

PPAR γ and PGC-1 α activators protect against diabetic nephropathy by suppressing the inflammation and NF- κ B activation

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Abstract

Background: Inflammation played critical roles in the progression of various kidney diseases and led to irreversible kidney fibrosis. Peroxisome proliferator-activated receptor gamma (PPAR γ) and its coactivator PPAR γ coactivator-1 alpha (PGC-1 α)

negatively regulated mitochondrial biogenesis, cellular energy metabolism, and inflammation. But the cooperative molecular mechanism of them in kidney remained unclear. The aim of present study was to investigate this issue.

Methods: Human proximal tubular HK-2 cell line was stimulated by inflammatory factors, and the expression of PPAR γ and its coregulators were determined via reverse transcription-quantitative polymerase chain reaction and western blotting, and DNA binding capacity was measured by EMSA. Furthermore, db/db mice were used to establish a diabetic nephropathy model and administrated with PPAR γ and PGC-1 α activator. Kidney injury was evaluated microscopically, and inflammatory mechanism was assessed by western blotting.

Results: Our results revealed that either TNF- α or IL-1 β could significantly decrease PPAR γ and PGC-1 α expression in vitro. Cytokines also obviously inhibited PPAR γ DNA binding activities. Meanwhile, we detected rapid activation of NF- κ B pathway under the same experimental conditions. PPAR γ and PGC-1 α activators effectively protect against diabetic nephropathy and suppress NF- κ B expression in db/db mice.

Conclusions: PPAR γ and its coactivator PGC-1 α actively participated in the protection against renal inflammation through regulating NF- κ B pathway, which highlighted a potential therapeutic target for renal diseases.

Introduction

Inflammation is one of the most important factors in the genesis of various complications in renal diseases and leads to renal injury and renal insufficiency. Pro-inflammatory cytokines like tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) play a major role in the onset of acute and chronic inflammatory response in kidney through activating NF- κ B pathway, and further induce a wide array of genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines in human and experimental models of kidney diseases (1, 2). Our previous studies have described the enrichment in inflammation-related pathways of differential RNAs between diabetic nephropathy and normal control, which indicated the importance of pro-inflammatory mechanisms in diabetic nephropathy (3).

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear transcription factors and are proved to play key roles in the regulation of lipid metabolism, inflammation, cellular growth and differentiation. Among the various subtypes of PPARs, PPAR γ is the best characterized receptor in humans (4) and is widely expressed in the nuclei of mesangial and epithelial cells in glomeruli, vasculatures, proximal and distal tubules, the loop of Henle, and medullary collecting ducts (5). PPAR γ

also has been verified to exert reno-protective effects through an anti-inflammatory mechanism in glomerulosclerosis, glomerulonephritis, microvasculature nephropathy and interstitial inflammation (6-8). PPAR γ regulate the expression of genes through a complex set of mechanisms, and corepressors inhibit and coactivators stimulate PPAR γ activity by binding to the PPAR γ and changing its conformation without directly binding to the DNA (9, 10). Previous researches reported PPARs and peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α) regulate mitochondrial biogenesis, cellular energy production, thermogenesis, and lipid metabolism (11). We also demonstrated the renoprotective effects against diabetic nephropathy of PPAR γ and its coactivator, PGC-1 α , through metabolic and antioxidative mechanisms (12).

Investigations have verified that expression levels of coregulators are crucial for nuclear receptor-mediated transcription and part of them have been demonstrated to be targeted for diverse intracellular signaling pathways and post-translational modifications (13-16). Moreover, recent studies suggest some coactivators are regulated together with nuclear receptors (such as liver X receptor, retinoid X receptor) in the inflammatory response induced by TNF- α and IL-1 β in the heart, liver, brain and adipose tissue (17-18). But the molecular mechanism in anti-inflammation of PPAR γ and PGC-1 α in kidney remain to be fully understood.

In the present study, we investigated the expression changes of PPAR γ as well as its coactivators, including steroid receptor coactivators (SRCs) and PGC-1 α under treatment with TNF- α and IL-1 β in vitro, as well as NF- κ B pathway activities to primarily explore the mechanism that underlies these changes. And we further verified the effects of activating PPAR γ and PGC-1 α on diabetic nephropathy and the mechanisms related to NF- κ B.

Materials And Methods

Materials

Cytokines (human TNF- α and human IL-1 β) and PPAR γ activator (rosiglitazone) were purchased from Sigma-Aldrich (St. Louis, MO); Keratinocyte serum free medias for cell culture were obtained from Invitrogen Co.Ltd (United States) supplemented with bovine pituitary extract (BPE) and epidermal growth factor (EGF). PGC-1 α activators (ZLN005) from Selleck Co.Ltd (China), which was diluted with 0.5% sodium carboxymethylcellulose Sangon Biotech Co.Ltd (China).

Cell culture

HK-2 (the immortalized human proximal tubular cell line, CRL-2190) were purchased from American Type Culture Collection (Rockville, MD) and maintained in keratinocyte serum-free media mentioned above. The cells were cultured in a 37°C incubator with 5% CO₂ and subcultured at 80% confluence using 0.05% trypsin-0.02% EDTA (Invitrogen).

Animal experiments

A total of 18 male db/db diabetic mice weighing 32-34 g with a C57BL/KsJ (BKS.Cg-Dock7m+/+Leprdb/Nju) background and 6 male non-diabetic littermate control db/m mice weighing 16-18g (6 weeks old) were obtained from Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China). They were bred in the laboratory animal center at

Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, China) as previously described (12). Mice were separated into three groups (n=6/group) as follows: db/m (C group); db/db (D group) and db/db mice administered 20 mg/kg/day rosiglitazone (R group) or 15 mg/kg/day ZLN005 (Z group) by gavage for 8 weeks. Mice were housed in a specific pathogen-free room at a constant temperature of 22±2°C and a constant humidity of 50±5%, normal air CO₂ and a 12-h light/dark cycle and kept with free access to chow and water. Mice were sacrificed after the treatment for 8 weeks. Surgeries were performed under general anesthesia with isoflurane. All procedures were performed in accordance with the guidelines established by National Research Council Guide for the Care and Use of Laboratory Animals and approval of our Institute Animal Care and Use Committee (IACUC). Experiments were carried out in accordance with ARRIVE guidelines (<https://arriveguidelines.org>), the relevant guidelines and regulation.

RNA isolation and real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR)

The total RNA from renal cortical tissues was extracted by using TRIzol (Applied Biosystems, Waltham, MA, USA). The RNA concentration was measured by an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The first strand of cDNA synthesis was carried out by using a reverse transcription system kit according to the instructions of the manufacturer (Promega, Madison, WI). Real-time PCR amplification was performed using the SYBR Green master mix (Toyobo, Japan) and the Opticon Real-time PCR Detection System (Bio-rad). Primers of GAPDH, PPAR γ , SRC-1, SRC-2, and PGC-1 were designed using Primer software and the sequences were as follows: GAPDH, 5'-CAG-GGC-TGC-TTT-TAA-CTC-TGG-TAA-3' (sense) and 5'-GGG-TGG-AAT-CAT-ATT-GGA-ACA-TGT-3' (antisense); PPAR γ , 5'-GGG-CCC-TGG-CAA-AAC-ATT-3' (sense) and 5'-AAG-ATC-GCC-CTC-GCC-TTT-3' (antisense); SRC-1, 5'-TGG-GTA-CCA-GTC-ACC-AGA-CA-3' (sense) and 5'-GAA-TGT-TTG-CGT-TTC-CAC-CT-3' (antisense); SRC-2, 5'-GAC-AGA-TCG-TGC-CAG-TAA-CAC-AA-3' (sense) and 5'-TTC-AGC-TGT-GAG-TTG-CAT-GAG-G-3' (antisense); PGC-1, 5'-CCA-AGA-CCA-GCA-GCT-CCT-A-3' (sense) and 5'-CCA-CTG-TCA-AGG-TCT-GCT-CA-3' (antisense); MCP-1, 5'-CAG-CCA-GAT-GCA-ATC-AAT-GC-3' (sense) and 5'-GTG-GTC-CAT-GGA-ATC-CTG-AA-3' (antisense). Relative amounts of mRNA were normalized by GAPDH and calculated using comparative Ct ($\Delta\Delta Ct$) method.

