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PPARgamma Mediated Tumor Necrosis Factor alpha Regulation in Human Neural Stem Cells

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Abstract. Peroxisome proliferator-activated receptor gamma (PPARγ) belongs to a family of ligand-activated transcription factor and its ligands are known to control many physiological and pathological states. Therefore, we investigate the hypothesis that the PPARγ agonist (rosiglitazone) could mediate tumor necrosis factor alpha (TNFα) related regulation of human neural stem cells (hNSCs), by which TNFα possibly fulfill important roles in neuronal impairment. In the study, we show that rosiglitazone (5 μM) rescued the TNFα (100 ng/ml) decreased hNSCs viability via the PPARγ pathway. The stimulation of mitochondrial function by PPARγ was associated with a activation of the PPAR coactivator-1 alpha (PGC-1α) gene by up-regulation of mitochondrial systems. The above protective effects appeared to be exerted by a direct activation of the rosiglitazone because it protected the hNSCs from TNFα evoked mitochondrial deficiency (lower ATP level and mitochondrial mass). These findings extend our understanding of the central role of PPARγ in TNFα induced excitotoxicity, which probably increase risks of neuronal impairment in neurodegeneration.

Keywords: PPARγ, TNFα, hNSCs

1. Introduction

PPARγ is a member of ligand-activated transcription factor, which binds to the PPAR responsive element within the promoter of the target genes (Kersten et al., 2000, Rosen and Spiegelman, 2001). In addition, accumulating data suggest a possible role for PPARγ ligands in suppressing inflammatory response in brain system (Ji et al., 2010, Morgenweck et al., 2010). TZD compounds such as rosiglitazone is common oral insulin sensitizing anti-diabetic agents mediated by their interaction with PPARγ. TNFα is induced in damaged and inflamed tissues, and plays a key role in post injury organ dysfunction by inducing various inflammatory cytokines and chemokines (Locksley et al., 2001). Although TNFα is known to inhibit the ligand-dependent transcriptional activity of PPARγ, the precise mechanism remains to be fully understood (Hu et al., 1996, Zhang et al., 1996, Ruan et al., 2002, Ruan et al., 2003, Suzawa et al., 2003). In addition, rosiglitazone is reported to be important anti-inflammatory mediators and may be useful in the treatment of neurodegenerative diseases, the mechanism regarding how rosiglitazone inhibit the inflammatory response including PGC-1α pathway expression in hNSCs is still unclear. In this study, we addressed this issue by analyzing the molecular mechanism of TNFα action on PPARγ. These findings improve the development of hNSCs-based therapeutically strategies in the treatment of neurodegenerative disorders and malignancies as well.

2. Materials and Methods

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Cell culture: GIBCO® human neural stem cells (H9 hESC-derived) were originally obtained from the NIH approved H9 (WA09) human embryonic stem cells (hESCs). Medium used Complete StemPro® NSC SFM (serum free medium) was used for optimal growth and expansion of GIBCO® hNSCs, and to keep the NSCs undifferentiated. StemPro® NSC SFM complete medium consists of KnockOut™ D-MEM/F-12 with 2% StemPro® Neural Supplement, 20 ng/mL EGF, 20 ng/mL bFGF, and 2 mM GlutaMAX™-I.

Evaluation of cell growth: Cell viability was assayed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) absorbance and cell count. After synchronized hNSCs were treated with TNFα (100 ng/ml), PPARγ agonist rosiglitazone (5 μM), or GW9662 (10 μM) for 2 days, MTT solution (Sigma) was added to the culture medium and the cells were incubated, and absorbance at 570 nm was measured in solubility cells using an ELISA plate reader.

RNA isolation and quantitative real-time polymerase chain reaction (QPCR): A real-time quantitative 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). Sequences of primers are listed below: for PGC1α (5'-TGAGAGGGCCAAGCAAAG-3' and 5'-ATAAATCACACGGCGCTCTT-3'), and for GAPDH (5'-TGCACCACCAACTGCTTAGC-3' and 5'-GGCATGGACTGTGGTCATGAG-3'). Independent reverse-transcription PCRs were performed as described elsewhere (Chiang et al., 2011). The relative transcript amount of the target gene, which was calculated using standard curves of serial RNA dilutions, was normalized to that of GAPDH of the same RNA.

