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Rosiglitazone 抑制高糖促進細胞外間質堆積之作用機轉

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中文摘要

研究背景 腎絲球硬化症(Glomerulosclerosis)是進行性腎臟疾病重要的病理變化。腎膈細胞(mesangial cell, 簡稱 MC)不當增生及所造成的細胞外間質(Extracellular matrix, 簡稱 ECM)堆積是造成腎絲球硬化及纖維化的前趨變化。Peroxisome proliferator-activated receptor γ , (簡稱 PPAR γ)是細胞核受體超級家族的一員, 調控著脂肪細胞分化及體內葡萄糖恆定, 是胰島素促感受性藥物作用的分子標的。Thiazolidinedione (簡稱 TZD), 為人工合成的 PPAR γ 配體, 用以控制第二型糖尿病, 在動物實驗中已證實可以減少腎膈細胞區域的擴張, 因此, 吾人擬在細胞培養層次, 探討 PPAR γ 配體是否可以抑制大鼠腎膈細胞(rat mesangial cell, 簡稱 RMC)增生及減少細胞外間質基因之製造與合成。

研究設計與方法 RMC 取自公 Sprague-Dawley 大鼠, 以酵素消化萃取後, 進行培養。用修正過的 MTT 法觀察 RMC 增生情形。位研究 RMC 增生及被抑制的狀況, 吾人利用流式細胞儀分析 RMC 的細胞週期分佈情形。本人另採用北方墨點法分析各種狀況下, $\alpha 1(I)$ 膠原蛋白, fibronectin 及結締組織生長因子 (connective tissue growth factor, 簡稱 CTGF) 基因的表現情形。

結果 在細胞培養實驗中, 吾人證實 PPAR γ 配體(1~100 μ M 的 rosiglitazone, 簡稱 RSG; 1~10 μ M 的 15-deoxy-delta^{12, 14}-prostaglandin J₂, 簡稱 15d-PGJ₂) 抑制血清(10% FCS)及 PDGF(platelet-derived growth factor)RMC 增生的效果, 進而發現使 RMC 生長停滯在 G1 期。除上述抑制 PDGF 刺激 RMC 增生效果外, PPAR γ 配體也會抑制血清或 TGF- β 刺激 RMC 合成 $\alpha 1(I)$ 膠原蛋白, fibronectin 及 CTGF 基因的表現。

討論 吾人的細胞培養實驗之初步結果顯示, PPAR γ 配體(RSG 及 15d-PGJ₂)可能有預防腎絲球硬化症的效果。PPAR γ 配體可以抑制 RMC 細胞的增生, 減少血清 TGF- β 刺激的 $\alpha 1(I)$ 膠原蛋白, fibronectin 及 CTGF) 基因的表現。

(關鍵字: PPAR γ , 大鼠腎膈細胞, 細胞週期, 細胞增生, 細胞外間質, 結締組織生長因子, 腎小球硬化症)

Abstract

Background. Glomerulosclerosis is a central pathologic feature of progressive renal diseases. Mesangial cell proliferation and extracellular matrix (ECM) deposition are regarded as main processes predisposing to glomerulosclerosis. The peroxisome proliferator-activated receptor γ (PPAR γ) is a member of nuclear receptor superfamily that regulates fat-cell differentiation and glucose homeostasis and is the molecular target of a class of insulin-sensitizing agents used for the management of type II diabetes mellitus. Thiazolidinedione (TZD), a synthetic ligand of PPAR γ , was shown to ameliorate mesangial expansion in vivo. Thus, we investigated the effect of PPAR γ ligands in inhibiting cell proliferation and ECM gene expression of rat mesangial cells (RMCs) in vitro.

Methods. RMCs were cultured from Sprague-Dawley rats by a modified enzyme digestion method. Cell proliferation was measured by the methyltetrazolium assay. Cell-cycle distribution of RMC was analyzed by flow cytometry. Expression of type I (α 1) collagen, fibronectin and connective tissue growth factor (CTGF) mRNA level were analyzed by Northern blotting.

Results. Treatment of cultured-RMCs with ligands for PPAR : [rosiglitazone (RSG), 1 ~50 μ M or 15-deoxy-delta^{12, 14}-prostaglandin J₂ (15d-PGJ₂), 1 ~10 μ M] inhibited serum and platelet-derived growth factor (PDGF)-stimulated RMCs proliferation without affecting the cell viability. The PPAR γ activation suppressed PDGF-stimulated RMCs proliferation by cell-cycle arrest at the G1 phase. PPAR agonists suppressed serum and transforming growth factor- β (TGF- β)-induced extracellular matrix gene and CTGF gene expression in RMCs cultures.

Conclusions. PPAR γ ligands, RSG and 15d-PGJ₂, inhibit RMC proliferation, type I (α 1) collagen, fibronectin and CTGF mRNA expression. These results indicate that PPAR γ ligands have therapeutic potential in progressive renal disease.

(Key Word: peroxisome proliferator-activated receptor γ , rat mesangial cell, cell proliferation, cell cycle, extracellular matrix, connective tissue growth factor, glomerulosclerosis)

Introduction

Glomerulosclerosis, a central pathologic feature of progressive renal diseases, is considered as the final common response to injury, and the scarring process involving ECM accumulation and structural collapse is further demonstrated in several kidney diseases [1]. Mesangial cell (MC) proliferation has also been regarded as the antecedent of glomerulosclerosis [2,3]. The decrease of MC replication was associated with a marked reduction of mesangial ECM accumulation and deposition [4]. Because of the perceived importance of MC proliferation and ECM deposition in glomerular diseases, pharmacological interventions has been undertaken to target against these processes.

The peroxisome proliferator-activated receptor γ (PPAR γ), a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors, has recently been cloned and functional in rat and human mesangial cells [5-7]. PPAR γ forms a heterodimeric complex with retinoic X receptor- α (RXR α). Activation of PPAR γ /RXR α heterodimer by PPAR γ ligands and/or RXR α ligands result in a conformational change of the receptors, allowing the heterodimers to bind PPAR response element (PPREs) in target genes and modulate transcription of target genes [8]. PPAR γ is a key regulator of lipid and glucose metabolism and plays an important role in adipocyte differentiation [9,10]. PPAR γ ligands have been proven to be potent antidiabetic agents in human [11,12]. Accumulating evidences suggest that PPAR γ activation plays an important role in insulin sensitization which affects diabetes and atherogenesis.

Thiazolidinedione (TZD), including troglitazone, rosiglitazone (Avandia), and pioglitazone (Actos), has been identified as a synthetic ligand for PPAR γ and the prostaglandin J₂ metabolite, 15-deoxy-delta^{12,14}-prostaglandin J₂, served as a natural ligand for PPAR γ [13,14]. In recent reports, the suppressive effects of troglitazone and 15d-PGJ₂ on thymidine incorporation and cell viability have been demonstrated in rat and rabbit MCs [5,6]. To our knowledge, there has been no report investigating the level of cell cycle arrest by PPAR γ ligands in MCs.