Western blot analysis

Nuclear and cytosolic fractions of HK-2 cells were separated using NE-PER™ Nuclear and Cytoplasmic Extraction reagents (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and renal tissues were lysed in radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology, Haimen, China) containing protease inhibitor cocktail (Bimake, Houston, TX, USA) to extract the total protein. The concentration of proteins was determined by Bicinchoninic Acid (BCA) method using BCA Protein Assay kit (Shanghai

Epizyme Biotechnology, China). Western blotting was performed as previously described (19). The primary antibodies were obtained from the following sources: anti-PPAR γ (Santa Cruz Biotechnology), anti-NLRP3 (Novus Biologicals), anti-PGC-1 α (EMD Millipore), anti-Nephrin and anti-NF- κ B (Abcam). Anti-LaminB and anti- β -actin (Abcam) were used as the loading controls for nuclear and total protein expression, respectively.

MCP-1 assays

Culture supernatants were collected from 6-well plates and concentration of MCP-1 measured by enzyme linked immunosorbent assay (ELISA) using protocols supplied by the manufacturer (R&D Systems, Abingdon, UK) and normalized to cell protein concentrations.

Electrophoretic mobility shift assay (EMSA)

Procedures were performed following standard steps (20). Nuclear extracts containing 10 μ g protein were added to DNA-binding reaction buffer. Resultant nucleoprotein complexes were analyzed by loading samples on a 6% native polyacrylamide gel in 0.5 \times TBE buffer, dried, and visualized by autoradiography. In each set of experiments, a self-competition was performed by adding excess of the same unlabeled oligonucleotide probe or a corresponding mutant oligonucleotide probe to the binding reaction at the preincubation step. The following oligonucleotides were used: peroxisome proliferator response element (PPRE), 5'biotin- GAT-CCT-CCC-GAA-CGT-GAC-CTT-TGT-CCT-GGT-CCA -3', 3'biotin-CTA-GGA-GGG-CTT-GCA-CTG-GAA-ACA-GGA-CCA-GGT-5'; mutant-PPRE, 5'biotin- GAT-CCT-CCC-GAA-CGC-AGC-TGT-CAG-CTG-GGT-CCA-3', 3'biotin-CTA-GGA-GGG-CTT-GCG-TCG-ACA-GTC-GAC-CCA-GGT-5'; NF- κ B response element, 5'biotin-AGT-TGA-GGG-GAC-TTT-CCC-AGG-C-3', 3'biotin-TCA-ACT-CCC-CTG-AAA-GGG-TCC-G-5'; mutant-NF- κ B response element, 5'biotin-AGT-TGA-GGC-GAC-TTT-CCC-AGG-C-3', 3'biotin-TCA-ACT-CCG-CTG-AAA-GGG-TCC-G-5'.

Biochemical analysis of serum and urine samples

Protein concentration in urine was detected using BCA Protein Assay kit (Shanghai Epizyme Biotechnology, China), and glucose concentration in serum was detected by Glucose LiquiColor $^{\circledR}$ test kit (EFK Diagnostics, Inc., Boerne, TX, USA). After 8 weeks of treatment, urine was collected over 24 h in metabolic cages to measure and calculate the urinary protein excretion (UAE). Blood from the caudal vein was collected for monitoring serum glucose.

Kidney histopathology

Kidney histopathology was performed as previously described (12). Kidneys removed from euthanized mice and were immediately cut in half, fixed in 10% formaldehyde in 0.1 mol/L PBS (pH 7.2) at 4 $^{\circ}$ C for 24 h, then embedded in paraffin and sectioned at 4 μ m. The 4- μ m-sections were dewaxed in xylene, rehydrated through decreasing concentrations of ethanol and washed in PBS. Subsequently, for each sample, one section was stained with periodic acid-Schiff (Goodbio Technology CO., LTD, Wuhan, China). Following staining, sections were dehydrated through increasing concentrations of ethanol and xylene.

The general histological alterations in glomerular and tubular structures were evaluated under a light microscope.

Transmission electron microscopy

Renal cortical tissues were fixed in 2% glutaraldehyde in phosphate-buffered solution (pH 7.4). Samples were further incubated with 2% osmium tetroxide in phosphatebuffered solution (pH 7.4) for 2 h at 4 °C. Ultrathin sections were stained with lead citrate and uranyl acetate and viewed on a HT770 transmission electron microscope (Hitachi, Japan) at an accelerating voltage of 80 kV as previously described (21).

Statistical analysis

Data are expressed as mean±SEM. Multiple comparisons were examined for significant differences using analysis of variance (ANOVA). Statistical significance was set at P<0.05.

Results

TNF- α and IL-1 β decrease PPAR γ mRNA and protein levels.

As shown in Fig. 1, we firstly investigated the dose-response curves and time course of TNF- α or IL-1 β on PPAR γ expression in HK-2 cells. At the 24h time point, TNF- α induced a dose-dependent decrease in PPAR γ mRNA levels, with a nearly 60% decrease at 10ng/ml compared to control group (Paragraph A). IL-1 β also decreased PPAR γ mRNA level by nearly 50% at the same concentration (Paragraph C). Thereafter, we chose 10ng/ml TNF- α and 10ng/ml IL-1 β as the working concentration for subsequent experiments in this study.

Furthermore, at different time points, both TNF- α and IL-1 β at 10ng/ml were able to decrease PPAR γ mRNA expression as early as 4 h after treatment (Paragraph B and D). We also examined PPAR γ protein levels. As shown in Fig. 2, TNF- α or IL-1 β at 10ng/ml induced a decrease in PPAR γ protein levels at 4h or even earlier at 2h after treatment. Together, these results demonstrated that TNF- α and IL-1 β could rapidly suppress PPAR γ expression and the decrease is a much sustained cytokine response.

TNF- α and IL-1 β decrease the binding of nuclear extracts to PPAR γ response elements.

To determine if the decrease of PPAR γ induced by TNF- α and IL-1 β influenced binding of nuclear extracts to the peroxisome proliferator response elements (PPRE), we carried out electrophoretic mobility shift assays (EMSA). As shown in Fig. 3, PPAR γ binding activities were significantly reduced by 10ng/ml IL-1 β (Figure C and D) as early as 2h after treatment and sustained to 24 h. TNF- α (Figure A and B) also decreased PPAR γ binding activities at 2 h after treatment, but recovered gradually since 4h. These data demonstrates that TNF- α and IL-1 β could reduce PPAR γ binding activity to known cognate response elements for their target genes, especially at early stage of stimulation.

TNF- α and IL-1 β influence levels of coactivators SRC-1, SRC-2 and PGC-1.

Since TNF- α and IL-1 β could greatly decrease expression levels of PPAR γ , we examined whether cytokines treatment also have an effect on the expression of coactivators(including SRC-1, SRC-2 and PGC-1). We found the mRNA levels of SRC-1 and SRC-2, which belonged to the p160 family coactivators, were decreased by 35% and 41% within 2 hours after TNF- α (10ng/ml) treatment (Fig. 4A). The expression of PGC-1, another coactivator of PPAR γ , was also markedly decreased by 46% as early as 1 hour after TNF- α stimulation (Fig. 4A). Similarly, treatment with IL-1 β (10ng/ml) also reduced mRNA level of SRC-1, SRC-2 and PGC-1 α by 43%, 58% and 48% separately within 2 hours (Fig. 4B). These results proved that the mRNA levels of the coactivators SRC-1, SRC-2 and PGC-1 could be influenced together with PPAR γ after TNF- α and IL-1 β treatment, which may contribute to cytokines-induced renal interstitial inflammation.

