Original Article
Telmisartan counteracts TGF-β1 induced epithelial–to–mesenchymal transition via PPAR-γ in human proximal tubule epithelial cells

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Abstract: Chronic renal failure (CRF) mainly results from kidney fibrosis. Epithelial-to-mesenchymal transition (EMT) occurs in stressed tubular epithelial cells and contributes to renal fibrosis. Transforming growth factor-β1 (TGF-β1) has been shown to initiate and complete the whole EMT process. Peroxisome proliferators-activated receptor-γ (PPAR-γ) exerts anti-inflammatory, anti-fibrotic and vaculo-protective effects on different renal diseases. Telmisartan is a member of angiotensin II (Ang II) receptor blocker (ARB) family. Recent studies show that Telmisartan has a partial agonistic effect on PPAR-γ. Therefore, we tested the hypothesis that Telmisartan reverses the progression of induced EMT by TGF-β1 in cultured human renal proximal tubular epithelial (HK-2) cells. Cultured HK-2 cells were treated with TGF-β1 (3 ng/ml), a combination of TGF-β1 and Telmisartan (10-200umol/L) and a combination of TGF-β1, Telmisartan and GW9662, a PPAR-γ antagonist for 48 hours. EMT was determined by quantitative real-time PCR analysis of E-cadherin (E-cad), Connective Tissue Growth Factor (CTGF) and PPAR-γ transcript expression and immunocytochemical analysis of E-cad, α-Smooth Muscle Actin (α-SMA) and PPAR-γ protein expression. TGF-β1 induced phenotypic EMT in cultured HK-2 cell line via significantly reduced E-cad expression and significantly increased CTGF, α-SMA expression in association with the loss of epithelial morphology. Telmisartan reversed all EMT markers in a dose-dependent manner which was inhibited by PPAR antagonist GW9662. In the present study, it was suggested that Telmisartan attenuated TGF-β1 induced EMT by agonistic activation of PPAR-γ.

Keywords: PPAR-γ, telmisartan, epithelial-to-mesenchymal transition, proximal tubular epithelial cells, TGF-β1, GW9662

Introduction

Epithelial-mesenchymal transition (EMT) is a process of epithelial cells losing their phenotypic markers and characteristics and acquiring the phenotypic features of mesenchymal cells. The occurrences of EMT are the results of persistent vicious stimuli as injuries and reactions after injuries. EMT is hot topic recently since it involved in many pathologic processes as inflammation, fibrosis tumorigenesis and metastasis.

For instance, EMT occurred in kidney is one of the key mechanisms arousing kidney fibrosis [1-3]. In details, tubular EMT happens in losing epithelial markers such as E-cadherin (E-cad) and acquiring mesenchymal features such as α-smooth muscle actin (α-SMA) and connective tissue growth factor (CTGF) [4-7]. Also, tubular epithelial cells lose their epithelial phenotype and become elongated myofibroblast-like phenotype, as well as obtain the capacity of migrating into the peritubular interstitium via the damaged tubular basement membrane (TBM). EMT is thought to be driven by extracellular stimuli, transformation growth factor β1 (TGF-β1) is one of the major player [8-11]. Interestingly, if the surrounding pathogenic factors are removed timely EMT has potential possibility to be reversed [12]. Beyond delaying the inevitable onset of end-stage kidney disease cessation and regression of EMT are ideal schemes of clinical treatment. Realizing the process of EMT is essential in

Table 1. Primers used for RT-PCR amplification in the present study*

Target	Sequence	Size (bp)	Annealing (°C)	Cycles
CTGF	5'-TCATCAAGACCTGTGCCTGCCA-3' 5'-GGGTGGGAATCTTTTCCCCCAGT-3'	230bp	60	30
E-cad	5'-CCCCCATACCAGAACCTCGAA-3' 5'-TTCTGGTTATCCATGAGCTTGAGA-3'	247bp	60	40
PPAR-γ	5'-TGGTGACTTTATGGAGCCCAA-3' 5'-GGCAAACAGCTGTGAGGACTCAG-3'	220bp	60	30
GAPDH	5'-CTCCGGGAAACTGTGGCGTG-3' 5'-ACAAAGTGGTCGTTGAGGGCA-3'	351bp	60	30

*CTGF: Connective Tissue Growth Factor; E-cad: E-cadherin; PPAR-γ: Peroxisome Proliferators Activated Receptor γ; GAPDH : glyceraldehyde-3-phosphate dehydrogenase

establishing therapeutic strategies for progressive renal failure, the pathway to counteract and reverse EMT seems to be focused.