A number of prosclerotic responses in MCs, including up-regulation of the expression of type I collagen, fibronectin, TGF- β and PDGF, appear to be a pivotal contributor to glomerulosclerosis. *In vivo*, PPAR γ ligand was shown to ameliorate albuminuria, peripheral neuropathy, mesangial expansion, ECM and TGF- β expression in diabetic and nondiabetic glomerulosclerosis in rats, regardless of blood glucose levels [15-18]. *In vitro*, PPAR γ activation attenuated the expression of type I collagen and elastin gene in lung fibroblast, hepatic stellate cells and mice MCs [19-21]. CTGF may mediate many of the pro-fibrotic actions of TGF- β , it has recently been demonstrated in experimental and human renal fibrosis where its expression appears to correlate with the degree of tubulointerstitial fibrosis [22-23]. We postulated that the effect of PPAR on ECM gene expression not only through its inhibition of TGF- β but also the downstream CTGF gene.

We found that PPAR γ ligands inhibited RMCs proliferation and arrested RMCs at the G1 phase. Besides, the gene expression of type I collagen, fibronectin and CTGF were also attenuated by PPAR γ ligands. Our data show that PPAR γ ligands might have potential to be an anti-proliferative

and anti-fibrotic agent in progressive disease treatment.

Materials and Methods

Materials

Fetal calf serum (FCS) was obtained from Biochrome KG (Berlin, Germany). Cultured flasks and plates were purchased from Corning (Corning, NY, USA) and precoated with $1.6\mu\text{g}/\text{cm}^2$ of Vitrogen 100® (Celtrix Lab, Polo Alto, CA, USA) before cell coating. Trypsin-ethyl-enediaminetetraacetic acid (EDTA), RPMI-1640 medium, glutamine, and trypan blue were obtained from GIBCO (Grand Island, NY, USA). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT), recombinant PDGF-BB, and other tissue culture reagents were purchased from Sigma (St. Louis, MO, USA). TGF- β 1 was obtained from R&D laboratory. Human type I (α 1) collagen and human fibronectin-1 cDNA was purchased from American Type Culture Collection (Rockville, MD, USA). Agents used to isolate the total RNA and Northern blot analysis were obtained from Boehringer Mannheim (Mannheim, Germany) unless otherwise specified. 15-deoxy-delta^{12, 14}-prostagalindin J₂ (15d-PGJ₂) was obtained from Cayman Chemical (Ann Arbor, MI, USA). Rosiglitazone used in this study was a generous gift from GlaxoSmithKline (GSK). All other chemical used were of analytical grade.

Rat Mesangial Cell Culture

RMCs were cultured from male Sprague-Dawley rats weighting about 200 g with method modified from that reported by Groggel et al. [24] In brief, the kidneys were removed and the cortex minced. After passing through glomerular sieves, the glomerular suspension was digested with type IV collagenase (1 mg/ml) for 20 min. The resulting glomerular cores were washed with RPMI medium, and then plated down in 75 cm² flasks. The RPMI medium contained penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) and *L*-glutamine (2mM). Initially, epithelial cells grew but after 3-4weeks mesangial cells replaced them. The mesangial cells were tested by their resistance to puromycin (50 $\mu\text{g}/\text{ml}$) and also by *D*-valine containing medium to rule out fibroblast contamination [25]. Mesangial cells were identified by their stellate or elongated morphology with prominent intracellular fibrils. They showed positive immunofluorescence with anti-alpha smooth muscle actin and anti-thy-1-1 antibody, but negative immunofluorescence with antifactor VIII antibody. Experiments were performed using cells in 8-20 passages.

Cell Proliferation Assay

A modified MTT assay and Coulter cell counting were used. MTT uptake was based on the ability of the living cells to reduce MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide, Sigma] to the blue formazan product by their mitochondria. MTT uptake has been shown to be consistent with the H³-thymidine uptake test [26]. MTT uptake (absorbance at 570 nm) for the mesangial cells was found to vary linearly with cell numbers ranging from 4000 cells/well to 12.8×10^4 cells/well in 96-well plates[27]. Therefore, we loaded 5,000 cells/well for each MTT assay. In studying the inhibitory effect on serum and PDGF-stimulated cell growth, cells were initially plated

down in medium containing 10% FCS. The PDGF-stimulated group was then shifted to 0.5% FCS-containing RPMI. PDGF at a concentration of 20ng/ml was added to all wells in conjunction with various concentration of rosiglitazone and 15d-PGJ₂. After an additional incubation of 24-120h, 20µl of MTT solution (5mg/ml in PBS) were added to the culture medium. After a further 4-hour incubation, the medium was replaced by 100µl of ethanol. An ELISA reader measured absorbance at the reference wavelength of 630 nm and test wavelength of 570 nm. All samples were processed in triplicate. The inhibition of RMC growth was calculated as follows [28]: Percent inhibition = $1 - [\text{absorbance of (test well - initial plating)} / \text{absorbance of (control - initial plating)}] \times 100\%$.

Flow cytometry analysis of cell cycle

Three x 10⁵ RMCs were seeded into 10 cm diameter dishes with RPMI medium containing 10% FCS and arrested growth with 0.5% FCS RPMI for 24 hours. Flow cytometry analysis of cell-cycle distribution of RMCs was performed at the 15th hours after treatment with indicated condition. Briefly, cells were washed twice with PBS, harvested by trypsinization, centrifuged and resuspended with 1ml cold PBS, and then fixed in methanol overnight. After washing with PBS, the fixed cells were incubated in 1 mg/ml RNase (Calbiochem, San Diego, CA) at room temperature for 30 min, followed by staining of the DNA with 1 µg/µl propidium iodide (Sigma) at room temperature for 30 minutes in the dark; 1 x 10⁴ cells of each sample were analyzed with Coulter EPICS 753 flow cytometer, and the percentage of cells within the G1, S and G2/M phases of the cell cycle were determined [29].

Northern blot analysis

To determine the effect of PPAR ligands on extracellular matrix gene expression, RMCs were cultured in RPMI supplemented with 10% FCS until subconfluency. RMCs were then replaced with 10% FCS-containing RPMI with various concentration of RSG and 15d-PGJ₂. At various time intervals, the total RNA was isolated using the acid guanidinium thiocyanate-phenol-chloroform method, as described by Chomczynski and Sacchi [30]. The procedure is briefly described as follows: The concentration of each sample was determined using spectrophotometry with the absorbance at 260 nm (A₂₆₀). The purity of each sample was determined based on the ratio of A₂₆₀ to A₂₈₀. Ten micrograms of RNA were electrophoresed on a 1.0% agarose gel containing 1 mol/L formaldehyde in MOPS buffer (0.2 mol/L morpholinopropanesulfonic acid, 0.05 mol/L Na acetate, 0.01 mol/L EDTA). Equivalency of sample loading and lack of degradation were verified by ethidium bromide staining of the 28 S and 18 S rRNA bands. The RNA then was transferred to nylon membranes by overnight capillary action and followed by fixation in a UV cross-linker.

