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Rosiglitazone activation of PPARγ-dependent pathways is neuroprotective in human neural stem cells against amyloid-beta–induced mitochondrial dysfunction and oxidative stress

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ABSTRACT

Neuronal cell impairment, such as that induced by amyloid-beta (Aβ) protein, is a process with limited therapeutic interventions and often leads to long-term neurodegeneration common in disorders such as Alzheimer's disease. Interestingly, peroxisome proliferator–activated receptor gamma (PPARγ) is a ligand-activated nuclear receptor whose ligands control many physiological and pathologic processes, and may be neuroprotective. We hypothesized that rosiglitazone, a PPARγ agonist, would prevent Aβ-mediated effects in human neural stem cells (hNSCs). Here, we show that rosiglitazone reverses, via PPARγ-dependent downregulation of caspase 3 and 9 activity, the Aβ-mediated decreases in hNSC cell viability. In addition, Aβ decreases hNSC messenger RNA (mRNA) levels of 2 neuroprotective factors (Bcl-2 and CREB), but co-treatment with rosiglitazone significantly rescues these effects. Rosiglitazone co-treated hNSCs also showed significantly increased mitochondrial function (reflected by levels of adenosine triphosphate and Mit mass), and PPARγ-dependent mRNA upregulation of PGC1α and mitochondrial genes (nuclear respiratory factor-1 and Tfam). Furthermore, hNSCs co-treated with rosiglitazone were significantly rescued from Aβ-induced oxidative stress and correlates with reversal of the Aβ-induced mRNA decrease in oxidative defense genes (superoxide dismutase 1, superoxide dismutase 2, and glutathione peroxidase 1). Taken together, these novel findings show that rosiglitazone-induced activation of PPARγ-dependent signaling rescues Aβ-mediated toxicity in hNSCs and provide evidence supporting a neuroprotective role for PPARγ activating drugs in Aβ-related diseases such as Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is the most frequent degenerative neurologic disorder affecting more than 30 million people worldwide (Ferri et al., 2005). The clinical characteristics of AD include gradual memory loss, cognitive impairment, emotional disturbances and progressive deterioration of language skills, psychiatric issues, and dementia (Blennow et al., 2006). Neurodegenerative diseases such as AD are characterized by the progressive region-specific loss of neurons in the brain, including the hippocampus, cortex, and amygdala (Bobinski et al., 1997; Whitehouse et al., 1982). Neuronal dysfunction begins in the hippocampus and cortex, leading to early stage hypofunction, and eventually to long term-cognitive deficits and dementia commonly associated with AD. The disorder is characterized by neuropathologic markers such as amyloid-beta (Aβ) protein, which trigger a complex cascade

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leading to the neurodegenerative profiles observed in AD (Hardy and Selkoe, 2002; Lesne et al., 2006). AD is positively correlated with accumulation in the brain of pathogenic Aβ that progressively abolishes neuronal networks and promotes synaptic loss, microglial activation, mitochondrial dysfunction, oxidative stress, and neuroinflammation, which ultimately results in neuronal death (Butterfield, 2002; Heneka and O'Banion, 2007; Landreth and Heneka, 2001; Zhu et al., 2007).

Impaired neurogenesis in the brain is correlated with the pathogenesis of AD, suggesting that AD progression is linked with the levels of cognitive impairments and neurodegeneration present in the brain (Rodriguez et al., 2008; Verret et al., 2007). It is suggested that neurologic dysfunction associated with AD may be due to impairment of hippocampal neural stem cell (NSC) function (Li et al., 2008). Zheng et al. (Zheng et al., 2013) demonstrated that intrahippocampal injection of Aβ decreased hippocampal neurogenesis in the adult mouse brain. Indeed, Aβ was toxic and increased the percentage of apoptotic cells in treated human neural progenitor cells (Haughey et al., 2002) and human neural stem cells (hNSCs) (Chiang et al., 2013). Therefore, strategies to improve NSCs and neural progenitor cells survival and function may be beneficial for the treatment of neurodegenerative diseases such as AD.

As a member of the nuclear receptor superfamily of ligand-dependent transcription factors, peroxisome proliferator–activated receptor (PPAR)γ plays an important role in the control of glucose homeostasis, insulin sensitivity, lipogenesis, cell differentiation, anti-oxidative stress, and neuroprotection (Kariharan et al., 2015; Landreth and Heneka, 2001; Lehrke and Lazar, 2005; Sato et al., 2011; Wang et al., 2012). Importantly, PPARγ is implicated in AD (Landreth, 2006, 2007; Landreth and Heneka, 2001; Landreth et al., 2008; Nicolakakis and Hamel, 2010). In this study, the effect of a PPARγ agonist on hNSC survival, mitochondrial dysfunction, and oxidative stress was assessed. Our findings provide novel evidence that PPARγ is a promising drug target for the treatment of AD.

2. Materials and methods

2.1. Cell culture

GIBCO hNSCs were originally obtained from National Institutes of Health–approved H9 (WA09) human embryonic stem cells. The medium Complete StemPro NSC serum-free medium (SFM) was used for optimal growth and expansion of GIBCO hNSCs, as well as kept the NSCs undifferentiated as described previously (Chiang et al., 2012b). StemPro NSC SFM complete medium consists of KnockOut D-MEM/F-12 with 2% StemPro Neural Supplement, 20 ng/mL of epidermal growth factor, 20 ng/mL of basic fibroblast growth factor, and 2-mM of GlutaMAX-I.

2.2. Evaluation of cell growth

Cell viability was assayed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide) absorbance and cell count as reported elsewhere (Chiang et al., 2012b). Synchronized hNSCs were treated as described with or without Aβ1–42 synthetic peptide (Millipore Billerica, MA, USA; 5 μM), PPARγ agonist rosiglitazone (10 μM), or PPARγ antagonist GW9662 (20 μM; both from Cayman Chemicals, Ann Arbor, MI, USA) for 3 days. MTT solution (Sigma, Austin, TX, USA) was added to the culture medium for incubating the cells, and absorbance at 570 nm was measured in solubilized cells using an EZ Read 400 ELISA Reader (Biochrom, Holliston, USA). The cell growth rate was expressed as a percentage of values obtained in vehicle control (CON).

2.3. Caspase activity assay

Caspase activity assay was carried out using caspase-3-like (DEVD-AFC) Fluorometric Protease Assay Kit (Chemicon, MI, USA). In brief, hemisected fresh cells were homogenized in the lysis buffer for 10 minutes. The cellular lysate (standardized to protein concentration) was incubated with an equal volume of 2 × reaction buffer (with 0.01 M of dithiothreitol) for an additional 1 hour at 37 °C with caspase-3 substrates (DEVD-AFC) at a final concentration of 50 μM. Fluorescence was measured by a microplate reader with an excitation filter of 390 ± 22 nm and an emission filter of 510 ± 10 nm.