Peroxisome proliferators-activated receptors (PPARs) are members of nuclear receptor superfamily with 3 isotypes existed in mammals: PPARα, PPARβ and PPAR-γ [13]. Steroid, thyroid and retinoid hormones are ligands for the receptors. PPAR-γ is highly expressed in adipose tissue; its activation plays a key role in increasing systemic insulin sensitivity. PPAR-γ agonists are clinically used in the treatment of type2 diabetes mellitus and metabolic syndrome. PPAR-γ has been found constitutively expressed in many other tissues like in kidney, for example, of the inner medullary collecting ducts, proximal tubules, thick ascending limb of Henle's loop, it has been thought to play a protective role for the organ [14]. Also PPAR-γ expression in macrophages and lymphocytes suppresses inflammatory responses and PPAR-γ agonists inhibit the production of proinflammatory cytokines and regulate the process of inflammation by activating this nuclear receptor [15]. More interested, PPAR-γ mediates cellular differentiation, antitumorigenic activity in various tumor types [16, 17] and EMT [18, 19].

Telmisartan is a highly selective angiotensin II type 1 (AT1) receptor blocker (ARB) for treatment of cardiovascular diseases as hypertension. In 2004, it was first identified Telmisartan as partial agonist of PPAR-γ in treatment of hypertension and diabetes [20, 21]. Later on, Telmisartan was reported to connected its functions with PPAR-γ in inflammation [22, 23], cancer treatment [24, 25], as well as used for treating patients with chronic renal diseases

[26-28]. Also, it was reported recently special agonist of PPAR-γ activates PPAR-γ in prevention EMT in alveolar epithelial cells [18]. Now, we address the question whether Telmisartan has the capacity of reversing the process of EMT in cultured human kidney proximal tubule epithelial cell HK-2 to counteract EMT-related pathological change as renal fibrosis via PPAR-γ pathway.

Materials and methods

Cell culture and treatment

Immortalized human proximal tubule epithelial cells (HK-2) were obtained from Shenzhen Chinese medicine hospital and maintained in DMEM/F12 medium containing 10% fetal boving serum (FBS, HyClone, USA) and 1% penicillin/streptomycin solution (HyClone, USA) at 37 °C in 5% CO_2 atmosphere. Cells were used between passages 3 and 20. The HK-2 cells were seeded on standard 6-well culture plates at a density of 150,000 cells/well to approximately 60% to 70% confluence 48 hours prior to treatment. Twenty-four hours prior to treatment, cells were maintained in medium without any additives. TGF-β1(R&D System, USA), TGF-β1+telmisartan (Sigma, USA) , TGF-β1+telmisartan+GW9662 (Merck, USA) were then applied to the cells for 48 hours before being harvested for real-time polymerase chain reaction (RT-PCR) or immunofluorescence.

RT-PCR and gene expression analysis

Total RNA was extracted from cells using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. cDNA was generated by reverse transcription 500ng of
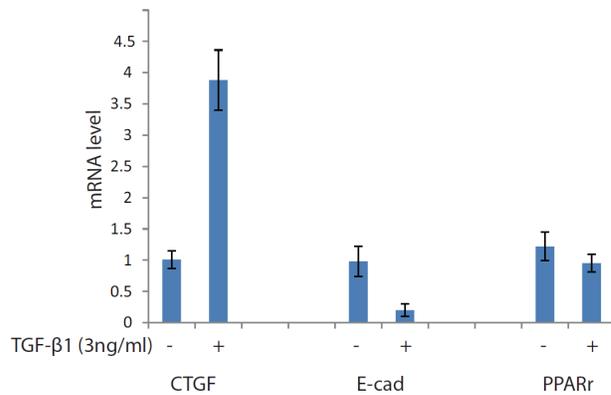
Figure 1. Measurement of mRNA level for CTFG, E-cad and PPAR-γ in absence or presence of TGF-β1 stimulation. Shown quantification of mRNA level of CTFG, E-cad and PPARr by RT-PCR measurement in absence or presence of TGF-β1 (3ng/ml) stimulation. Data were represented in triplicate experiments ± S.D.