For synthesis of collagen and fibronectin RNA probes, cDNAs for human type I (α1) collagen and human fibronectin-1 were purchased from American Type Culture Collection (Rockville, MD, USA). A 1.5-kb *EcoRI* fragment of type I (α1) collagen and a 1.2-kb *EcoRI* fragment of fibronectin cDNAs were subcloned, respectively, into the pBSII/SK⁻ vector (Stratagene, La Jolla, CA, USA). For

synthesis of rat CTGF riboprobe, cDNA fragments were first generated by reverse transcription-polymerase chain reaction (RT-PCR) from glomerular RNA of male Sprague-Dawley rats, using the following specific primer pairs: upstream, 5'-AGTCCTTCCAAAGCAGTTGC-3' (corresponding to bases 559 to 578) and downstream, 5'-GCCTAAAATTGCCAAGCCTG-3' (corresponding to base 995 to 1014). The products were subsequently subcloned into the pGEM-dT vector (Promega, Madison, WI, USA). The cloned cDNAs were then linearized and used as templates for *in vitro* transcription of antisense digoxigenin-conjugated riboprobes, following the supplier's instructions (Boehringer Mannheim). The blots were developed using CSPD (Boehringer Mannheim) as the substrate for alkaline phosphatase, as described by the supplier. The signal intensity recorded on x-ray film was then quantified with computerized densitometry and normalized against the signal of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) messages.

Statistical analysis

The results are expressed as mean \pm SEM, unless otherwise stated. These statistical analyses were carried out using GraphPad Prism® 3.0 on a personal computer. Statistical significance ($P < 0.05$) was evaluated using the Student's *t*-test or one-way ANOVA with modified *t*-test performed using the Bonferroni correction.

Results

PPAR γ ligands inhibit serum and PDGF-stimulated proliferation of RMCs

RSG and 15d-PGJ₂ inhibited serum and PDGF-stimulated proliferation of RMCs. The MTT uptake experiments were performed to evaluate the effect of Rosiglitazone and 15d-PGJ₂ on serum-stimulated RMC proliferation. In time-response study (Figure 1A and 1B), RSG and 15d-PGJ₂ at concentrations above 10 and 3 μ M caused a dose-dependent inhibition of RMC. In dose-response study of increasing concentrations of PPAR γ ligands on serum-stimulated RMC proliferation is shown in Figure 1C. When compared to the control group (10% FCS-RPMI), growth inhibition was significant ($P < 0.05$) starting at 10 μ M of RSG and 3 μ M of 15d-PGJ₂ at the fifth days after treatment. The percentages of inhibition by RSG at concentrations of 10, 30 and 100 μ M were 8, 21 and 41%, respectively. The percentages of inhibition by 15d-PGJ₂ at concentrations of 3 and 10 μ M were 19 and 65%, respectively. The dose-dependent study of PPAR γ ligands on PDGF-stimulated RMC proliferation is shown in Figure 2. When compared with the effect of PDGF alone, significant inhibition of RMC proliferation was observed at the dose greater than 10 μ M of RSG and 3 μ M of 15d-PGJ₂. RSG, at concentrations of 10, 30 and 100 μ M, were PDGF-stimulated RMC proliferation by 14, 24 and 51%, respectively. 15d-PGJ₂, at concentrations of 3 and 10 μ M were 16 and 68%, respectively. Therefore, we used a concentration of RSG (10~50 μ M) and 15d-PGJ₂ (1~10 μ M) in the following experiments to study their antifibrotic effects on RMCs. To exclude the possible toxic effect of PPAR γ ligands on proliferative HPMC, a viability test was performed using the trypan blue exclusion method and by measuring the lactate dehydrogenase (LDH) activity of the supernatant as we previously described [27]. No differences were found in the numbers of dead cells in the supernatant and adherent RMC fractions between control and PPAR γ ligands-treated wells (data not shown).

PPAR Ligands and PD98059 Block the Progression of RMC into S phase

To investigate the cellular mechanism of the inhibitory effect of RSG, 15d-PGJ₂, and a MAPK inhibitor PD98059 on RMCs growth, cell cycle was determined by flow cytometry. Subconfluent RMCs accumulated in G₁ phase after serum starvation for 48 h (82.8% in G₀/G₁ phase and 15.4 % in S phase; Figure. 3A). Quiescent RMC were induced to enter S phase by stimulation with competence factor PDGF-BB (20 ng/ml). The population of G₀/G₁ cells decreased substantially (60.5 %; Figure 3B) with a concomitant increase in RMC in S phase (37.6 %; Figure 1B). RSG inhibited G₁ \rightarrow S progression as reflected by the higher percentage of G₀/G₁ cells (75.2%; Figure 3C) and by the lower percentage of S phase (23.7%; Figure 3C). Movement of cells from G₁ \rightarrow S was also inhibited by 15d-PGJ₂ and PD98059, with an increase in the population of G₀/G₁ cells (78.0% in Figure 3D and 80.5% in Figure 3E, respectively) and with a concomitant decrease in S phase cells (19.8 % in Figure 1D and 16.1% in Figure 3E, respectively). Inhibition of 62.5, 79.7 and 96.8% was observed at 50 μ M for RSG, 10 μ M for 15d-PGJ₂, and 50 μ M for PD98059 respectively.

Effect of PPAR γ ligands on the RMC mRNA levels of type I (α 1) collagen and fibronectin

The effects of RSG and 15d-PGJ₂ on the expression of type I (α 1) collagen and fibronectin mRNA level were analyzed in RMC. The results of densitometry showed that the maximum expression of type I (α 1) collagen and fibronectin mRNA was observed 72 hours after serum-stimulation (data not shown). Therefore, we choose this time point for additional experiments. Treatment of RMC with RSG and 15d-PGJ₂ substantially reduced the serum-stimulated type I (α 1) collagen and fibronectin mRNA expression in a dose-dependent manner (Fig. 4,5 and 6). Maximal decrease of type I (α 1) collagen and fibronectin mRNA levels was observed after the treatment of 50 μ M RSG (73 and 79% decrease respectively) and 10 μ M 15d-PGJ₂ (69 and 55% decrease respectively). The effect of TGF- β 1 on the expression of type I (α 1) collagen and fibronectin mRNA level were also analyzed in RMC. TGF- β 1 (2.5 ng/mL) maximally increased type I (α 1) collagen and fibronectin mRNA expression by 1.49 and 3.06-fold above baseline at 24 hours. PPAR γ activation by RSG (50 μ M) suppressed basal and TGF- β 1 activated type I (α 1) collagen mRNA expression. Fibronectin mRNA expression was also abolished by PPAR γ activation as compared with that treated with TGF- β 1 alone (Figure 9 A and B).

Effect of PPAR γ ligands on the CTGF gene expression in RMC

The effects of RSG and 15d-PGJ₂ on the expression of CTGF mRNA level were analyzed in RMC. The results of densitometry showed that the maximum expression of CTGF mRNA was observed 72 hours after serum-stimulation (data not shown). Therefore, we choose this time point for additional experiments. Treatment with RSG and 15d-PGJ₂ attenuated serum-stimulated CTGF mRNA expression dose-dependently while GAPDH mRNA was not affected (Figure 7 and 8). Besides, the effect of TGF- β 1 on the expression of CTGF mRNA level was also analyzed in RMCs. Similar to previous report [30], TGF- β 1 induces CTGF mRNA expression was prolonged and lasted for more than one day (data not shown). Pretreatment of RMC with 50 μ M RSG substantially reduced TGF- β 1-stimulated CTGF mRNA by approximately 50%. Similar results were obtained following pretreatment with 10 μ M 15d-PGJ₂. (Figure 9C). In conclusion, these results indicated that PPAR γ activation inhibited both serum and TGF- β 1-stimulated CTGF gene expression.