2.4. RNA isolation and quantitative real-time polymerase chain reaction

Total RNA was isolated and complementary DNA synthesis reactions were performed as described previously (Chiang et al., 2011). A real-time quantitative polymerase chain reaction (PCR) was performed using a TaqMan kit (PE Applied Biosystems, Foster City, CA, USA) on a StepOne quantitative PCR machine (PE Applied Biosystems) using heat-activated TaqDNA polymerase (AmpliTaq Gold; PE Applied Biosystems). The sequences of primers were as follows: PPARγ (Forward (F): 5′-AAAGAAGCCGACACTAAACC-3′ and Reverse (R): 5′-CTTCCATTACGGAGAGATCC-3′); Bcl-2 (F: 5′-ACTTTGCAGAGATGTCCAGT-3′ and R: 5′-CGGTTCAGGTACT CAGTCAT-3′); CREB (F: 5′-CCAAGCT- TATGGATCCTCCTGGAGAGAA GATGG-3′ and R: 5′-GCCTCGAGAAGCACAT- TGACGCTCCTGAC-3′); PGC1α (F: 5′-TGAGAGGGCCAAGCAAAG-3′ and R: 5′-ATAAATCA CACGGCGCTCTT-3′); NRF1 (nuclear respiratory factor-1; F: 5′-CCAT CTGGTGGCCTGAAG-3′ and R: 5′-GTGCCTGGGTCCATGAAA-3′); mitochondrial transcription factor A (Tfam) (F: 5′-GAACAA CTACCCATATT- TAAAGCTCA-3′ and R: 5′-GAATCAGGAAGTTCCCTC CA-3′); superoxide dismutase (SOD1; F: 5′-AAGGCCGTGTGCGTG CTGAA-3′ and R: 5′-CAGGTCTCCAACATGCCTCT-3′); SOD2 (F: 5′-GC ACATTAACGCGCAGTCA-3′ and R: 5′-AGCCTCCAGCAACTCTCCTT-3′);

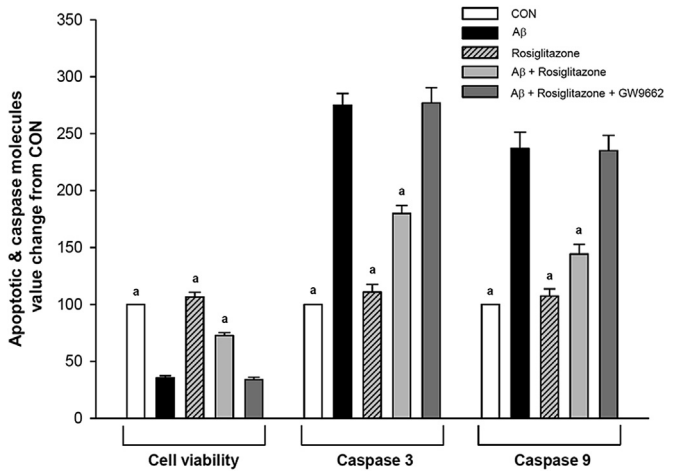

Fig. 1. Rosiglitazone rescue of the Aβ-mediated decrease in hNSCs viability is PPARγ dependent. hNSCs were treated with 5-μM Aβ for 24 hours then treated with the indicated reagents (10-μM rosiglitazone or 20-μM GW9662) for another 48 hours, and cell viability was detected by MTT assay. All reactions were run in triplicate in 3 independent experiments. Cell viability is expressed as a percentage of the indicated transcript in CON and is presented as the mean ± SEM values. Caspase activities were detected by a fluorometric protease assay using substrates for caspase-3-like (DEVD-AFC) and caspase-9-like (LEHD-FMK). Caspase 3 and 9 are expressed as the mean ± SEM values. [a]Specific comparison to the indicated hNSCs with Aβ ($p < 0.001$; one-way ANOVA). Abbreviations: Aβ, amyloid-beta; ANOVA; analysis of variance; hNSCs, human neural stem cells; PPARγ, peroxisome proliferator-activated receptor gamma; SEM, standard error of the mean.