total RNA in a reaction volume of 10ul random hexamers as a priming agent with PrimeScript RT Master Mix (TAKARA, CHN). One microliters of cDNA was used as template in 20ul PCR reaction. Quantitative real-time PCR was performed using Roche LightCycler 480 Real-Time PCR System with SYBR Premix Ex Taq (TAKARA, CHN). The number of PCR cycles used was determined to be within the linear range of the reactions. Sequences of primers and predicted PCR product sizes were shown as **Table 1**. Reactions were performed in at least triplicate and analyzed via relative quantitation with data as average fold change compared to no treatment of TGF-β1 after normalization to GAPDH.

Immunofluorescence labelling of α-SMA, E-cad and PPAR-γ

HK-2 cells were grown to confluence 60%-70% on sterile cover slips (18×18 mm) under the same conditions described above before the treatment. After 48 hours' incubation with different drugs, glass slides with cells were washed three times with phosphate-buffered saline (PBS) before fixing in 4% paraformaldehyde/PBS for 30 minutes at room temperature. Next, cells were rinsed in PBS three times and blocked with peroxidase inhibitor (MAIXIN, CHN) for 10 minutes. After blocking, the cells were rinsed in PBS three times and reacted with non-immune serum of intact goat for 10 minutes. Then cells were incubated in diluted primary antibodies separately-mouse anti-human α-SMA (1:50) (R&D Systems) in PBS, mouse anti-human E-cad (1:50) (R&D Systems) in PBS and mouse anti-human PPAR-γ (1:100) (R&D Systems) in PBS overnight at 4 °C followed by incubation in

secondary antibody donkey anti-mouse Alexa Fluor 594 (1:40) (Invitrogen) for α-SMA and PPAR-γ or goat anti-mouse Alexa Fluor 488 (1:40) (Invitrogen) for E-cad at 37 °C in the dark for one hour. Between each step, cells were washed with PBS adequately. After washing, cells were double-stained with DAPI (1:20) at 37 °C in the dark for 30 minutes. The slides were mounted using antifade mounting medium and viewed with Leica DM4000 fluorescent microscope (Germany).

Statistical analysis

Data are expressed as mean ± standard derivation. Unless stated otherwise, statistical significance was determined using Student›s t-test and statistical significance was achieved when the p value is <0.05.

Results

TGF-β1 increased mRNA level of CTFG and decreased mRNA level of E-cad in HK-2 cells

HK-2 cells were grown and treated with TGF-β1 (3ng/ml) 48 hours; it had been found that the mRNA level of CTGF increased but the mRNA level of E-cad decreased. The results was consistent the pattern of TGF-β1 induced EMT. The mRNA level of PPAR-γ had not difference between non-treatment and treatment of TGF-β1 (**Figure 1**).