Discussion

Because proliferation of MCs in the normal adult kidney is tightly regulated with a growth rate of less than 1% [32], an imbalance in the control of MC proliferation appears to play an early and crucial role in the pathogenesis of progressive glomerular injury and glomerulosclerosis [33]. In experimental models of nephritis, MC proliferation frequently precedes and is linked to the increase of ECM in the mesangium and glomerulosclerosis [34,35]. In the present study, the antiproliferative effects of PPAR γ ligands on mesangial cells were confirmed *in vitro*. We demonstrated an inhibitory effect of PPAR γ ligands, RSG and 15d-PGJ₂, in serum and PDGF-stimulated RMC proliferation (Figure 1 and 2). Previous studies of vascular smooth muscle cells [36] indicated that inhibition of cell proliferation by PPAR γ ligands occurred at a specific arrest point late in the G1 phase of the cell cycle, about four to six hours prior to S phase. Similarly, PPAR γ ligands treated RMCs were arrested in the Go/G1 phase, suggesting that the transition from late G1 to S phase may be blocked by PPAR γ ligands in MC (Figure 3). The influence of PPAR γ ligands on growth regulation of RMC, with a focus on cell-cycle machinery such as cyclin D1, P21 and P27, is currently being pursued in our laboratory. The observation that PPAR γ ligands inhibit cell cycle processes activated by growth factors produced in response to glomerular injury may provide a new oral therapeutic approach for proliferative glomerular disease such as several types of glomerulonephritis.

The deposition of extracellular matrix proteins in the glomerular mesangium plays a prominent role in the development of progressive glomerulosclerosis [1,37,38]. This study demonstrated that PPAR γ ligands, RSG and 15d-PGJ₂, significantly attenuated the serum-stimulated mRNA levels of type I (α 1) collagen and fibronectin at 72 hours (Figure 4, 5 and 6). Previous literatures have shown that, by inducing the expression of type I (α 1) collagen and fibronectin mRNA in RMCs, TGF- β 1 may be associated with an increased risk of glomerulosclerosis. Our current study demonstrated that PPAR γ activation inhibited the basal and TGF- β -induced expression of type I (α 1) collagen mRNA (Figure 9A). PPAR γ ligands also attenuated the fibronectin mRNA expression (Figure 9B). Because MCs are the major source for various matrix proteins in the glomerulus [39,40], the decrease in the matrix mRNA levels might reduce the number of activated proliferating MCs in the progression renal disease. These *in vitro* effects of PPAR γ activation indicate that PPAR γ ligands have therapeutic potential for progressive glomerulosclerosis *in vivo*.

Connective tissue growth factor is a cysteine-rich, heparin-binding 38 kD growth factor protein [41], that is secreted by fibroblasts, endothelial cells, and mesangial cells after stimulation with TGF- β [42-44]. It mediates at least some of the downstream effects of TGF- β , including cell proliferation and collagen synthesis [45-47]. In fibroblasts, CTGF may mediate some of the TGF- β -induced increase in ECM protein production by autocrine modes of action [47,48]. Moreover, incubation of these cells with high glucose increases CTGF expression by TGF- β -dependent mechanisms [49]. Currently, the CTGF expression has been reported in normal kidney and in a variety of renal diseases [22]. In mesangial cell, TGF- β induced CTGF mRNA expression was prolonged and lasted for more than one day [31]. Thus, CTGF is implicated in the pathogenesis of

diabetic nephropathy and glomerulonephritis [46,49]. In this study, PPAR γ ligands attenuated the expression of serum and TGF- β 1-stimulated CTGF mRNA expression (Figure 7, 8 and 9). The relevance of the findings demonstrates that PPAR γ ligands may operate the antifibrotic effect of serum and TGF- β 1-induced ECM accumulation partially through inhibiting the expression of CTGF gene. Further investigation of the TGF- β downstream pathways modulated by PPAR γ activation may elucidate the pleiotropic effects of PPAR γ ligands in RMCs.

In conclusion, PPAR γ ligands, RSG and 15d-PGJ₂, inhibited RMC proliferation, modulate cell-cycle progression, decreased matrix accumulation and CTGF gene expression in vitro. Moreover, the inhibitory effect of PPAR γ ligands on TGF- β 1-induced ECM gene and CTGF gene expression was also demonstrated. These results suggested that PPAR γ activation might have a potential therapeutic value in preventing or treating glomerular diseases, such as glomerulosclerosis and glomerulonephritis in progressive renal disease.

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FIGURE LEGENDS

Figure 1. Effects of PPAR ligands, RSG and 15d-PGJ₂ on serum-stimulated RMC proliferation.

Time-response curve (A and B): Various concentrations of RSG and 15d-PGJ₂ were treated after overnight plating of RMC and further incubated with 10% fetal calf serum (FCS) for one to five days. Dose-response study (C): RMCs were treated with various concentrations of RSG and 15d-PGJ₂ for 5 days. Data represent the mean ± SEM from three independent experiments performed in triplicate. *P < 0.05 and **P < 0.01, compared to control.

Figure 2. PPAR ligands, RSG and 15d-PGJ₂ inhibits platelet-derived growth factor-stimulated RMC growth.

Dose-response study: RMC were incubated for 5 days after addition of various concentrations of RSG or 15d-PGJ₂, and a 20ng/ml of PDGF in 0.5% FCS-RPMI medium. Data represent the mean ± SEM from three independent experiments performed in triplicate. I, initial plating at day 0; C, control. **P < 0.01, compared to control.

Figure 3. PPAR ligands prevent mitogen-induced G1 → S progression in RMC.

Subconfluent RMCs were growth arrested by 0.5% FCS for 48 hours (A), then changed into mediums in the presence of PDGF (20 ng/ml) alone. (B), or PDGF plus (C) 50 μ M RSG, (D) 10 μ M 15-PGJ₂, (E) 50 μ M PD98059. The x and y axes represent the DNA content and cell number, respectively. The data are representative of three separate experiments.

Figure 4. Effect of RSG on serum-stimulated type I (α1) collagen mRNA expression.

RMCs were treated with various concentrations of RSG for 72 hours. Representative Northern blots of type I (α1) collagen mRNA are shown in the top panel. Densitometric analysis of blots from 3 separate experiments is shown in the bottom panel. Values in the graph are shown as fold over control. *P < 0.05 vs. Control alone (lane 1).

Figure 5. Effect of 15d-PGJ₂ on serum-stimulated type I (α1) collagen mRNA expression.

RMCs were treated with various concentrations of 15d-PGJ₂ for 72 hours. Representative Northern blots of type I (α1) collagen mRNA are shown in the top panel. Densitometric analysis of blots from 3 separate experiments is shown in the bottom panel. Values in the graph are shown as fold over control. *P < 0.05 vs. Control alone (lane 1).

Figure 6. Effects of RSG and 15d-PGJ₂ on serum-stimulated fibronectin mRNA expression.

(A) Lane 1, control; lanes 2-4, were treated with RSG, 10 , 30 and 50 μ M for 72 hours, respectively. (B) Lane 5, control; lanes 6-8, treatment of cells with 15d-PGJ₂, 1, 5 and 10 μ M for 72 hours, respectively. Values in the graph are shown as fold over control. The data are representative of three separate experiments.