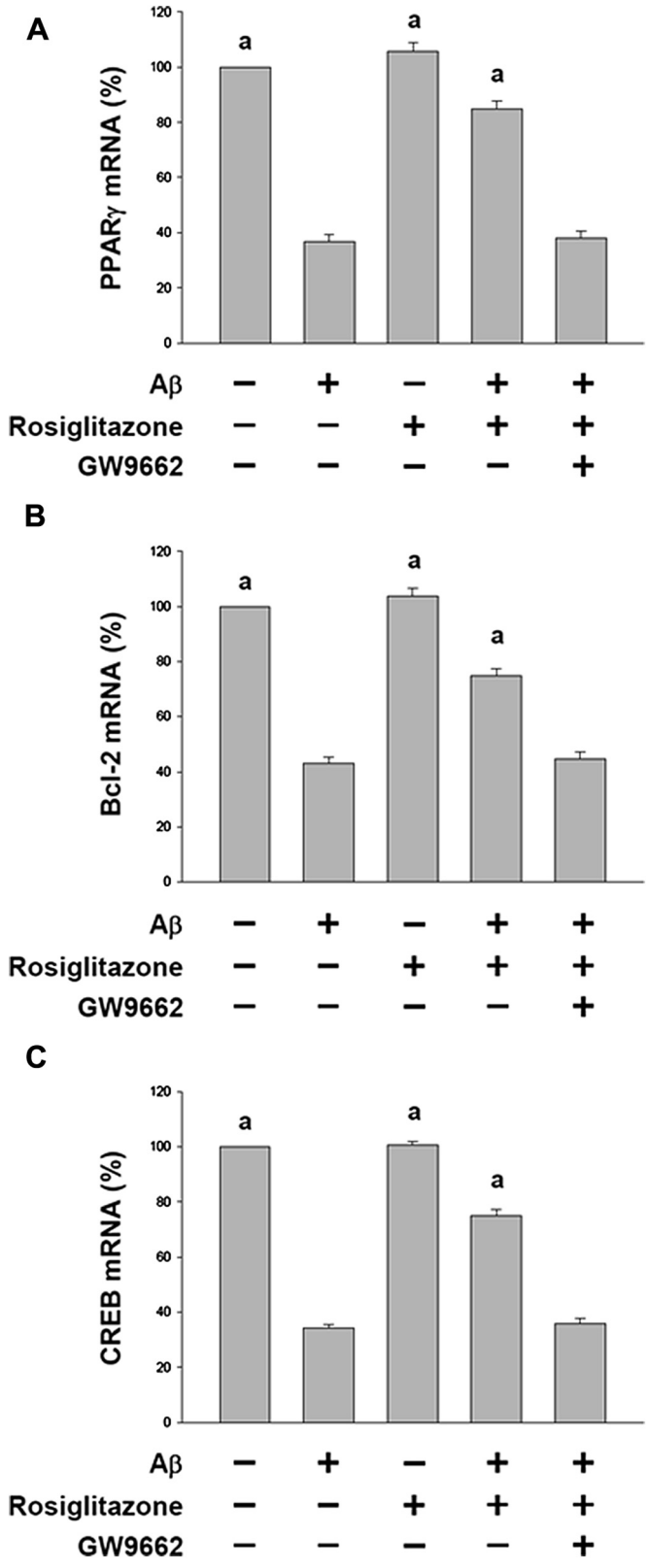

Fig. 2. Rosiglitazone increased mRNA expression levels of PPARγ, Bcl-2, and CREB transcripts in hNSCs treated with Aβ. hNSCs were treated with 5-μM Aβ for 24 hours then treated with the indicated reagents (10-μM rosiglitazone or 20-μM GW9662) for another 48 hours. The PPARγ (A), Bcl-2 (B), and CREB (C) transcripts in the indicated hNSCs were analyzed using the Q-PCR technique. RNA of the indicated hNSCs was collected and reverse-transcribed into cDNA. Q-PCR technique of the indicated gene was performed and normalized to GAPDH levels. Values are expressed as percentages of the indicated level in CON and are presented as the mean ± SEM values from 3 independent experiments. [a]Specific comparison to the indicated hNSCs with Aβ ($p < 0.001$; one-way ANOVA). Abbreviations: Aβ, amyloid-beta; ANOVA, analysis of variance; cDNA, complimentary DNA; hNSCs, human neural stem cells; mRNA, messenger RNA; PPARγ, peroxisome proliferator-activated receptor gamma; SEM, standard error of the mean.

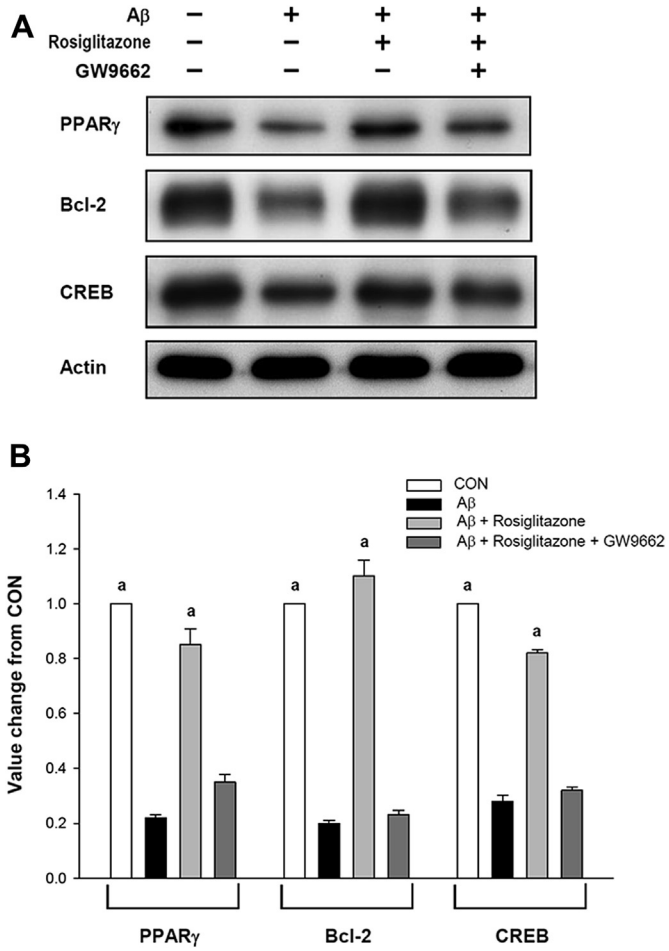

Fig. 3. Rosiglitazone enhances protein expression of PPARγ, Bcl-2, and CREB in hNSCs treated with Aβ. hNSCs were treated with 5-μM Aβ for 24 hours then treated with the indicated reagents (10-μM rosiglitazone or 20-μM GW9662) for another 48 hours. Lysates (20 μg) were collected from the indicated treatment group and subjected to a western blot analysis. (A) Levels of PPARγ, Bcl-2, and CREB proteins were normalized with the corresponding internal control (actin). All reactions were run in triplicate in 3 independent experiments. (B) Values are expressed as a percentage of the indicated protein level in CON and are presented as the mean ± SEM values. [a]Specific comparison to the indicated hNSCs with Aβ ($p < 0.001$; one-way ANOVA). Abbreviations: Aβ, amyloid-beta; ANOVA, analysis of variance; CON, controls; hNSCs, human neural stem cells; PPARγ, peroxisome proliferator-activated receptor gamma; SEM, standard error of the mean.

glutathione peroxidase 1 (Gpx1; F: 5′-CCTCAAGTACGTCCGACCTG-3′ and R: 5′-CAATGTCGTTGCGGCACACC-3′) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (F: 5′-TGCACCACCAACTGCTTAGC-3′ and R: 5′-GGCATGGACTGTGGTCATGAG-3′). Independent reverse-transcription PCRs were performed as described previously (Chiang et al., 2011). The relative transcript amount of the target gene, calculated using standard curves of serial RNA dilutions, is normalized to GAPDH expression in the same RNA sample.