TNF- α and IL-1 β effectively induce NF- κ B activation and MCP-1 production.

Finally, we explored the mechanism of the inhibitory effects of TNF- α and IL-1 β on PPAR γ and its coactivators. We focused on NF- κ B pathway which plays critical regulatory roles in inflammation and could be activated by TNF- α and IL-1 β directly. Previous investigations have proved that NF- κ B pathway has a great relationship with PPAR γ and could directly repress PPAR- γ and related genes expression.

We examined the activity of NF- κ B after TNF- α and IL-1 β stimulation. By EMSA, we found TNF- α or IL-1 β could significantly promote NF- κ B DNA binding activities even after 2 hours treatment, which increased 2.5-fold (Fig. 5A and 5B) or 2.3-fold over baseline (Fig. 5C and 5D), respectively. Furthermore, we examined monocyte chemoattractant protein-1 (MCP-1) production after NF- κ B pathway activation, which is a downstream target gene of NF- κ B pathway and plays important roles in transmigration of inflammatory cells. MCP-1 protein secretion was determined by ELISA as stated in Materials and Methods. We found TNF- α significantly increased MCP-1 protein secretion from 15.52 ± 1.05 pg/ml at baseline to 40.47 ± 0.97 pg/ml after 24 hours treatment (Fig. 6 and Table 1). Meanwhile, IL-1 β also greatly induced MCP-1 secretion from 19.33 ± 2.33 pg/ml at baseline to 160.56 ± 2.8 pg/ml even after 8 hours treatment (Fig. 6 and Table 2). These data verified TNF- α and IL-1 β effectively induced the activation of NF- κ B signaling pathway under same experimental conditions, which mediated down-regulation of PPAR γ and coactivators expression.

Table 1
Influence of TNF- α on MCP-1 secretion in HK-2 cells

Time points (TNF- α 10ng/ml)	MCP-1 Concentration (pg/ml)
1 Control	15.52 ± 1.05
2 1h	26.69 ± 0.53
3 2h	25.95 ± 0.21
4 4h	29.17 ± 0.56
5 8h	32.72 ± 2.14
6 24h	40.47 ± 0.97

Table 2
Influence of IL-1 β on MCP-1 secretion in HK-2 cells

Time points (IL-1 β 10ng/ml)	MCP-1 Concentration (pg/ml)
1 Control	19.33 \pm 2.23
2 1h	32.89 \pm 0.3
3 2h	33.76 \pm 1.47
4 4h	57.46 \pm 1.86
5 8h	160.56 \pm 2.8
6 24h	50.82 \pm 1.25

PPAR γ and PGC-1 α activators protect against diabetic nephropathy and suppress NF- κ B expression in db/db mice

The body weight and serum glucose levels of the 14 week old db/db mice were significantly higher compared with the db/m control group (Fig. 7A and 7B). The rosiglitazone administration but not ZLN005 reduced serum glucose levels to control levels, and both aginists showed no effects on the body weight of db/db mice after 8 week of administration (Fig. 7A and 7B). For 14 week old mice, the 24-hour urinary protein levels of the D group was significantly increased compared with the C group, while 8 weeks of either rosiglitazone or ZLN005 treatment reduced the 24-hour urinary protein of db/db mice significantly (Fig. 7C).

PAS staining revealed obvious mesangial cell proliferation and mesangial matrix expansion in the D group compared with the C group, while either rosiglitazone or ZLN005 treatment aliminated the pathological characteritics markedly and showed only minor differernces compared with the C group (Fig. 7D). Transmission electron microscopy (TEM) micrographs revealed that podocyte foot process effaced and glomerular basement membrane (GBM) thickened in glomeruli and the density and quantity of mitochondials in tubule cells decreased in db/db mice compared with that in the control mice, and morphological injuries were aliminated after the treatment of rosignitazone or ZLN005(Fig. 7E and 7F).

The total protein of kidney tissues were extracted and the western blotting results showed a significantly reduced expression of nephrin and PGC-1 α (Fig. 8A, 8B, 8C, 8D) but significantly increased levels of NF- κ B and NLRP3 in db/db mice compared with the control mice (Fig. 8E, 8F), and rosignitazone or ZLN005 treatment recovered these changes. These results indicated the renoprotective role of both PPAR γ and PGC-1 α in diabetic nephropathy with inducing PGC-1 α and reducing NF- κ B.

Discussion

Extensive studies have confirmed that protective roles of nuclear receptor PPAR γ and coactivators in heart, liver, brain and adipose tissue, with the well-defined mechanism of regulation of inflammatory response, energy metabolism and mitochondrial biogenesis. Our present work revealed that TNF- α or IL-1 β administration could induce a rapid and marked decrease in the expression of negative inflammatory protein PPAR γ and its coactivators (including SRC-1, SRC-2 and PGC-1) expression in human proximal tubular cells. Activation of NF- κ B pathway may be the potential mechanism. Activators of PPAR γ and PGC-1 α contributed to attenuated the damage in both glomeruli and tubules of diabetic nephropathy in vivo through downregulating the expression of NF- κ B and repressing inflammation.

Our data are consistent with previous studies which showed PPARs and coregulators were actively involved in inflammation courses in different human organs. In acute renal failure, Portilla D et al found cisplatin deactivated PPAR α by reducing its DNA binding activity and expression of its specific coactivator PGC-1 (20). Feingold K and colleagues verified either administration of LPS in vivo or TNF- α in vitro could obviously decrease the expression of PPAR α , - γ and - δ as well as the coactivators (including SRC-1, SRC-2, SRC-3 and PGC-1) in the heart and adipose tissue (22, 23). Kim MS group and Rita S group demonstrated similar inflammatory stimulation (including LPS, TNF- α and IL-1 β) also decreased the expression of PPAR γ , SRC-1, SRC-2 and PGC-1 in the liver cells and uterine smooth muscle cells, but do not influence expression of SRC-3 and corepressors NCoR and SMRT (24, 25). Moreover, Rame JE et al found conditions that induced myocardial steatosis, such as pressure overload, resulted in the decrease of PPAR α and PGC-1 levels, which leading to disorders of glycerolipid/free fatty acid cycling (26). Inflammation has also been identified as a risk factor for cancer and is associated with poor clinical outcome. Investigators found levels of PPAR γ , SRC-1 and PGC-1 were obviously lower in breast and colon tumor tissues (27, 28), and treatment with anti-tumor drugs could up-regulate SRC-1 and PGC-1 expressions (29, 30). All these studies strongly suggested that down-regulation of type II nuclear hormone receptors PPAR γ , together with its coactivators, contributed to the progression of inflammatory injuries.

We also explored the molecular mechanism of these effects. NF- κ B is a critical transcription factor for maximal expression of many cytokines that are involved in the pathogenesis of inflammatory diseases (31). Moreover, the NF- κ B signaling pathway has been represented to be a major avenue for TNF- α or IL-1 β inhibition of PPAR γ function (32). Under normal conditions, NF- κ B is present in the cytoplasm in an inactive state by binding to an inhibitory protein, I κ B. In response to activating signals such as TNF- α and IL-1 β , I κ B is phosphorylated and degraded, which leads to the activation and nuclear translocation of NF- κ B (33). Previous studies have certified NF- κ B activation could inhibit PPAR γ function through several approaches. First, NF- κ B could cause a direct phosphorylation of serine residues in PPAR γ protein, such as Ser112 or Ser82, results in a reduction of its transcriptional activity (34). Second, in acute manner, I κ B degradation could cause PPAR γ corepressor HDAC3 (Sin3 deacetylase and histone deacetylase) nuclear translocation, and up-regulated binding activities between corepressor complex SMRT/NCoR/HDAC3 with PPAR γ , which not only influences interaction between PPAR γ and coactivators, but leads to suppression of the transcriptional activity of PPAR γ in the nucleus (34). Moreover, recent studies verified NF- κ B has direct interaction with PPAR γ and its coactivators and resulted in deprivation of the transcriptional coactivators from PPAR γ (35, 36). In the present study, we also found NF- κ B activity was greatly up-

regulated along with reduced expression of PPAR γ as well as its coactivators and provided new evidences for these inhibitory roles of NF- κ B. But the precise mechanism and other possible contributors still need to be explored further.