Figure 7. Effect of RSG on serum-stimulated CTGF mRNA expression.

RMCs were treated with various concentrations of RSG for 24 hours. Representative Northern blots of CTGF mRNA are shown in the top panel. Densitometric analysis of blots from 3 separate experiments is shown in the bottom panel. Values in the graph are shown as fold over control. *P<0.05 vs. Control alone (lane 1).

Figure 8. Effect of 15d-PGJ₂ on serum-stimulated CTGF mRNA expression.

RMCs were treated with various concentrations of 15d-PGJ₂ for 72 hours. Representative Northern blots of CTGF mRNA are shown in the top panel. Densitometric analysis of blots from 3 separate experiments is shown in the bottom panel. Values in the graph are shown as fold over control. *P<0.05 vs. Control alone (lane 1).

Figure 9. Inhibitory effects of PPAR ligands on TGF- β 1-induced type I (α 1) collagen, fibronectin, and CTGF mRNA expression.

RMCs were preincubated with RSG or 15d-PGJ₂ for one hour. After these additions, the cells were incubated with TGF- β 1 (2.5 ng/mL) for 6 (C) and 24 (A and B) hours in the presence of the same additions. A: type I (α 1) collagen, B: fibronectin, C: CTGF. Values in the graph are shown as fold over control. This experiment result was typical of 3 experiments.

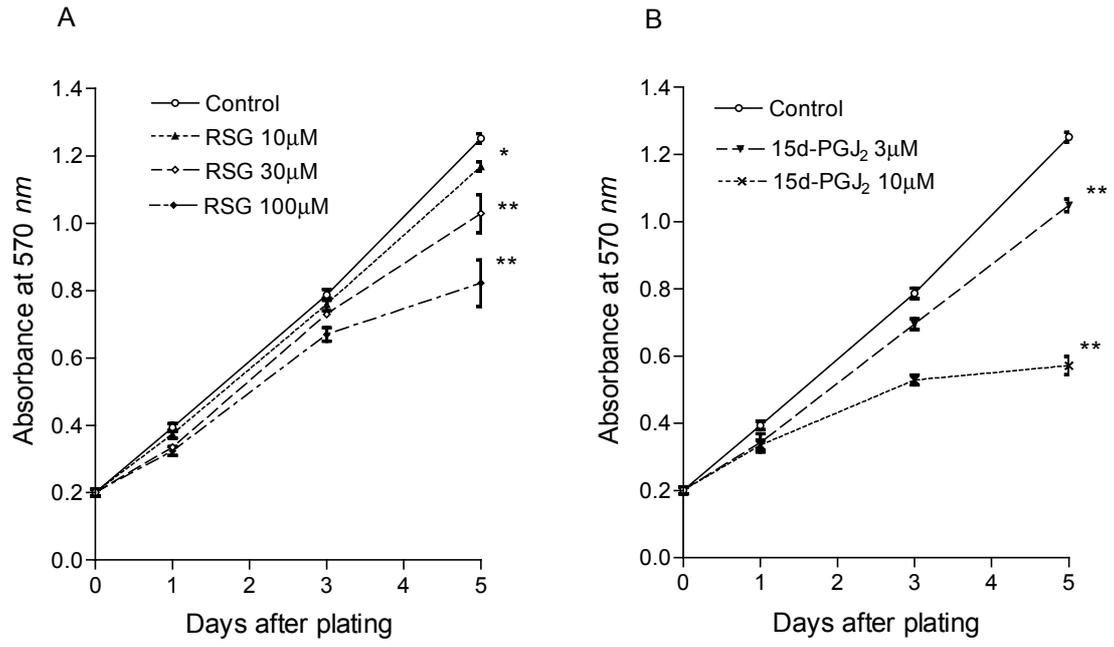


Figure 1 Chiang et al.

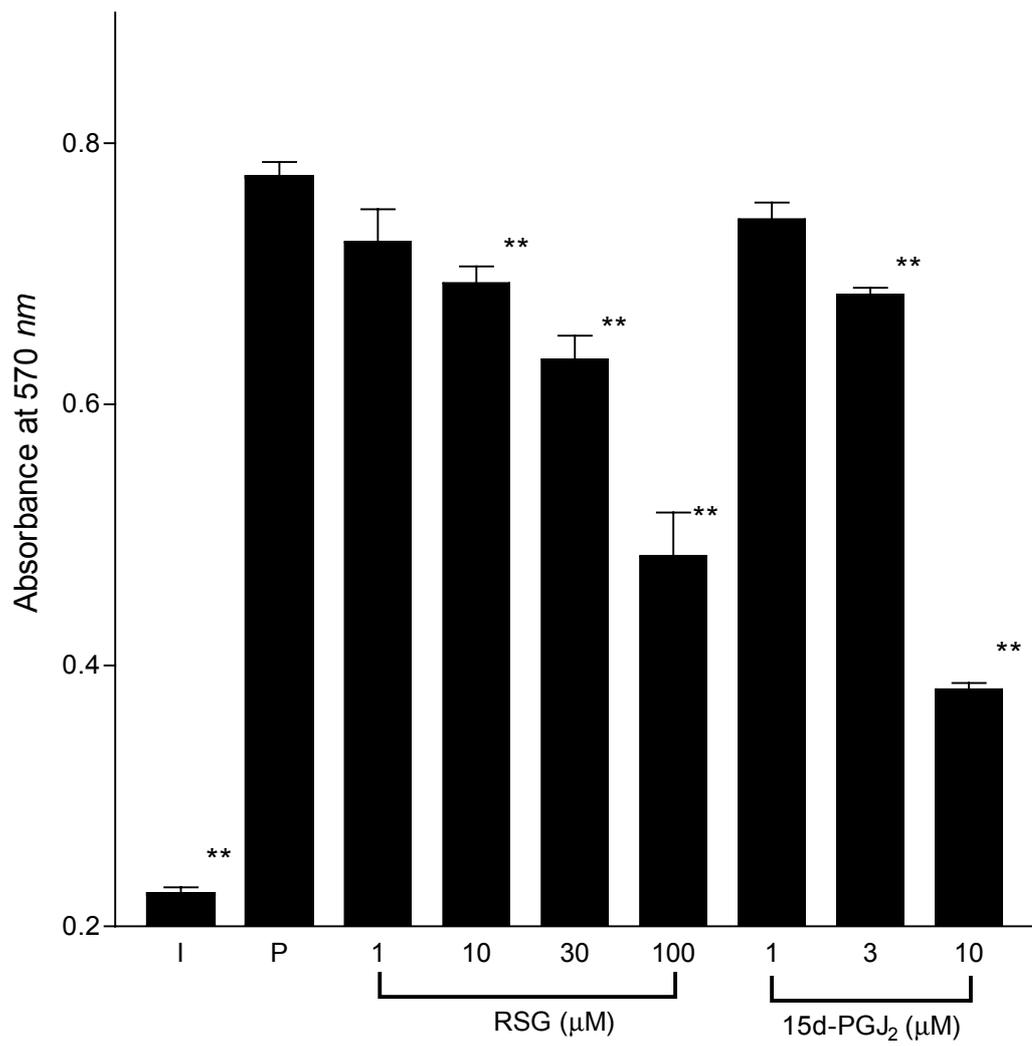


Figure 2 Chiang et al.