2.5. Western blot assays

Equal amounts of protein were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis using 10%

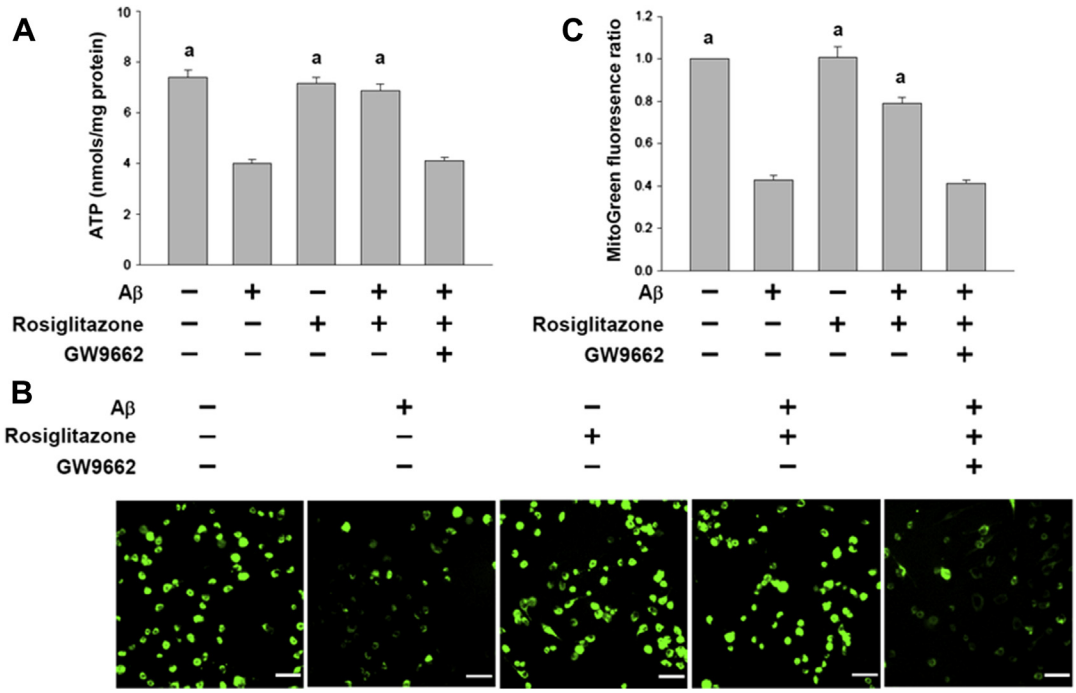

Fig. 4. Rosiglitazone enhances ATP levels and mitochondrial mass in Aβ-treated hNSCs. hNSCs were treated with 5-μM Aβ for 24 hours then treated with the indicated reagents (10-μM rosiglitazone or 20-μM GW9662) for another 48 hours. (A) Lysates harvested from the indicated condition were subjected to ATP assay. (B) The expression levels of mitochondrial mass were normalized to cell numbers. Data are expressed as the mean ± SEM values from 3 independent experiments. (C) hNSCs were collected to determine the level of mitochondrial mass using Mitotracker Green dye (green). Scale bar: 100 μm. [a]Specific comparison to the indicated hNSCs with Aβ ($p < 0.001$; one-way ANOVA). Abbreviations: Aβ, amyloid-beta; ANOVA, analysis of variance; hNSCs, human neural stem cells; SEM, standard error of the mean. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

polyacrylamide gels according to the method of Laemmli (Laemmli, 1970). The resolved proteins were electroblotted onto Immobilon polyvinylidene difluoride membranes (Millipore) for Western blot analyses as reported elsewhere (Chiang et al., 2012a). Primary antibodies of PPARγ (1:1000; Cell Signaling Technology), Bcl-2 (1:2000; Cell Signaling Technology), CREB (1:2000; GeneTex, Inc, Irvine, CA, USA), and actin (1:3000; GeneTex) were used as recommended by the respective manufacturers.

2.6. Measurement of intracellular ATP concentration

To determine adenosine triphosphate (ATP) levels, hNSCs were collected in a lysis buffer (0.1-M Tris, 0.04-M EDTA, pH 7.2) and boiled for 3 minutes. Samples were then centrifuged (112*g* for 5 minutes), and the supernatants were used for the luciferin/luciferase assay as described previously (Chiang et al., 2012b). The ATP levels were normalized to the protein content in the samples. Protein concentrations were determined by the Bradford analysis and used to calculate protein content in the number of samples used for the ATP assay (Promega, Madison, WI, USA). The reaction buffer for this assay contained 60 μM of luciferin, 0.06 μg/mL of luciferase, 0.01 M of magnesium acetate, 0.05% of bovine serum albumin, and 0.2 M of Tris (pH 7.5).

2.7. Mitochondrial mass

The fluorescent probe MitoTracker Green dye (MitoGreen, Invitrogen, Carlsbad, CA, USA) binds mitochondrial membrane lipids regardless of mitochondrial membrane potential or oxidant status. To determine the mass of mitochondria (Chung et al., 2015), cells were loaded with 0.2 μM/mL of MitoTracker Green dye in the medium for 30 minutes at 37 °C. Fluorescence measurements were made with excitation at 490 nm and emission at 516 nm using fluorescence microscopy (OPTIMA). Amounts were determined by comparing the means of the fluorescent signals.

2.8. Measurement of ROS

hNSCs were plated at a density of 5×10^4 per well in a 96-well culture plate. Cells were washed with phosphate-buffered saline and then with phosphate-buffered saline containing 5 μg/μL of dichlorofluorescein diacetate (Molecular Probes, Montlucon, France). Plates were read in a microplate reader over a 1-hour period with an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

2.9. DHE staining

hNSCs were plated at a density of 4×10^5 per well in a 6-well culture plate. Cells were incubated with phenol red free Complete StemPro NSC SFM containing 3-μM dihydroethidium (Molecular Probes Inc, Eugene, OR, USA). After 30 minutes, the cells were imaged under fluorescence microscopy (OPTIMA Biological Microscope). Amounts were determined by comparing the means of the fluorescent signal.

2.10. Statistical analysis

All data were expressed as means ± standard error of the mean from three independent experiments. To establish significance, data were subjected to unpaired one-way analysis of variance followed by Student Newman Keuls's post-hoc test to compare differences between groups using the SigmaStat 3.5 software statistical package (Systat SigmaStat V3.5.0.54 Software; San Jose, CA, USA). The criterion for significance was set at $p < 0.001$.

