per group. **p < 0.01, ***p = 0.005.

(B and C) Representative results from the in vivo ChIP assay showing binding on sites 1–4 (B) and on the Sp1, YY1, and HNF-3β sites (C) using the same 24 hr fasted mouse brains.

(D) Enhanced SIRT1-PGC-1 and PPARγ-PGC-1α interactions upon fasting. Fasted and control mouse forebrain lysates were immunoprecipitated by anti-PGC-1α antibody followed by western blot probing with either SIRT1 or PPARγ antibody.

of Tennessee Animal Care and Use Committee. High-fat, high-cholesterol (HFC) custom diet containing 21% fat and 1.25% cholesterol (D12079B, Teklad, Harlan Laboratories) was given to C57BL/6 mice from a 5-month-old group (n = 5/group) for 2 months and compared to a control chow diet. AAV2/AAV5 PGC-1α-small hairpin RNA (shRNA; 3.87 × 10^13 vg/ml, 5′-GGTGGATTGAAGTGGTGTAGA-3′) and AAV2/5-enhanced green fluorescent protein (EGFP; 2.56 × 10^13 vg/ml) were generated by the Virus Core Facility at Iowa University, and AAV2/1 viruses overexpressing PGC-1α were generated in-house (2 × 10^12 vg/ml). All AAV2 viruses were injected into the CA1 region of mouse hippocampus in 1 μl of volume, and mice were euthanized 21 days later for analysis.

Immunoprecipitation and Immunoblot
The procedures were performed as described (Chen et al., 2009; Kwak et al., 2011) using the following antibodies: mouse anti-BACE1 (3D5); mouse anti-Myc and mouse anti-β-actin (Sigma-Aldrich); Rabbit anti-SIRT1 (Millipore); mouse anti-PPARγ (81B8); rabbit polyclonal antibodies against p-AMPKα, AMPKα, acetylated lysine, p-eIF2α, and eIF2α, GGA3 (Cell Signaling Technology); and mouse anti-PGC-1α (H-300; Santa Cruz).

Quantitative RT-PCR on BACE1 and PGC-1 Messages
The procedures were performed as described using the same primers for rat and human bace1 (Chen et al., 2009; Kwak et al., 2011). Rat pgc-1α primers (forward 5′-AAAGGGCCAAGCAGAGAGA-3′ and reverse 5′-GTAAATCACACGGCGCTCTT-3′), rat pgc-1β primers (forward 5′-TTGACAGTGGGAGCTTTGTGG-3′ and reverse 5′-GGGCTTATATGGAGGTGTGG-3′), human pgc-1α primers (forward 5′-TTATTGGGAAATGCCTCCTG-3′ and reverse 5′-GGGTCATTTGGTGACTCTGG-3′), mouse pgc-1α primers (forward 5′-GAAAGGGCCAAACAGAGAGA-3′ and reverse 5′-GTAAATCACACGGCGCTCTT-3′), and mouse pgc-1β primers (forward 5′-CTCCAGTTCCGGCTCCTC-3′ and reverse 5′-CCCTCTGCTCTCACGTCTG-3′) were used in the present studies. Primers used for rat GAPDH, forward 5′-ACATTGTTGCCATCAACGAC-3′, reverse 5′-CTTGCCGTGGGTAGCGTCAT-3′; human GAPDH primers, forward 5′-AATCCCATCACCATCTTCC-3′ and reverse 5′-GGACTCCACGACGTACTCA-3′; mouse GAPDH primers, forward 5′-GGGTTCCTATAAATACGGACTGC-3′ and reverse 5′-CCATTTTGTCTACGGGACGA-3′.

Promoter Deletion Constructs and Luciferase Assay
Deletion constructs and luciferase assays were conducted as described (Chen et al., 2009). PGC-1α or SIRT1 expression vectors or PGC-1α shRNA plasmids were cotransfected into HEK293 cells; 24 hr later, cells were collected in passive lysis buffer and analyzed for luciferase activity. No-glucose treatment was performed 5 hr after transfection.

ChIP assays
Chromatin immunoprecipitation (ChIP) assays were performed using the kit from Upstate according to the manufacturer's instructions. The transfected

HEK293 cells were crosslinked with 1.0% formaldehyde and collected in lysis buffer. The following primers were used in PCR assays: for site 1, forward 5′-GAGTAATGTTGGTATGCCTC-3′ and reverse 5′-GGGATGAGAGTATGTCAGTC-3′; for site 2, forward 5′-GCTCCTCCAGTCTCTACTCC-3′ and reverse 5′-GACTACATAGAGAAACTCTG-3′. For detecting in vivo protein bindings, mouse forebrain lysates (20 mg/immunoprecipitation) were used.

Statistics
All quantitative data are presented as means ± SD. Comparisons between groups were analyzed with t test.

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