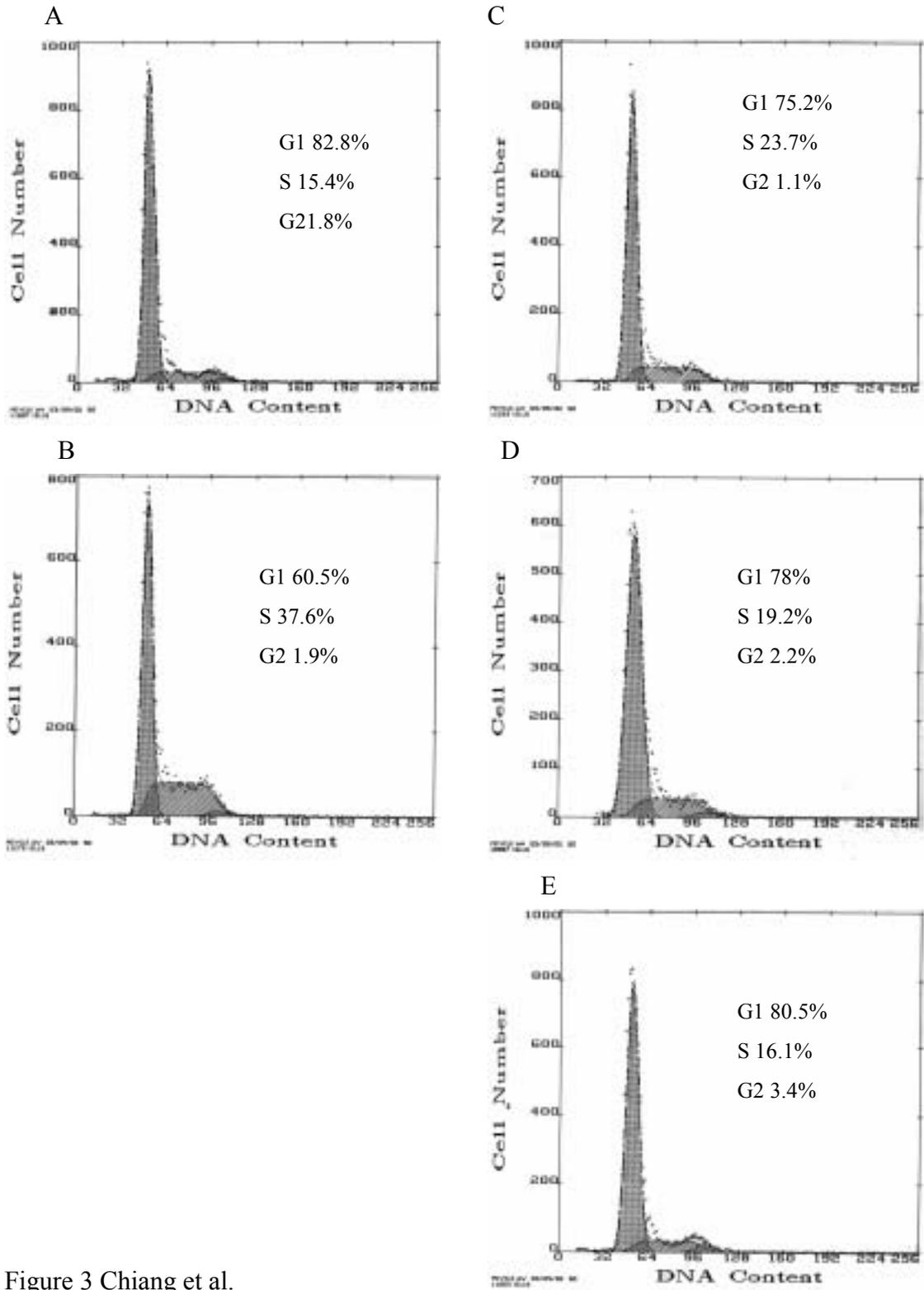


Figure 3 Chiang et al.