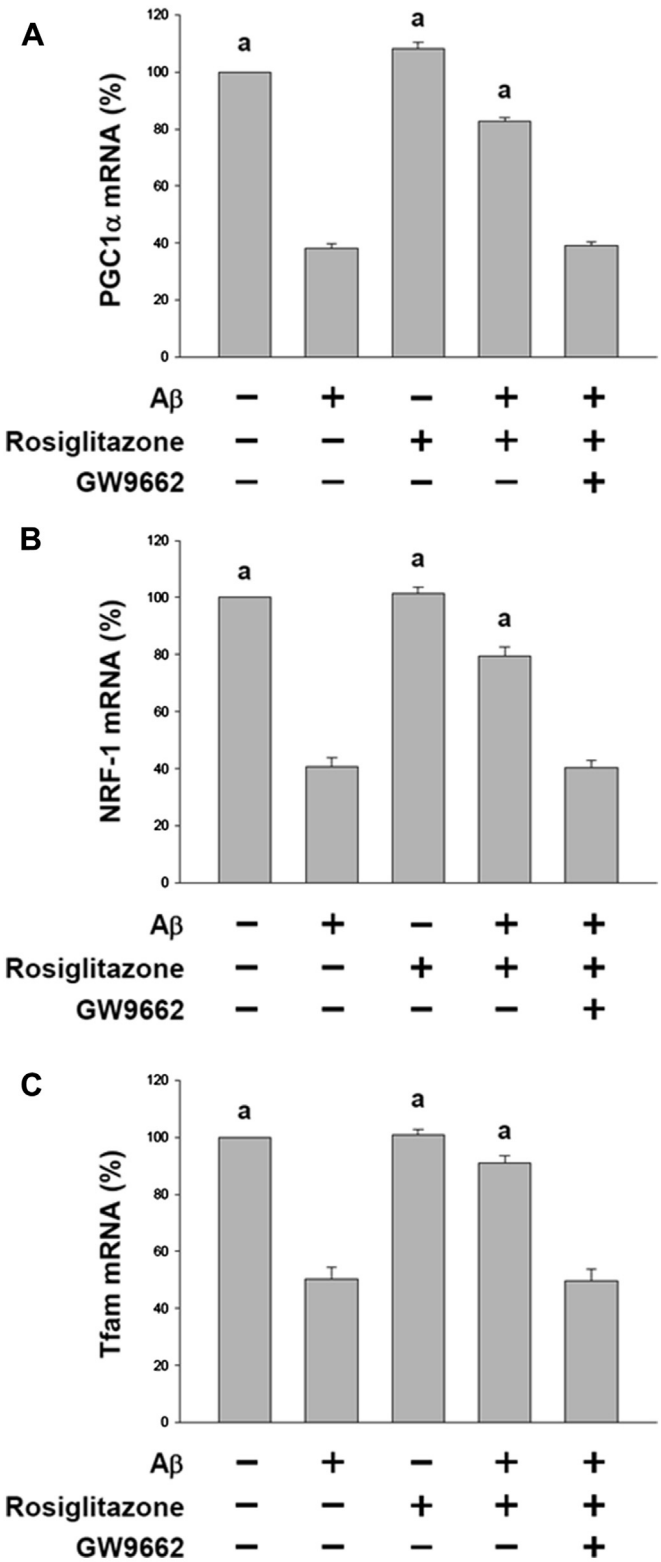

Fig. 5. Rosiglitazone reverses mitochondrial gene mRNA expression in Aβ-treated hNSCs in a PPARγ-dependent manner. hNSCs were treated with 5-μM Aβ for 24 hours then treated with the indicated reagents (10-μM rosiglitazone or 20-μM GW9662) for another 48 hours. The PGC1α (A), NRF-1 (B), and Tfam (C) transcripts in the indicated hNSCs were analyzed using the Q-PCR technique. RNA of the indicated hNSCs was collected and reverse-transcribed into cDNA. Q-PCR technique of the indicated gene was performed and normalized to that of GAPDH. Values A, B, and C are expressed as percentages of the indicated transcript in CON and are presented as the mean ± SEM values from 3 independent experiments. [a]Specific comparison to the indicated hNSCs with Aβ ($p < 0.001$; one-way ANOVA). Abbreviations: Aβ, amyloid-beta; ANOVA, analysis of variance; CON, controls; hNSCs, human neural stem cells; mRNA, messenger RNA; NRF-1, nuclear respiratory factor-1; PGC1alpha, PPAR coactivator 1 alpha; SEM, standard error of the mean; Tfam, mitochondrial transcription factor A.

3. Results

3.1. Rosiglitazone rescued of Aβ-induced hNSC changes in cell viability and caspase activity requires PPARγ

hNSCs were treated with 5-μM Aβ for 72 hours and evaluated for Aβ-induced changes on hNSC viability and caspase 3/9 activities (markers of caspase cascade activation). Cell viability was significantly decreased (Fig. 1), and caspase 3/9 activities were significantly increased after Aβ treatment for 72 hours (Fig. 1). Co-treatment with a PPARγ agonist (rosiglitazone) significantly normalized cell viability and caspase 3/9 activities, which was blocked by an antagonist of PPARγ (GW9662; Fig. 1).

3.2. Rosiglitazone reverses PPARγ, Bcl-2, and CREB expression in Aβ-treated hNSCs

Evidence from Fuenzalida et al. suggests that PPARγ increases expression of Bcl-2 anti-apoptotic protein in cells and protects against apoptosis (Fuenzalida et al., 2007). This is of particular interest because Bcl-2 and CREB are downstream targets of PPARγ (Butts et al., 2004; Chiang et al., 2014) and suggests the possibility that rosiglitazone leads to PPARγ-induced protection in neuronal cells exposed to Aβ by enhancing the survival markers (Bcl-2 and CREB) expression. hNSCs were then evaluated for gene expression changes resulting from Aβ treatment. Compared with respective vehicle CON, Aβ treatment significantly decreased messenger RNA (mRNA) levels of PPARγ and two important survival factors (Bcl-2 and CREB) known to be downstream targets of PPARγ (Butts et al., 2004; Chiang et al., 2014; Fuenzalida et al., 2007; Fig. 2A–C). In contrast, hNSCs treated with Aβ and rosiglitazone had significantly increased PPARγ, Bcl2, and CREB mRNA expression compared with Aβ treatment alone, but these effects were blocked by co-treatment with GW9662 (Fig. 2A–C). Fig. 3 demonstrates that the protein levels of PPARγ, Bcl-2, and CREB in hNSCs treated with Aβ were much lower than in vehicle-treated hNSCs. PPARγ, Bcl-2, and CREB protein levels were also significantly enhanced and blocked by the addition of rosiglitazone and GW9662 treatment, respectively (Fig. 3). Supplementary Fig. S1 exhibited that hNSCs treated with drug alone (rosiglitazone) for 72 hours showed no effect on the level of PPARγ, Bcl-2, and CREB, specific comparison between the hNSCs and hNSCs with rosiglitazone (Fig. S1).