Measurement of intracellular ATP concentration: To determine ATP levels, hNSCs cells were collected in a lysis buffer (0.1 M Tris, 0.04M EDTA, pH 7.2) and boiled for 3 minutes. Samples were then centrifuged (112*g* for 5min), and the supernatants were used in the luciferin/luciferase assay. 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).

Mitochondrial mass: The fluorescent probe Mitotracker GreenTM dye (MitoGreen, Invitrogen) binds to mitochondrial membrane lipids regardless of mitochondrial membrane potential or oxidant status (Chiang et al., 2012). To determine levels of mitochondria, cells being loaded with 0.2 μM/mL Mitotracker GreenTM dye in medium for 30 minutes at 37 °C using an cell observation system.

3. Results and Discussion

First, we assessed the effects of TNFα on cell viability in hNSCs. hNSCs were treated with TNFα (100 ng/ml) for 48 h, and cell viability was reduced significantly in TNFα (Fig. 1). In addition, treatment with a PPARγ agonist (rosiglitazone) significantly normalized cell viability which was blocked by an antagonist of PPARγ (GW9662). It has been demonstrated that PPARγ stimulation, through the induction of the PGC-1α, promotes mitochondrial biogenesis (Puigserver and Spiegelman, 2003, Wareski et al., 2009). We found that the transcript level of PGC-1α in the hNSCs with TNFα were much lower than those in the CON of hNSCs (Fig. 2). Treatment with rosiglitazone significantly enhanced PGC-1α, which were blocked by GW9662.

To analyze the consequence of defective mitochondrial biogenesis, hNSCs were employed for the ATP assay. We found that the ATP level in the hNSCs with TNFα was markedly lower than that in the CON of hNSCs (Fig. 3). Stimulation of PPARγ promotes mitochondrial biogenesis and remodelling via the induction of PGC-1α (Puigserver and Spiegelman, 2003, Wareski et al., 2009). Therefore, we evaluated whether TNFα compromised mitochondrial biogenesis via a PPARγ-dependent pathway in the hNSCs with TNFα. The mitochondrial biogenesis of hNSCs was assessed using MitoGreen, which was used to determine mitochondrial mass (Chiang et al., 2012). The mitochondrial mass in the hNSCs with TNFα was markedly lower than that observed in the CON of hNSCs (Fig. 4). Consistent with the beneficial effects of rosiglitazone on mitochondrial biogenesis (Chiang et al., 2011, Chiang et al., 2012), our results suggest that treatment by rosiglitazone enhanced ATP level and mitochondrial mass significantly, which was blocked by GW9662.

One possible way to promote extended viability of hNSCs *in vitro* is to modulate properties of the hNSCs, and this might be accomplished by the PPARγ pathway, which is known as a general mediator of cell death and mitochondrial dysfunction in the effects of TNFα. It was found that rosiglitazone significantly

elevated TNFα inhibited PGC-1α pathway expression through a PPARγ dependent pathway and acted via the direct inhibition of hNSCs' death and mitochondrial dysfunction signalling. The PPARγ has been primarily implicated in anti-inflammatory processes. Experiments carried out in hNSCs provide evidence that the neuroprotective effects of rosiglitazone are directly mediated by the PPARγ pathway.

4. Acknowledgements

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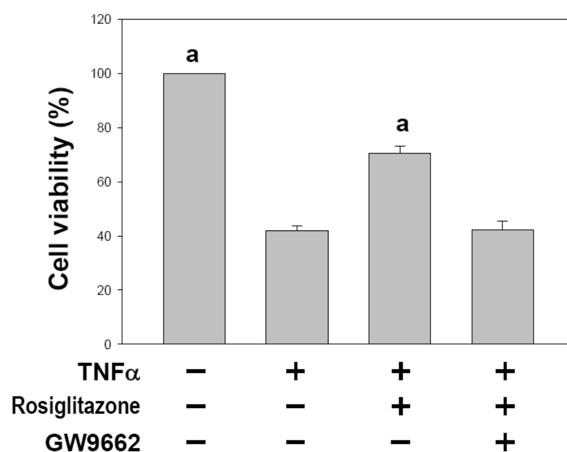

Fig. 1: Rosiglitazone rescued the TNFα decreased hNSCs viability via the PPARγ pathway. hNSCs were treated with TNFα (100 ng/ml) for 24 h, then treated with the indicated reagents (5 μM rosiglitazone or 10 μM GW9662) for another 24 h, and cell viability was detected. Data are expressed as the mean ± SEM values from three independent experiments. [a] Specific comparison to CON ($p < 0.001$; one-way ANOVA).