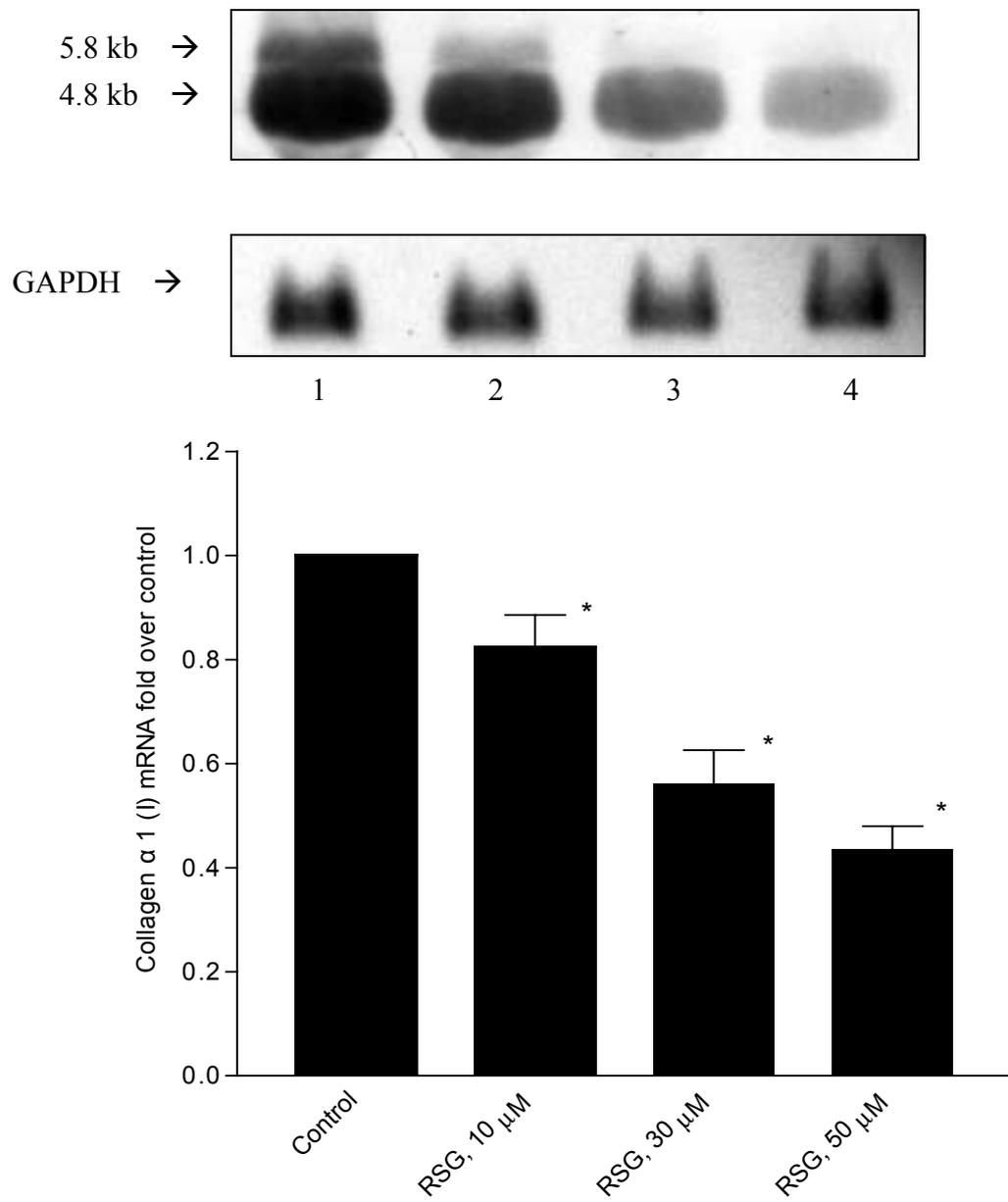


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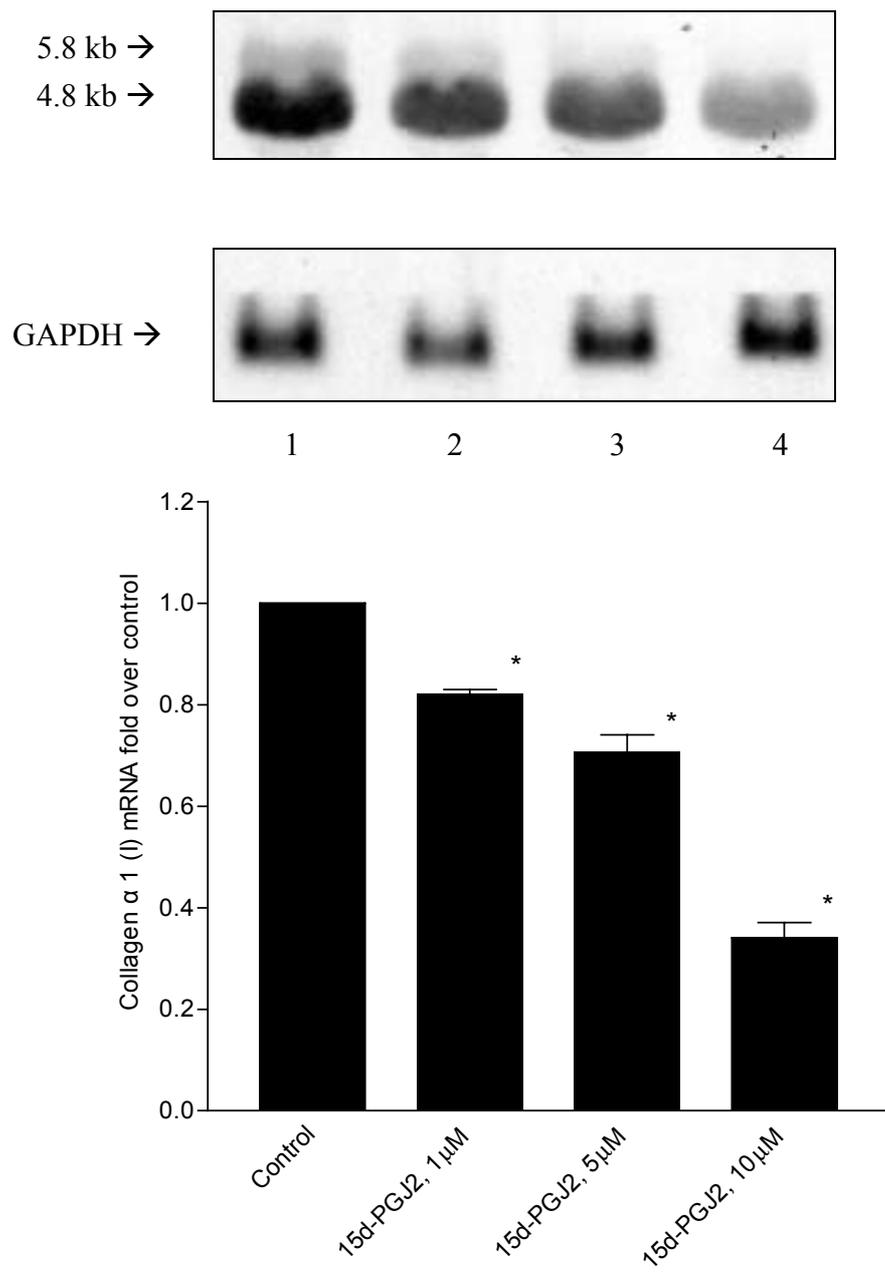


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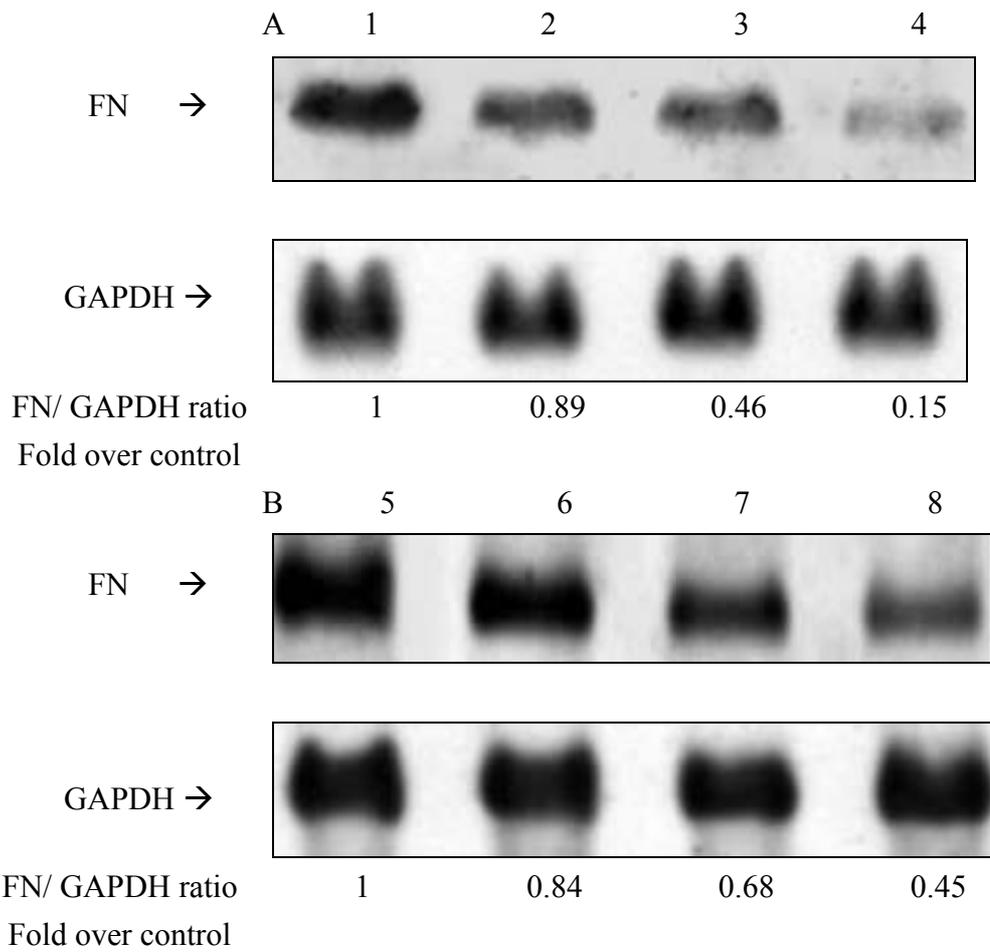