3.3. Rosiglitazone rescues mitochondrial dysfunction in Aβ-treated hNSCs

Ample evidence suggests that Aβ decreases mitochondrial activity and leads to energy depletion (de Arriba et al., 2007; Kuhla et al., 2004). First, an ATP assay was used to determine the consequence of Aβ treatment on hNSC mitochondrial biogenesis. The ATP levels in hNSCs treated with Aβ were significantly lower versus CON groups (Fig. 4A). Next, changes in hNSC mitochondrial mass were assessed using MitoGreen (Chiang et al., 2014; Quintanilla et al., 2008; Wilson-Fritch et al., 2003). The mitochondrial mass in the

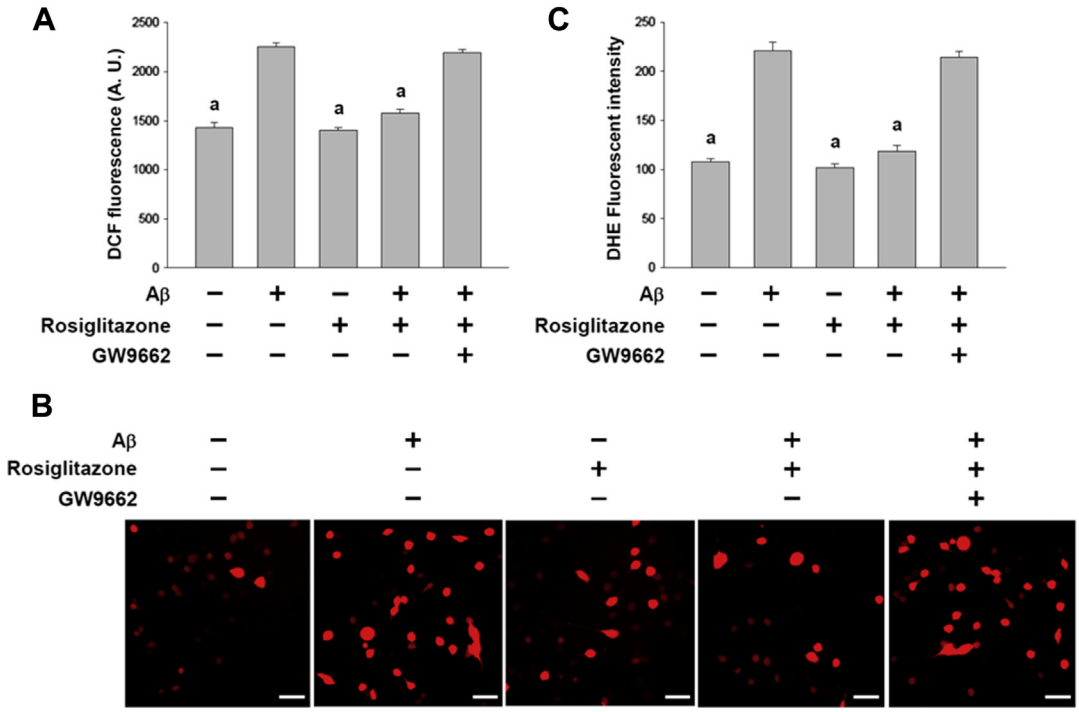

Fig. 6. Rosiglitazone normalizes ROS levels in hNSCs treated with Aβ. hNSCs were treated with 5-μM Aβ for 24 hours then treated with the indicated reagents (10-μM rosiglitazone or 20-μM GW9662) for another 48 hours. (A) Lysates harvested from the indicated condition were subjected to ROS assay by DCFH-DA. (B) hNSCs were collected to show typical microphotographs of using DHE dye (red). Scale bar: 100 μm. (C) Quantitative data of ROS generation evaluated by DHE fluorescent intensity then were normalized to cell numbers. Data are expressed as the mean ± SEM values from 3 independent experiments. [a]Specific comparison to the indicated hNSCs with Aβ ($p < 0.001$; one-way ANOVA). Abbreviations: Aβ, amyloid-beta; ANOVA; analysis of variance; DCFH-DA, dichlorofluorescein diacetate; DHE, dihydroethidium; hNSCs, human neural stem cells; ROS, reactive oxygen species; SEM, standard error of the mean. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

hNSCs treated with Aβ was significantly lower compared with hNSC CON groups (Fig. 4B and C). However, hNSC co-treatment with Aβ and rosiglitazone significantly increased ATP levels and mitochondrial mass and these effects were abrogated in the presence of GW9662 (Fig. 4A–C).

It was reported that PPARγ stimulation, through the induction of the PGC-1α, promotes mitochondrial biogenesis (Pagel-Langenickel et al., 2008; Puigserver and Spiegelman, 2003; Wareski et al., 2009; Wu et al., 1999). PGC-1α was also implicated in mitochondrial biogenesis through its ability to control a number of genes such as NRF-1 and Tfam (McGill and Beal, 2006; Puigserver and Spiegelman, 2003). Thus, expression of PGC-1α, NRF-1, and Tfam was also evaluated. Compared with hNSC controls, Aβ treatment significantly decreased mRNA expression of all three genes (Fig. 5A–C). Rosiglitazone co-treatment significantly increased PGC-1α, NRF-1, and Tfam transcript levels, but the addition of GW9662 blocked these effects (Fig. 5A–C).

3.4. Rosiglitazone normalized oxidative stress in the hNSCs with Aβ

It is suggested that Aβ increases reactive oxygen species (ROS) and oxidative stress leading to cytotoxicity in neuronal cells (Santos et al., 2005). Analyses of ROS levels using dichlorofluorescein fluorescence revealed that ROS levels in Aβ-treated hNSCs were significantly increased versus CON (Fig. 6A). Oxidative stress, assessed using dihydroethidium staining, was also significantly increased in Aβ-treated hNSCs versus CON (Fig. 6B and C). In contrast, rosiglitazone co-treatment significantly normalized ROS and oxidative stress levels in hNSCs, but only in the absence of the PPARγ antagonist GW9662 (Fig. 6A–C).

Additionally, expression changes of several anti-oxidative genes, SOD-1, SOD-2, and Gpx1, that facilitate cellular defense mechanisms against oxidative stress, and are known downstream targets of PGC1-α (St-Pierre et al., 2006), were evaluated. SOD-1, SOD-2, and Gpx1 mRNA levels were significantly decreased in the Aβ-treated hNSCs compared with respective CON groups (Fig. 7A–C). Rosiglitazone significantly increased their expression, but these effects were blocked by GW9662.