Increased mRNA level of CTFG by TGF-β1 was reversed by Telmisartan in HK-2 cells

In order to examine the function of PPAR-γ in the process, we used PPAR-γ agonist Telmisartan and antagonist GW9662 in the study. HK-2 cells were treated with TGF-β1(3ng/ml) 48 hours in presence of Telmisartan at different concentrations; it was interested that the mRNA level of CTGF decreased when Telmisartan concentration was increasing from 3 to 200μM. To confirm this finding, a PPAR-γ inhibitor, GW9662, was used in treatment. We
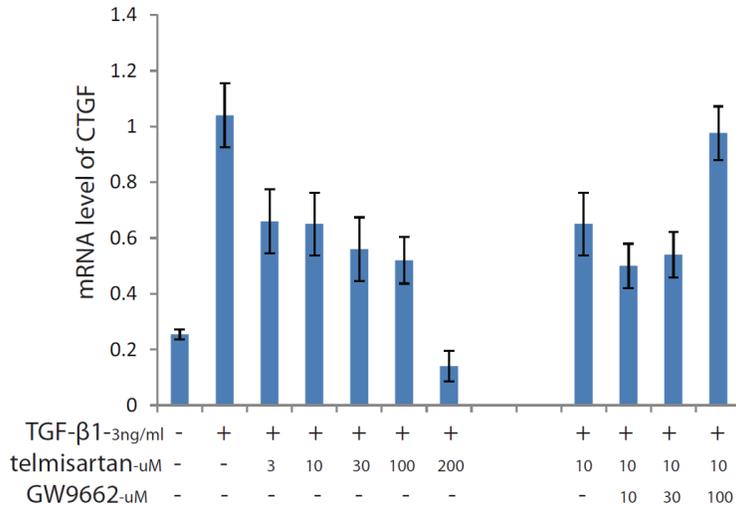
Figure 2. Measurement of mRNA level for CTFG by TGF-β1 plus Telmisartan stimulation in absent or present GW9662. Shown quantification of mRNA level of CTGF by RT-PCR measurement after stimulating with TGF-β1 (3ng/ml) plus Telmisartan at different concentrations (0, 3, 10, 30, 100 or 200μM) in absence or presence of GW9662 (0, 10, 30 or 100μM). Data were represented in triplicate experiments ± S.D.
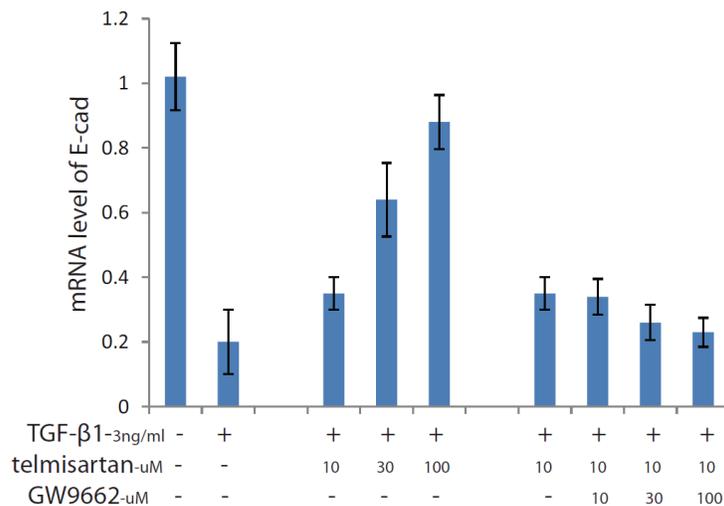

Figure 3. Measurement of mRNA level for E-cad by TGF-β1 plus Telmisartan stimulation in absence or presence of GW9662. Shown quantification of mRNA level of E-cad by RT-PCR measurement after stimulating with TGF-β1 (3ng/ml) plus Telmisartan at different concentrations (0, 10, 30 or 100μM) in absence or presence of GW9662 (0, 10, 30 or 100μM). Data were represented in triplicate experiments ± S.D.

found that GW9662 (100μM) rebounded the mRNA level of CTGF in presence of Telmisartan (10μM) it was indicated that Telmisartan inhibited CTGF expression in PPAR-γ dependent manner. (**Figure 2**)

Decreaced mRNA level of E-cad by TGF-β1 was reversed by telmisartan in HK-2 cells

Also, E-cad mRNA level decreased when HK-2 cells were treated with TGF-β1 (3ng/ml) for 48 hours, but the repression of E-cad mRNA level was inhibited when HK-2 cells were incubated in the presence of Telmisartan (100μM). While incubated with GW9662 (100μM), the effect of increasing E-cad mRNA level by using Telmisartan was blocked (**Figure 3**).