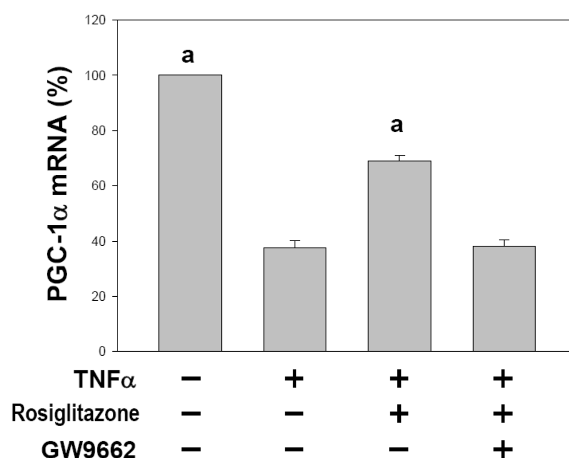

Fig. 2: Improvement in the expression of PGC-1α expression in the hNSCs with TNFα. hNSCs were treated with TNFα (100 ng/ml) for 24 h, then treated with the indicated reagents (5 μM rosiglitazone or 10 μM GW9662) for another 24 h. The PGC-1α transcript in the indicated hNSCs were analyzed using the Q-PCR 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 are expressed as percentages of the indicated level in CON and are presented as the mean ± SEM values from three independent experiments. [a] Specific comparison to CON ($p < 0.001$; one-way ANOVA).

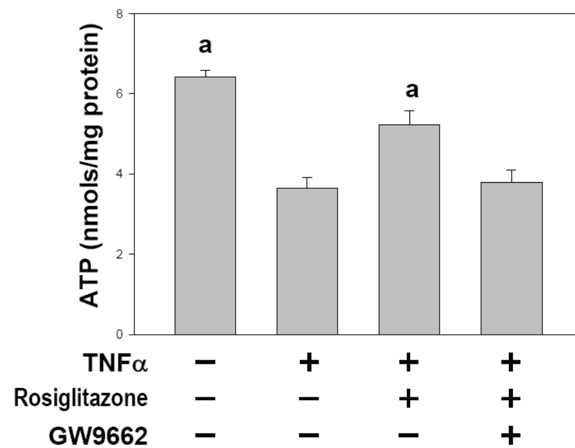

Fig. 3: Rosiglitazone normalized ATP level in the hNSCs with TNFα. hNSCs were treated with TNFα (100 ng/ml) for 24 h, then treated with the indicated reagents (5 µM rosiglitazone or 10 µM GW9662) for another 24 h. Lysates harvested from the indicated condition were subjected to ATP assay. Data are expressed as the mean ± SEM values from three independent experiments. [a] Specific comparison to CON ($p < 0.001$; one-way ANOVA).

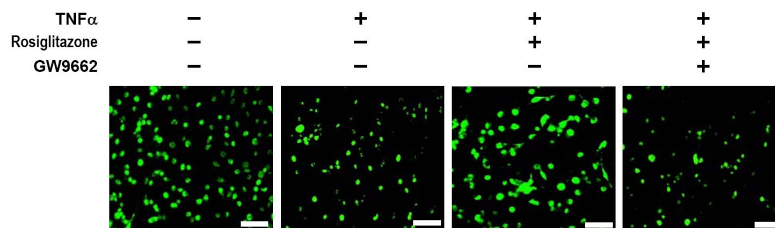

Fig. 4: Rosiglitazone enhanced mitochondrial mass in the hNSCs with TNFα. hNSCs were treated with TNFα (100 ng/ml) for 24 h, then treated with the indicated reagents (5 µM rosiglitazone or 10 µM GW9662) for another 24 h. hNSCs were collected to determine the level of mitochondrial mass using Mitotracker GreenTM dye (green). Scale bar: 100 µm.

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