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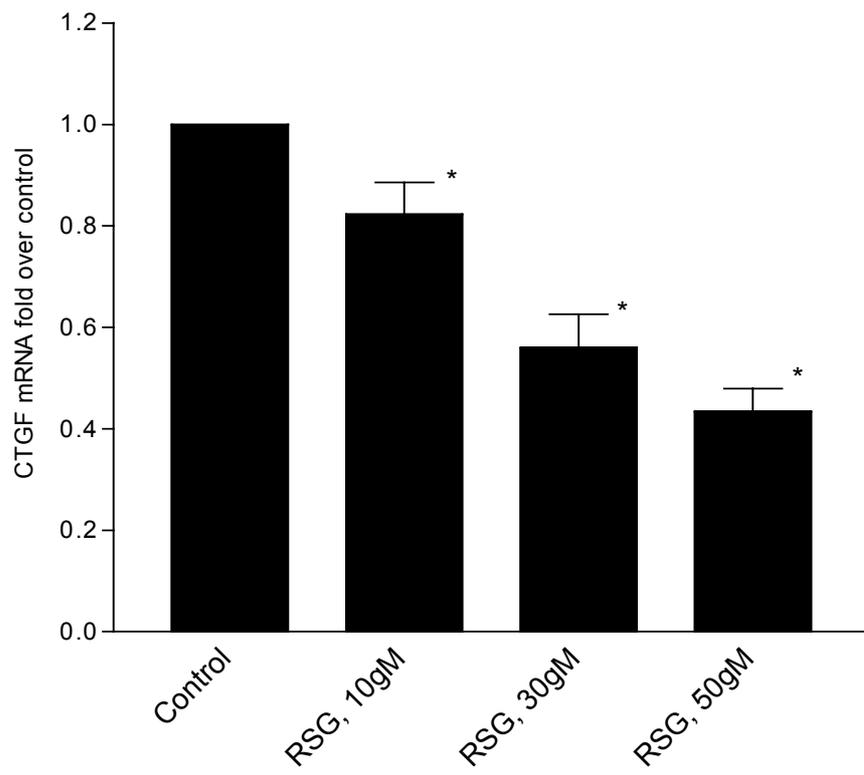
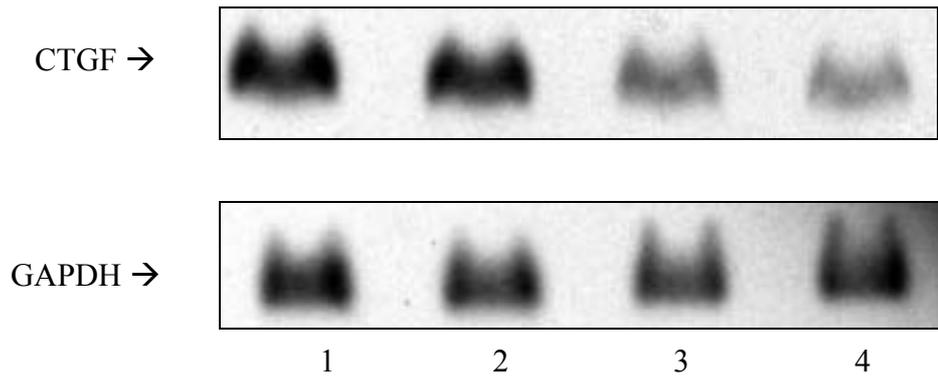


Figure 7 Chiang et al.

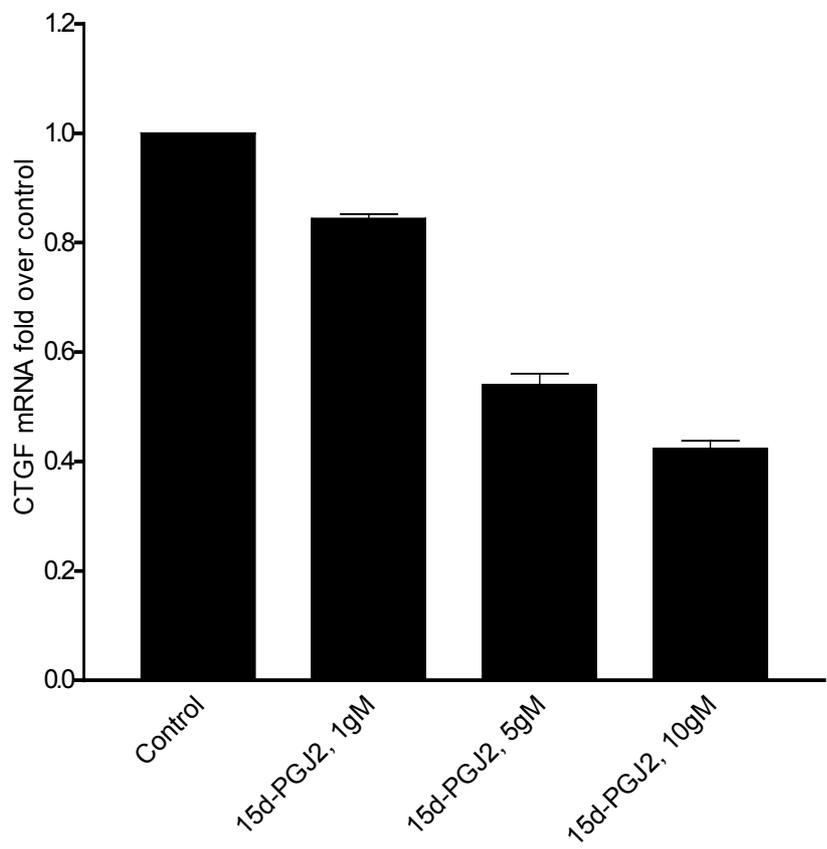
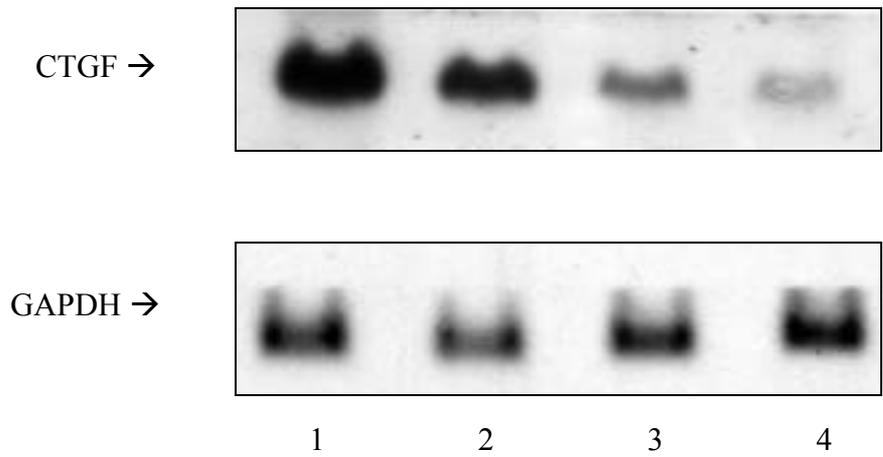


Figure 8 Chiang et al.

Figure 9 Chiang et al.