Since Zhang et al (37) reported ZLN005 as a small molecule inducing PGC-1 α expression in mRNA levels in 2013, it has been studied in nerve and cardiovascular tissues and cells on anti-oxidation and mitochondrial damage (38–41). But to our knowledge, there is no report about ZLN005 in kidney disease or anti-inflammatory effects. For the first time, we employed ZLN005 as the PGC-1 α activator in diabetic nephropathy and investigated its mechanism related to inflammatory regulation. These data strongly supported that ZLN005 induced the PGC-1 α expression in kidney tissue, which protected against renal inflammatory injury and improved diabetic nephropathy.

TNF- α and IL-1 β are systemic and renal local inflammatory mediators. The downregulation by TNF- α and IL-1 β of PPAR γ and PGC-1 α in this work is accompanied by the increase in expression and DNA binding activity of the proinflammatory transcriptional factor NF- κ B, which may suggest the molecular mechanism that systemic and renal local inflammation aggravating kidney disease. The renoprotective role of PPAR γ and PGC-1 α activators through the mechanism involving NF- κ B expression and inflammation reduction was verified in db/db diabetic nephropathy model mice. In summary, these results highlight that PPAR γ together with its coactivator PGC-1 α actively participates in the renal inflammation through NF- κ B related mechanism. Further exploration of detailed mechanisms may provide novel potential therapeutic targets for renal inflammatory diseases.

Declarations

Acknowledgments

Not Applicable.

Authors' contributions

Weiming WANG and Nan CHEN designed and supervised the study. Yuanmeng JIN, Liwen ZHANG and Ying ZHOU performed the experiments and data analysis. Liwen ZHANG and Yuanmeng JIN wrote the manuscript. Weiming WANG and Nan CHEN critically edited and revised the manuscript. All authors contributed to the preparation and approved the final draft of manuscript.

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Availability of data and materials

The datasets used for this work can be requested from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures used in animal experiments were conducted following the ARRIVE guidelines. This study was approved by the Animal Ethics Committee of Ruijin Hospital according to the provisions in the 1975 Declaration of Helsinki and its later amendments.

Consent for publication

Not applicable.

Competing interests

All the authors declared no competing interests.

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Figures

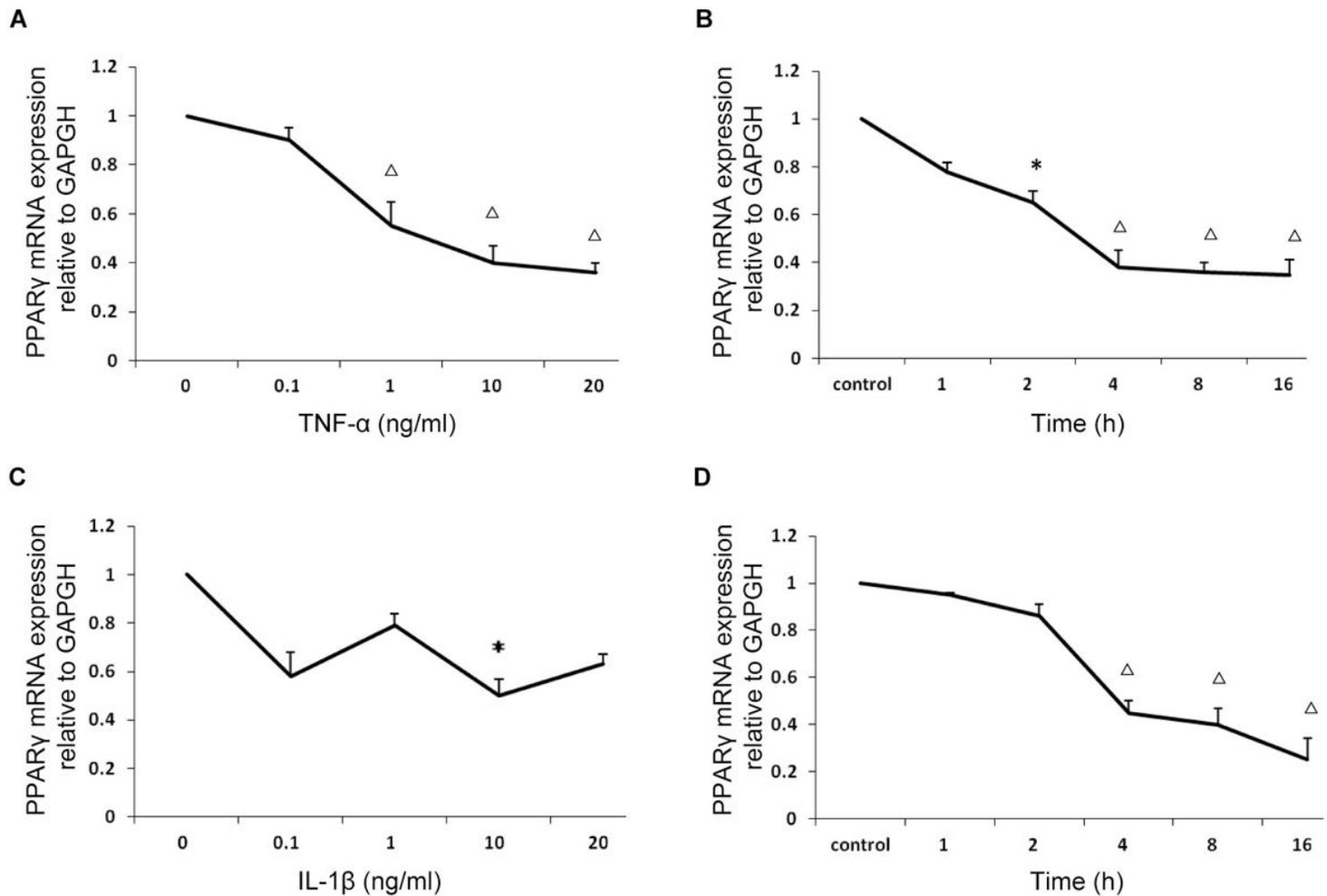


Figure 1

Time- and Dose-dependent changes in PPAR γ mRNA levels. HK-2 cells were cultured for 24 h with various concentrations of TNF- α (A) and IL-1 β (C) as indicated. Also, HK-2 cells were cultured with 10ng/ml TNF- α (B) or IL-1 β (D) at various time points as indicated. Total RNA was isolated and real-time PCR analysis was performed as described in Materials and Methods. Data (means \pm SEM,n=3) are expressed as percentages of control values.* $P < 0.05$ versus control, $\Delta P < 0.01$ versus control.