Non-significant changed mRNA level of PPAR-γ in TGF-β1 stimulation plus telmisartan or GW9662 treatment in HK-2 cells

Based on above finding, we investigated the transcript of PPAR-γ mRNA. As we found when HK-2 cells were stimulated by TGF-β1 (3ng/ml) 48 hours, the mRNA level of PPAR-γ was slightly decreased. After incubated HK-2 cells with TGF-β1 (3ng/ml) in presence of Telmisartan, the mRNA level of PPAR-γ did not change until to 100μM, at the concentration, the mRNA of PPAR-γ backed to the level before TGF-β1 stimulation. At last HK-2 cells were stimulated with TGF-β1 (3ng/ml) in presence of PPAR-γ agonist, Telmisartan (10μM) plus blocker of PPAR-γ (GW9662), the PPAR-γ mRNA level was unchanged (**Figure 4**).

Immunofluorescence labeling of HK-2 cells for expression of α-smooth muscle actin, E-cad and PPAR-γ

To deep understand the function of PPAR-γ, we were observing the protein expression pattern in stimulation of TGF-β1. As we had known the potential role of PPAR-γ regulating CTGF and E-cad in mRNA level, we questioned how E-cad
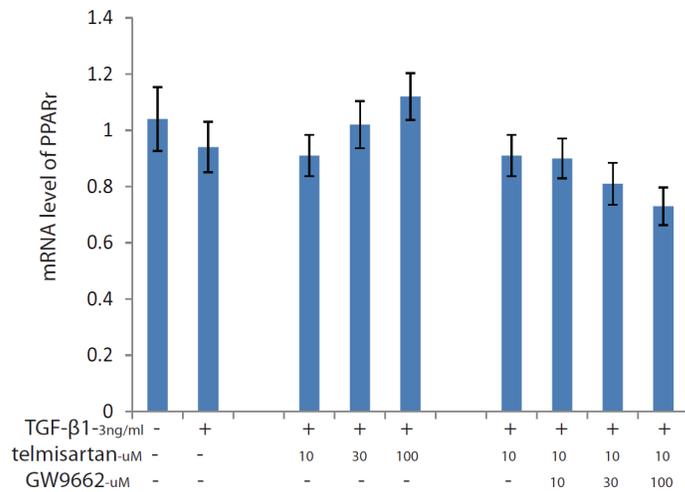
Figure 4. Measurement of mRNA level for PPAR-γ by TGF-β1 plus Telmisartan stimulation in absence or presence of GW9662. Shown quantification of mRNA level of PPAR-γ by RT-PCR measurement after stimulating with TGF-β1 (3ng/ml) plus Telmisartan at different concentrations (0, 10, 30 or 100μM) in absence or presence of GW9662 (0, 10, 30 or 100μM). Data were represented in triplicate experiments ± S.D.
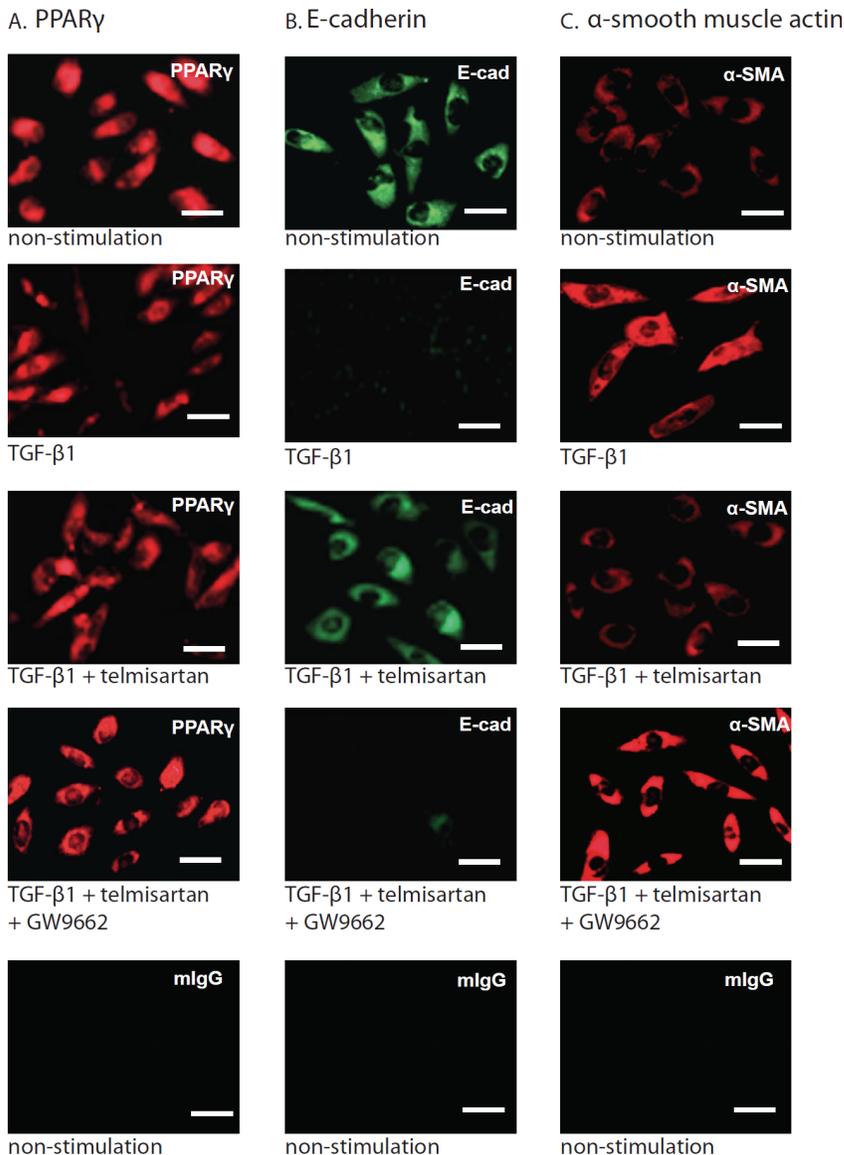