4. Discussion

Here, we summarize our understanding of the central role of PPARγ in hNSCs during $Aβ_{1–42}$-related neuronal impairment and discuss how PPARγ may protect against AD through attenuation of the Aβ-mediated toxicity (Figs. 1–3), mitochondrial dysfunction (Figs. 4 and 5), and oxidative stress (Figs. 6 and 7). Although the action of PPARγ is well characterized in terms of peripheral physiological and pathologic effects, a critical role for PPARγ in the brain is also emerging. In the past decade, drugs targeting PPARγ were shown to improve the pathophysiology of AD. Pedersen et al. (Pedersen and Flynn, 2004; Pedersen et al., 2006) demonstrated using a Tg2576 mouse model of AD that oral treatment with rosiglitazone for 4 months significantly ameliorated memory and decreased Aβ protein levels in the brains of treated mice. Similarly, three other Tg2576 AD mouse studies showed promising cognitive enhancements following rosiglitazone treatment, supporting PPARγ as a novel therapeutic target for the treatment of AD (Abisambra et al., 2013; Denner et al., 2012; Jahrling et al., 2014). Denner et al. (Denner et al., 2012) also demonstrated that rosiglitazone treatment improved hippocampal cognitive deficits in the AD-Tg2576 mice via activation of the extracellular signal regulated kinase (ERK) signaling cascade. Recently, Tg2576 mice treated with rosiglitazone for 30 days showed increased phosphorylated ERK levels, which was required for AD-related cognitive enhancement (Jahrling et al., 2014). In

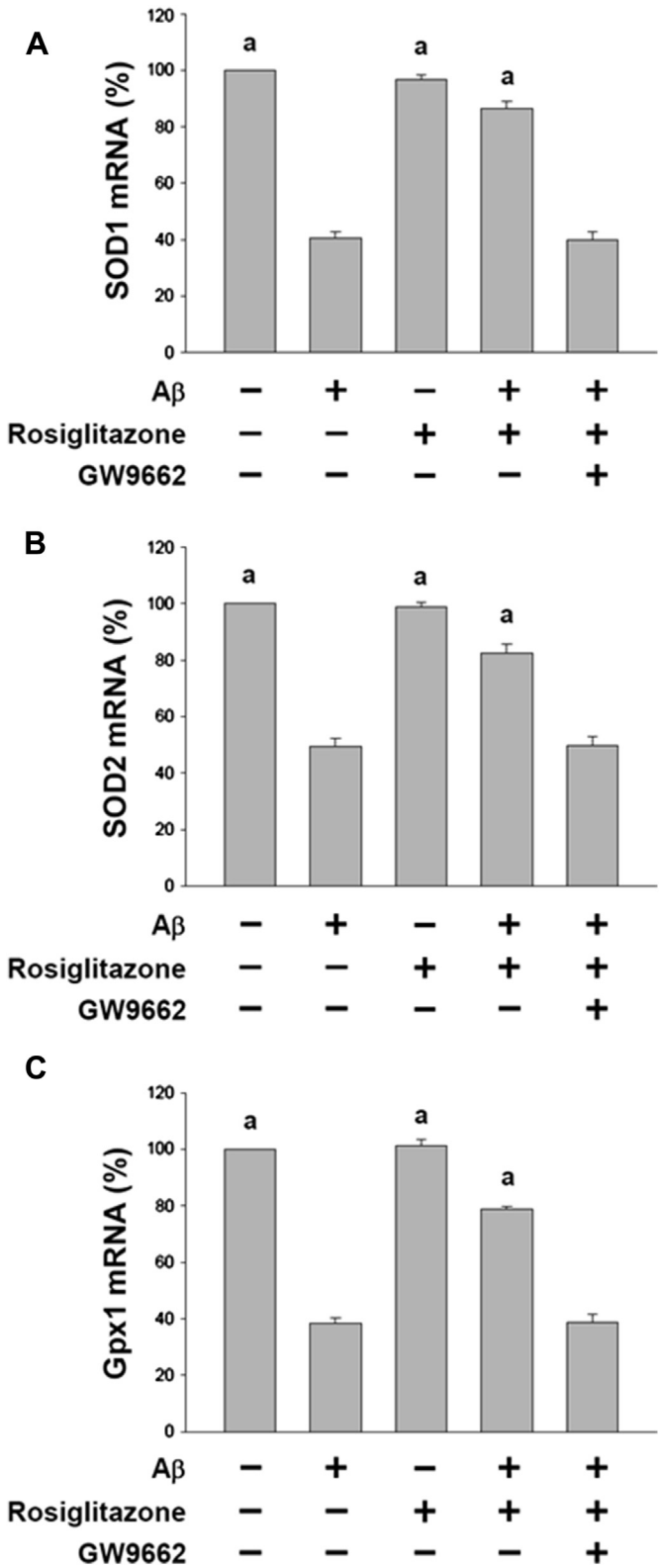

Fig. 7. Rosiglitazone improves oxidative defense gene mRNA expression in Aβ-treated hNSCs. hNSCs were treated with 5-μM Aβ for 24 hours, then treated with the indicated reagents (10-μM rosiglitazone or 20-μM GW9662) for another 48 hours. The SOD1 (A), SOD2 (B), and Gpx1 (C) transcripts in the indicated hNSCs were analyzed using the Q-PCR technique. RNA of the indicated hNSCs was collected and reverse-transcribed into cDNA. Q-PCR technique of the indicated gene was performed and normalized to that of GAPDH. Values A, B, and C are expressed as percentages of the indicated transcript in CON and are presented as the mean ± SEM values from 3 independent experiments. [a]Specific comparison to the indicated hNSCs with Aβ ($p < 0.001$; one-way ANOVA). Abbreviations: Aβ, amyloid-beta; cDNA, complimentary DNA; CON, controls; hNSCs, human neural stem cells; mRNA, messenger RNA; SEM, standard error of the mean; SOD, superoxide dismutase.

addition, Nenov et al. (2014) showed that PPARγ activation leads to hippocampal cognitive enhancement in Tg2576 mice through increased glutamatergic neurotransmitter release and synaptic plasticity (Nenov et al., 2014). Furthermore, amyloid precursor protein/presenilin 1 mice, another AD mouse model, showed improved performance in the Morris water maze when mice were treated with rosiglitazone (Toledo and Inestrosa, 2010), suggesting that activation of PPARγ signaling improves behavioral performance.