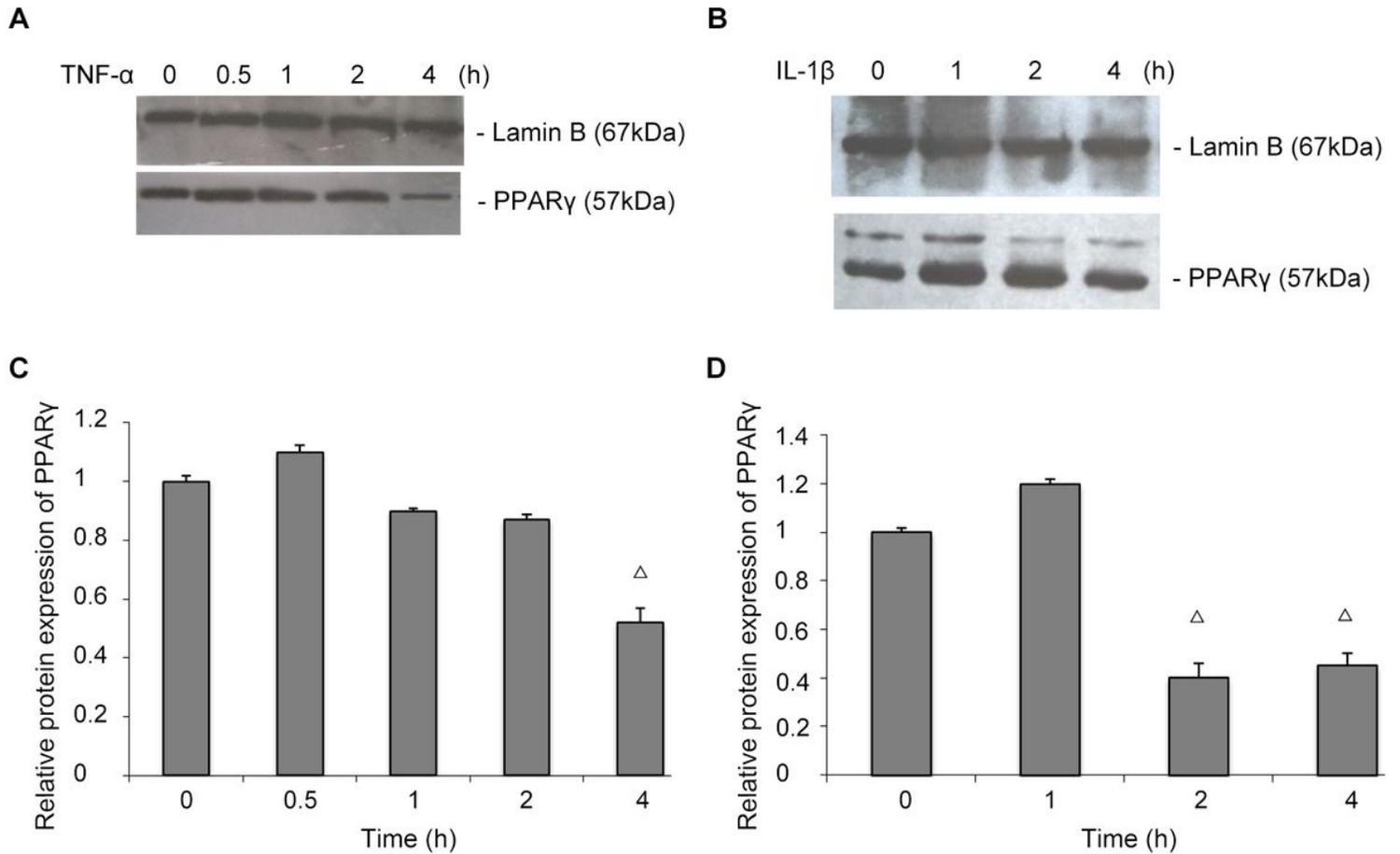


Figure 2

Effects of TNF- α and IL-1 β on protein expression of PPAR γ in HK-2 cells. HK-2 cells were treated for various times in the presence of 10ng/ml TNF- α or IL-1 β . (A and C) Nuclear extracts were prepared and Western blot analysis was done using antibodies recognizing PPAR γ as described in the Methods section. (B and D) Relative protein levels (% of controls) were reported after normalization with Lamin B, respectively.* P<0.05versus control, Δ P<0.01versus control.

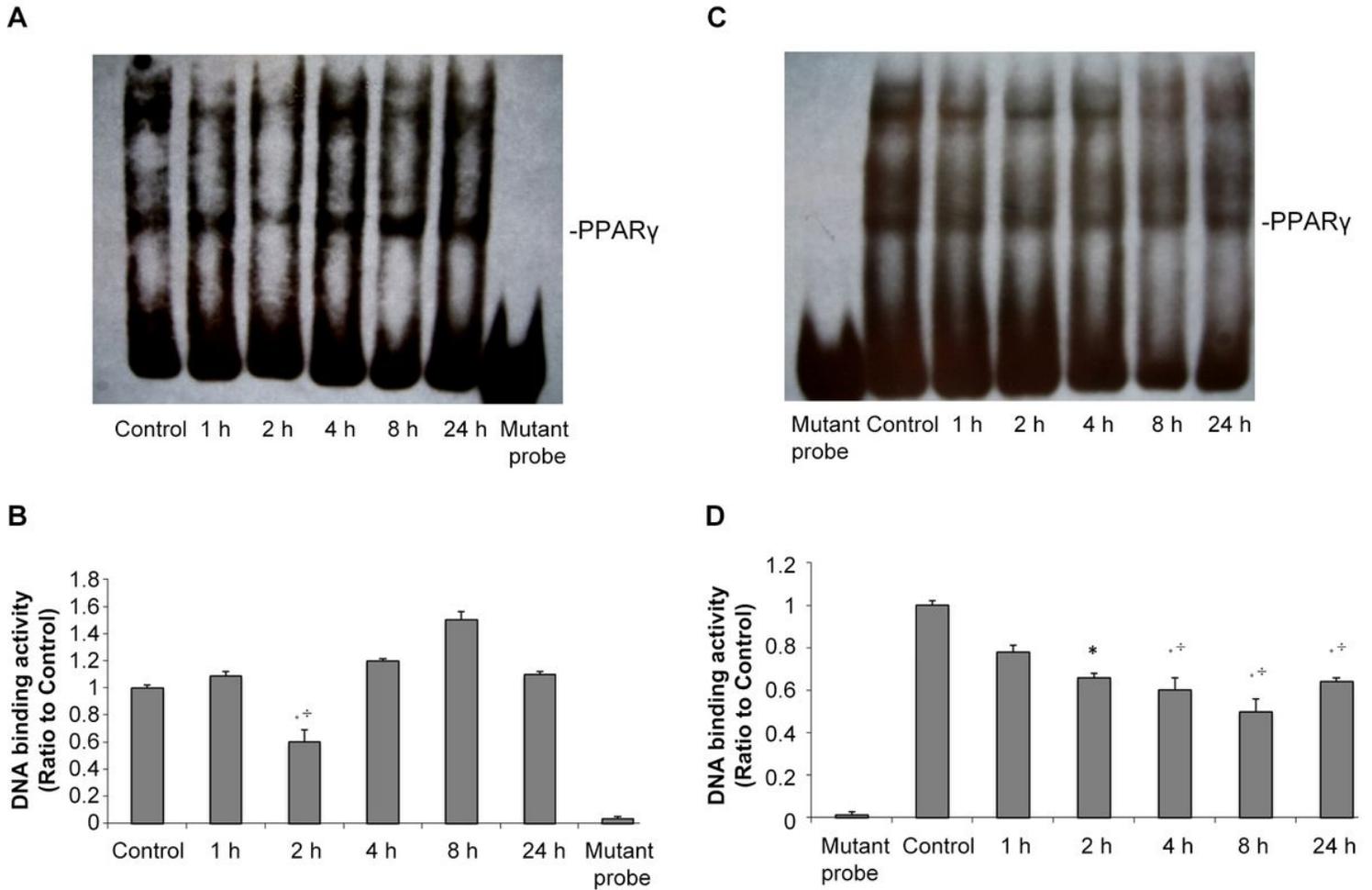


Figure 3

TNF- α and IL-1 β decrease the binding of nuclear extracts to PPAR γ . HK-2 cells were treated with TNF- α or IL-1 β at 10 ng/ml at various time points as indicated. Nuclear extracts were isolated and 10 μ g of the nuclear proteins were used for electrophoretic mobility shift assay (EMSA) with oligonucleotides for PPAR γ -specific response elements as described under "Materials and Methods". (A and C) Representative EMSA for nuclear receptors studied. (B and D) Quantification of EMSA from individual experiments. Data (means \pm SEM, n=3) are expressed as percentages of control values. * P<0.05 versus control, Δ P<0.01 versus control.