Figure 5. Immunofluorescence labelling of HK-2 cells for detecting expression of α-smooth muscle actin, E-cad and PPAR-γ Labelling HK-2 cells with mouse anti-human PPAR-γ (A), mouse anti-human E-cad (B) or mouse anti-human α-SMA (C) followed with donkey anti-mouse Alexa Fluor 594 for α-SMA and PPAR-γ or goat anti-mouse Alexa Fluor 488 for E-cad in non-stimulation, stimulation with TGF-β1 (3ng/ml), TGF-β1 plus Telmisartan (100μM) or TGF-β1 plus Telmisartan (100μM) and GW9662 (100μM). Mouse IgG (mIgG) labeling controls are shown. Bar 20μm.

was expressed in HK-2 cell with TGF-β1 stimulation plus PPAR-γ agonist or antagonist. We found E-cad protein level was regulated by Telmisartan and GW9662 as the same pattern as in its mRNA level, that was, Telmisartan rescued E-cad expression in EMT process and the rescuing were abolished by PPAR-γ inhibitor, GW9662 (**Figure 5B**). It has been accepted that

the decrease of E-cad expression is correlated with Epithelial-mesenchymal transition or transdifferentiation (EMT). To confirm our hypothesis, the expression of alpha-smooth muscle actin (α-SMA), commonly used as a marker of myofibroblast transdifferentiation, was checked. α-SMA expression level was also influenced by PPAR-γ agonist and antagonist (**Figure 5C**). Telmisartan blocked the TGF-β1 induced α-SMA expression, but the effect was attenuated by GW9662.

PPAR-γ expression was observed in HK-2 cells, the expression level did not change in presence of its agonist or blocker or both (**Figure 5A**). It suggested that the action of PPAR-γ might be performed by functional activation of protein rather than by cis-control of its own transcription and translation expression.

Discussion

In the present study we confirmed the efficacy of TGF-β1 inducing EMT in human proximal tubule epithelium HK-2 cell. The EMT was identified by the observation of dramatical down-regulation of E-cad which is expressed in epithelial cells only, and up-regulation α-SMA and CTGF as mensenchymal specific features. Additionally HK-2 cells acquired another myofibroblastic spindle-shaped morphologic phenotype. It is indicated that HK-2 cell lose it abilities for attachment and cell adhesion and possessed tendency of transition to myofibroblast cell. The protein expression patterns were attenuated by addition of Telmisartan and reversed by PPAR-γ blocker GW9662, supporting the idea that Telmisartan functions in PPAR-γ dependent manner.