The potential therapeutic benefits of rosiglitazone is further supported by the results of 2 clinical trials of AD patients (Risner et al., 2006; Watson et al., 2005). In 2005, a small clinical study (n = 20) demonstrated that 6 months of daily treatment with rosiglitazone, at a dose (4 mg) within the normal human therapeutic range, correlated with improvements in cognition and decreased plasma Aβ levels in AD patients (Watson et al., 2005). Risner et al. (2006) (Risner et al., 2006) found that rosiglitazone (2, 4, or 8 mg daily) had beneficial actions on the cognitive functions of 511 AD patients. The hopeful findings in these 2 studies add further evidence strongly supporting PPARγ as a significant therapeutic target for the treatment of AD. The phase II clinical trials of rosiglitazone suggested in AD observed a treatment benefit to cognition in patients. Clinical trials assessing the safety and efficacy of rosiglitazone in AD patients were importantly considered. To confirm the potential safety and efficacy of rosiglitazone, a 24-week phase III clinical trials was guided in AD patients. Unfortunately, Gold et al. (2010) (Gold et al., 2010) showed that no evidence of efficacy of 2- (n = 133) or 8-mg rosiglitazone (n = 127) in cognitive function was demonstrated in AD subjects. In 2010, a clinical trial demonstrated effects of 12 months treatment with rosiglitazone versus placebo in 80 mild-to-moderate AD patients (Tzimopoulou et al., 2010). This study suggests that rosiglitazone is correlated with an early elevate in whole brain glucose metabolism using [^{18}F] FDG-PET, but not with any clinical or biological effect for slowing progression in the symptomatic phases of AD. It is a critical concern that these clinical trials were treated with rosiglitazone dosages that were much lower than those required to observe useful results on physiological profile of neuropathology in animal models of AD. Although cognitive function was not demonstrated with rosiglitazone treatment in AD patients, recently results on the role of underlying PPARγ remains of critical concern in AD (Kummer et al., 2015; Skerrett et al., 2015; Xu et al., 2014), still as therapeutic options of further exploration.

Notwithstanding these promising findings, the mechanisms by which rosiglitazone may attenuate the pathogenesis and progression of AD remain elusive, not to mention defining the importance of PPARγ signaling in hNSCs throughout these processes. In 2005, a study revealed that PPARγ activation increases ubiquitination-mediated degradation of cellular amyloid precursor protein levels (d'Abramo et al., 2005). Moreover, Costello et al. (2005) (Costello et al., 2005) showed that PPARγ agonists inhibited Aβ-induced impairment in synaptic transmission in the hippocampus, suggesting that these agonists are involved in neuronal synaptic plasticity. PPARγ agonists also repress the neurotoxins induced by Aβ peptides and prevent neuronal cell death (Combs et al., 2000). However, the mechanisms by which PPARγ agonists perform these useful effects may be more complicated than it seems. Our previous study showed that activation of PPARγ by rosiglitazone protects hNSCs against $A\beta_{1-40}$-induced cytotoxicity, further suggesting that PPARγ may be involved in cell survival (Chiang et al., 2013).

Suppression of NSC proliferation and neurogenesis was observed in the hippocampus with AD (Demars et al., 2010; Rodriguez et al., 2008; Verret et al., 2007). Neuronal apoptosis was observed in AD experimental studies using rat hippocampal neurons (Jordan et al., 1997) and hNSCs (Chiang et al., 2013). As such, studies involving hNSCs have both etiologic and therapeutic utility for improving treatment outcomes of neurodegenerative disorders, such as AD (Lindvall and Kokaia, 2010). Likewise, NSCs are not only considered the main source of neurons but also useful for investigating brain development. Because to our knowledge no prior reports exist in the literature, the critical role of PPARγ in hNSCs with respect to Aβ-mediated toxicity was examined here.

Alterations in mitochondrial function were also implicated in neurodegenerative and metabolic diseases. Ample evidence suggests that Aβ decreases mitochondrial activity and leads to energy depletion (Atamna and Frey, 2007; Kuhla et al., 2004; Reddy, 2009). Aβ also decreases Ca^{2+} homeostasis and lowers mitochondrial membrane potential, which further impairs normal mitochondrial function (Fernandez-Morales et al., 2012). Several studies indicate that PPARγ signaling is important for normal mitochondrial function and that the latter might play an important role in NSC survival (Calingasan et al., 2008; Chiang et al., 2013). In the present study, rosiglitazone rescued mitochondrial mass, despite the Aβ-mediated toxicity induced in hNSCs. Although previous reports identified the beneficial effects of rosiglitazone on mitochondrial biogenesis in general (Guan et al., 2005; Hondares et al., 2006), our data provide the first evidence that activating PPARγ signaling in hNSCs reverses the neurodegenerative damage induced by Aβ and highlights the neurotherapeutic potential of PPARγ-activating drugs.

The stimulation of mitochondrial function by PPARγ with rosiglitazone is associated with activation of the mitochondrial biogenesis master regulator PPARγ-coactivator-1α (PGC-1α) pathway by upregulation of mitochondrial genes and function (Chiang et al., 2012a, 2013, 2014; Fuenzalida et al., 2007; Puigserver and Spiegelman, 2003). PGC-1α is also implicated in mitochondrial biogenesis through its ability to control a number of genes such as NRF-1, and Tfam (Puigserver and Spiegelman, 2003). NRF-1 and Tfam regulate nuclear-encoded mitochondrial genes such as mitochondrial enzymatic activities of COX (cytochrome c oxidase subunit) I-V of the mitochondrial electron transport chain pathway (Evans and Scarpulla, 1990). COX activity is also reduced in AD brain mitochondria (Swerdlow and Kish, 2002).

Here, we provide the first evidence that activation of PPARγ signaling within hNSCs is critically important to preventing Aβ-induced oxidative stress. Oxidative stress may be critical for hNSC survival (Chiang et al., 2013). Several researchers have suggested that Aβ could induce neurotoxicity via increased oxidative stress and associated to neurodegeneration in AD (Butterfield, 2002; Guglielmotto et al., 2010; Swerdlow, 2012; Tong et al., 2005; Varadarajan et al., 2000). These findings imply that oxidative stress is one of the major pathologic events because it contributes to neuronal cell death in AD (Cutler et al., 2004; Murakami et al., 2005; Nunomura et al., 2001; Perry et al., 2000; Tabner et al., 2005; Zhu et al., 2007). Importantly, Aβ is implicated as the major neurotoxic stressor in the pathogenesis of AD due to its ability to cause both neurotoxicity and oxidative stress (Breitner, 1996; Butterfield, 2002; Landreth and Heneka, 2001; Selkoe, 2001; Varadarajan et al., 2000). Most studies suggest PPARγ activation is neuroprotective because of the anti-oxidative pathways regulated by PPARγ (Bernardo and Minghetti, 2006; Chiang et al., 2013; Feinstein, 2003; Landreth and Heneka, 2001). Therefore, the suppression of Aβ-induced hNSC neurotoxicity and decreased oxidative stress support further clinical studies of PPARγ activators in the therapeutic management of AD patients for AD (Heneka and O'Banion, 2007; Inestrosa et al., 2005; Kalaria, 1999; McGeer et al., 1996; Watson and Craft, 2006).

Taken together, these novel findings show that rosiglitazone-induced activation of PPARγ-dependent signaling rescues Aβ-mediated toxicity in hNSCs. This study also provides evidence supporting a neuroprotective role for PPARγ-activating drugs in Aβ-related diseases such as AD.

Disclosure statement

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2016.01.132.

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