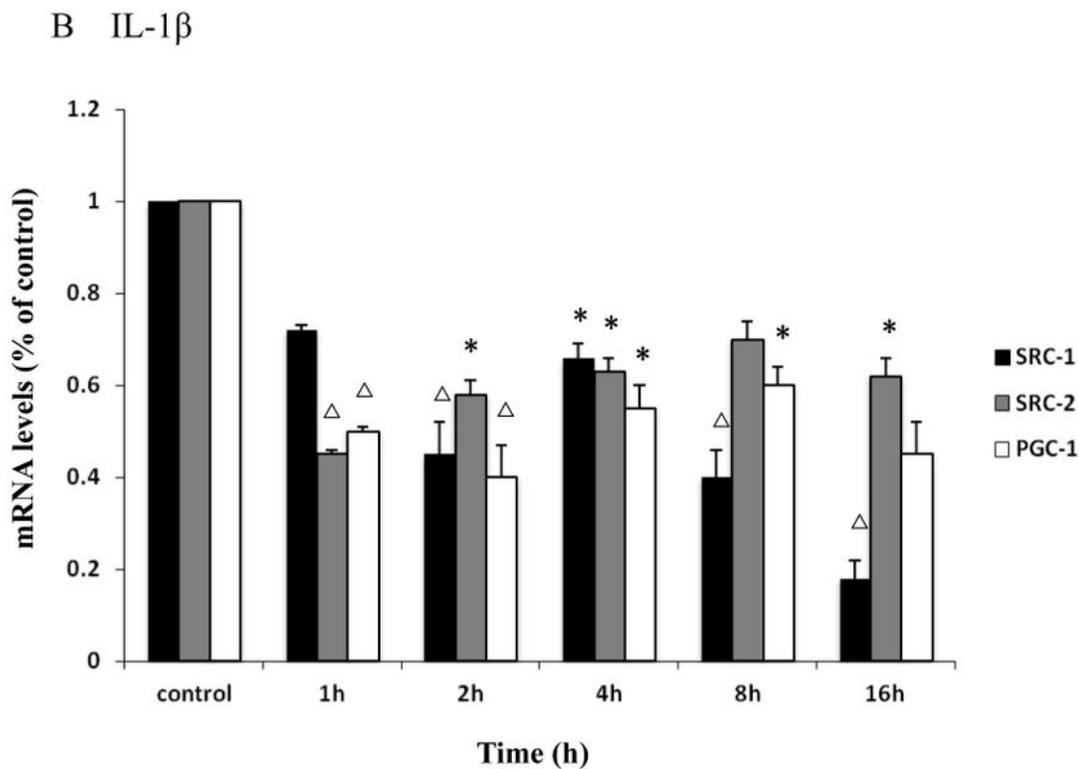
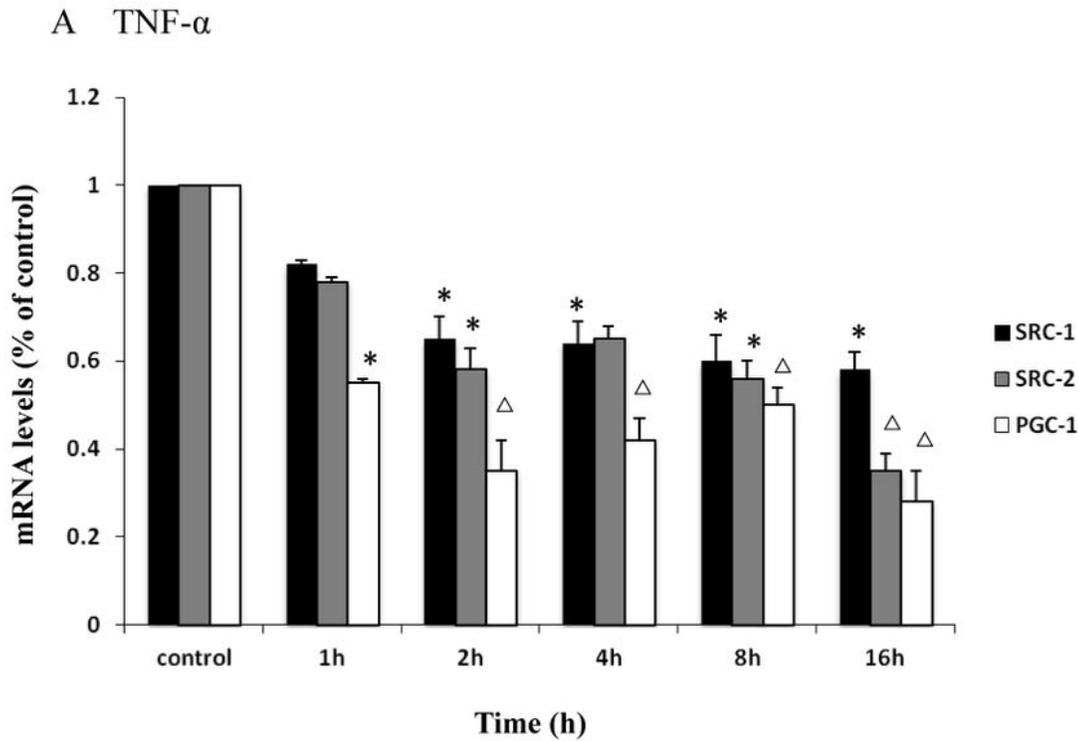


Figure 4

TNF- α and IL-1 β decrease the expression of SRC-1, SRC-2 and PGC-1 in HK-2 cells. HK-2 cells were treated for various times in the presence of 10ng/ml TNF- α (A) or IL-1 β (B). Total RNA was prepared and expression levels for SRC-1, SRC-2 and PGC-1 were determined by real-time PCR. Data (means \pm SEM, n=3) are expressed as percentages of control values.* P<0.05 versus control, Δ P<0.01 versus control.

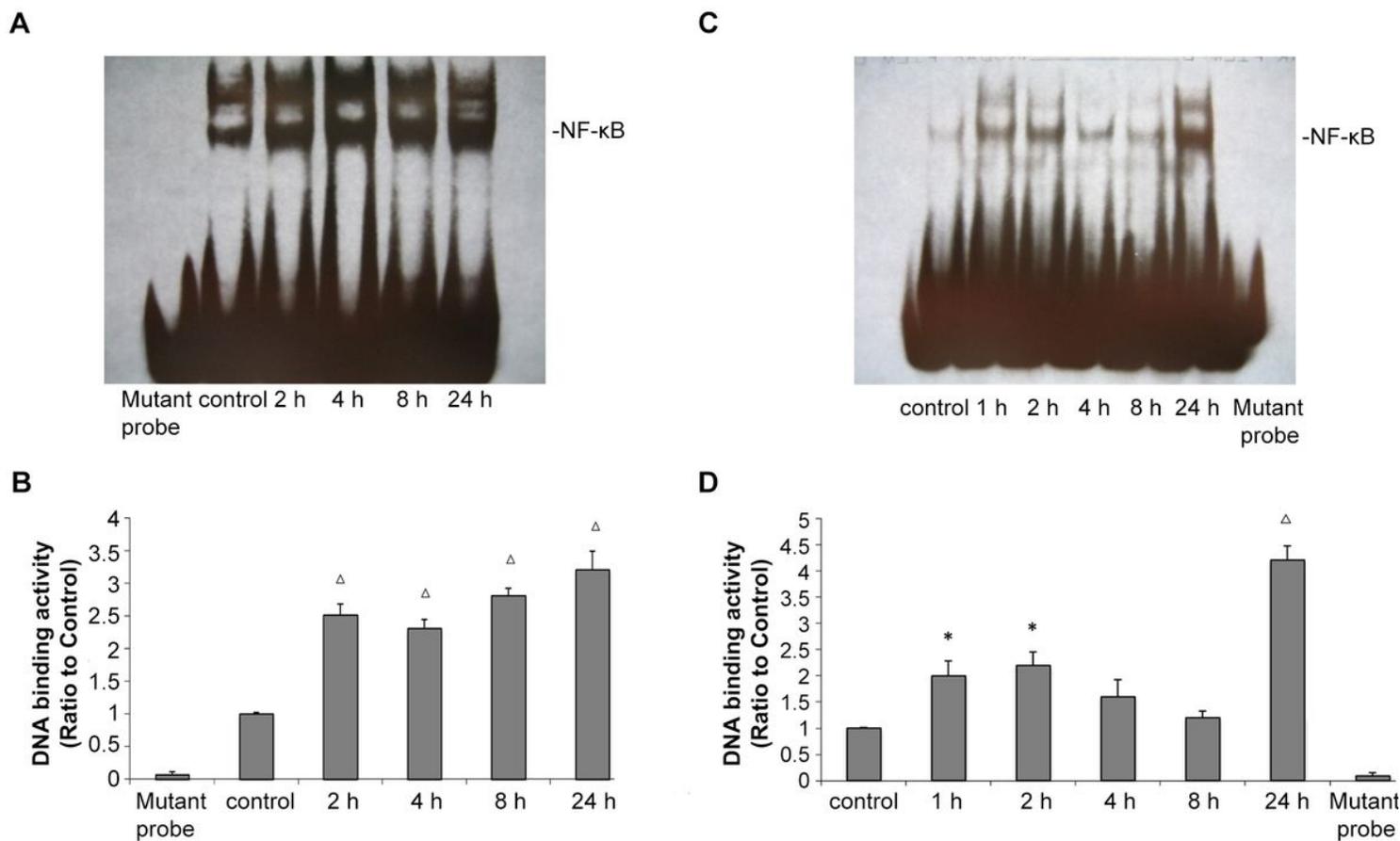


Figure 5

TNF- α and IL-1 β increase the binding of nuclear extracts to NF- κ B. HK-2 cells were treated with TNF- α or IL-1 β at 10ng/ml as indicated. Nuclear extracts were used for electrophoretic mobility shift assay (EMSA) with oligonucleotides for PPAR γ -specific response elements as described under "Materials and Methods". (A and C) Representative EMSA for nuclear receptors studied. (B and D) Quantification of EMSA from individual experiments. Data (means \pm SEM, n=3) are expressed as percentages of control values.* P<0.05 versus control, Δ P<0.01 versus control.

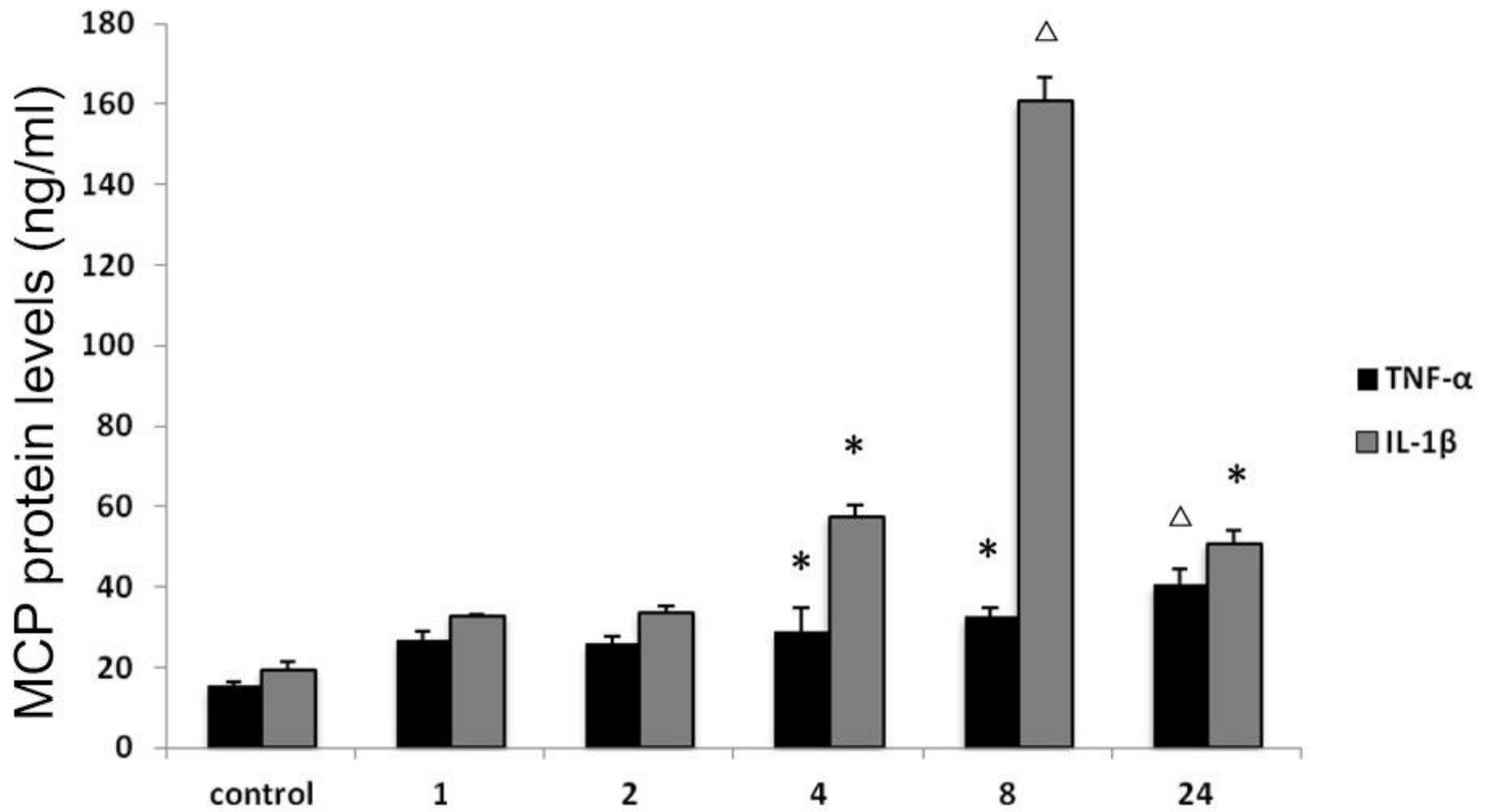


Figure 6

MCP-1 expression was up-regulated in 10ng/ml TNF- α and IL-1 β -treated HK-2 cells. MCP-1 protein levels in cell culture supernatant were determined by enzyme linked immunosorbent assay (ELISA) as described in Materials and Methods. Data (means \pm SEM) are from duplicate experiments. *P<0.05versus control, Δ P<0.01versus control.

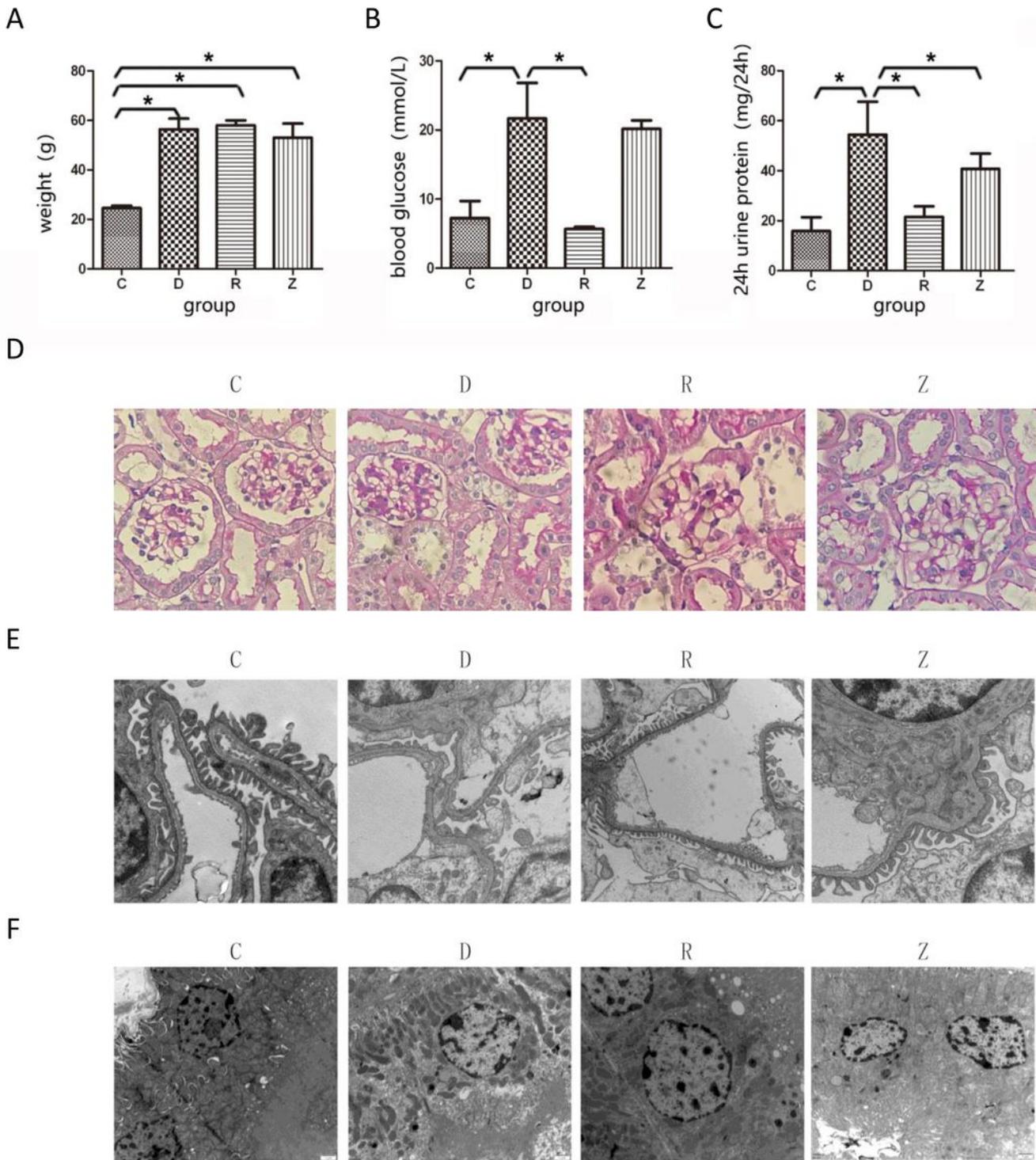


Figure 7

Treatment with rosiglitazone or ZLN005 exhibited protective effects against DN in mice. After 8 weeks of treatment, (A) body weight levels were detected in the C, D, R and Z groups. (B) Blood glucose levels were detected in the four mentioned groups. (C) 24 h urinary albumin excretion in mice in the four mentioned groups. Data are presented as the mean \pm sem (n=6 per group). *P<0.05 between the indicated groups. Representative photomicrographs depicting (D) PAS staining in the C, D, R and Z groups after the 8 week

experimental period. Original magnification, x400. Representative TEM micrographs of foot processes and GBM (E) and tubular cells (F) in the the C, D, R and Z groups. Original magnification, x10,000.

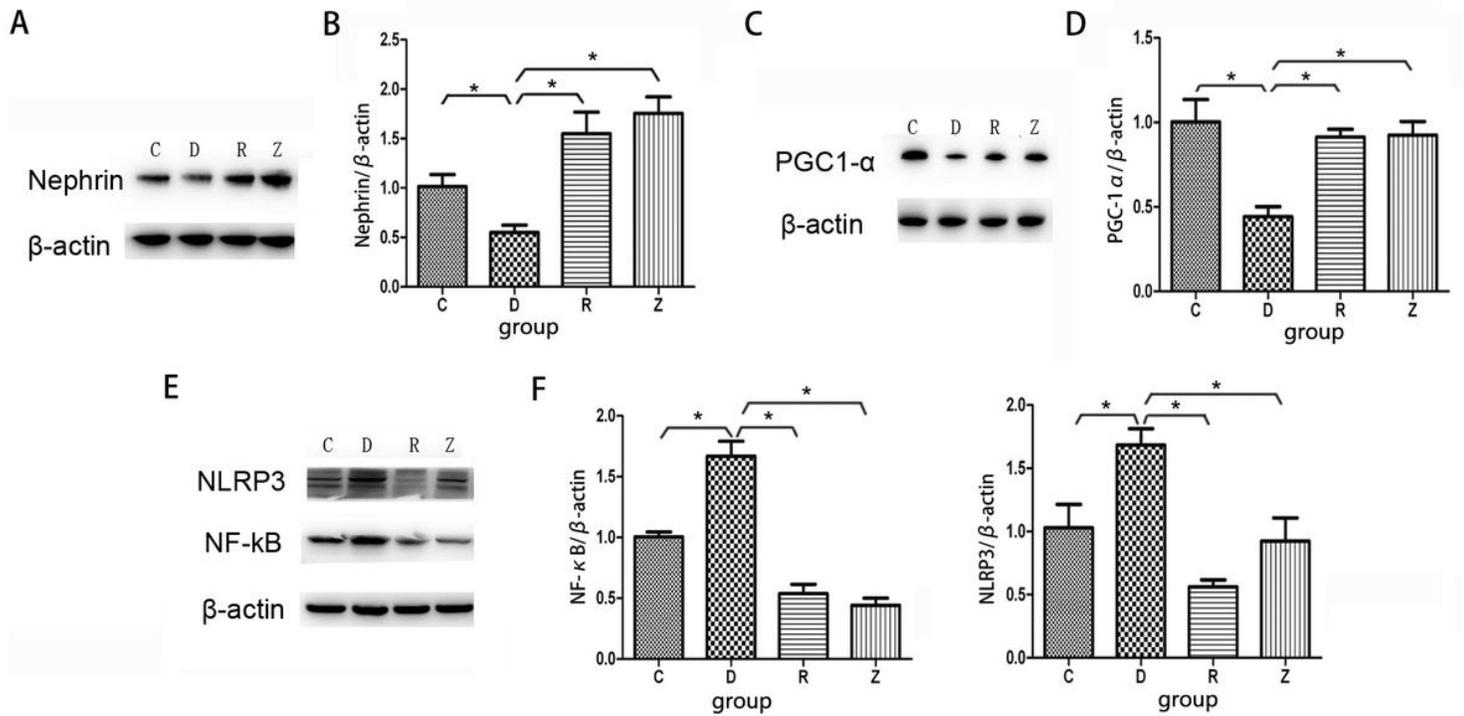


Figure 8

Rosiglitazone or ZLN005 treatment upregulated PGC-1 α and inhibited inflammation in db/db renal tissues. (A) Western blot analysis of nephrin and β -actin expression in the C, D, R and Z groups. (B) Densitometric analysis of western blot results. Relative band intensity was normalized to the intensity of the respective β -actin signal. (C) Western blot analysis of PGC-1 α and β -actin expression in the C, D, R and Z groups. (D) Densitometric analysis of western blot results. Relative band intensity was normalized to the intensity of the respective β -actin signal. (E) Western blot analysis of NF- κ B, NLRP3 and β -actin expression in the C, D, R and Z groups. (F) Densitometric analysis of western blot results. Relative band intensity was normalized to the intensity of the respective β -actin signal. Data are presented as the mean \pm standard deviation (n=6 per group). *P<0.05 as indicated.