PPAR-γ is a ligand-activated transcription factor belonging to the nuclear Hormone receptor superfamily. Like other nuclear receptors, PPAR-γ has a modular structure consisting of a DNA binding domain, and two ligand binding domains (LBD) [29]. PPAR-γ binding its agonist forms a complex with another nuclear receptor known as retinoid receptor (RXR), conformation change of the heterodimer receptors allows the PPAR-γ/RXR to recognize a specific DNA response elements (PPRE) in the promoter region of target genes thus to promote mRNA transcription.

The ligand dependent control of transcription of PPAR-γ target genes applies for the pharmacological functions in clinical. Conventional PPAR-γ agonist thiazolidinediones (TZD) are widely used in treatment in type 2 diabetes and metabolic syndrome. Since Telmisartan can bind LBD of PPAR-γ with a site that is different to TZD [30], it is not surprising that Telmisartan possesses unique effects not related to that of conventional TZD. Teimisartan was identified as PPARg partial agonist in 2004 [20, 21], since then it has been recognized recently that Telmisartan as PPAR-γ partial agonists has variety of therapeutic effects in insulin resistant type 2 diabetes, hypertension, injury protection in myocardial infarction and brain ischemia [31, 32], anti-inflammation [33], cancer treatment [24]. Through a PPAR-γ- dependent manner, Telmisartan also exerts some beneficial effects on the kidney, to decrease proteinuria, inflammation, renoprotection [34-37] although the molecular mechanism is not well understood.

In summary of the novelty of present study, Telmisartan reversed TGF-β1 induced EMT by blocking accumulation of CTGF and α-SMA and decrease of E-cad expression via activating PPAR-γ pathway. The findings might provide supportive evidence for the mechanism of chronic kidney diseases and for the potential usage of Telmisartan in clinical trials in reversal of human renal fibrosis.

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References

[1] Yoshino J, Monkawa T, Tsuji M, Inukai M, Itoh H and Hayashi M. Snail1 is involved in the renal epithelial-mesenchymal transition. Biochem Biophys Res Commun 2007; 362: 63-68.

[2] Hertig A, Anglicheau D, Verine J, Pallet N, Touzot M, Ancel PY, Mesnard L, Brousse N, Baugey E, Glotz D, Legendre C, Rondeau E and Xu-Dubois YC. Early epithelial phenotypic changes predict graft fibrosis. J Am Soc Nephrol 2008; 19: 1584-1591.

[3] Ding Z, Chen Z, Chen X, Cai M, Guo H and Gong N. Adenovirus-mediated anti-sense ERK2 gene therapy inhibits tubular epithelial-mesenchymal transition and ameliorates renal allograft fibrosis. Transpl Immunol 2011; 25: 34-41.

[4] Yang J and Liu Y. Dissection of key events in tubular epithelial to myofibroblast transition and its implications in renal interstitial fibrosis. Am J Pathol 2001; 159: 1465-1475.

[5] Carlisle RE, Heffernan A, Brimble E, Liu L, Jerome D, Collins CA, Mohammed-Ali Z, Margetts PJ, Austin RC and Dickhout JG. TDAG51 mediates epithelial-to-mesenchymal transition in human proximal tubular epithelium. Am J Physiol Renal Physiol 2012;

[6] Sigurdsson V, Hilmarsdottir B, Sigmundsdottir H, Fridriksdottir AJ, Ringner M, Villadsen R, Borg A, Agnarsson BA, Petersen OW, Magnusson MK and Gudjonsson T. Endothelial induced EMT in breast epithelial cells with stem cell properties. PLoS One 2011; 6: e23833.

[7] Yuan Q, Wang L, Zhang F, Wang R, Fu X, Peng Z, Ning W, Hu G, Wang Z and Tao L. Fluorofenidone suppresses epithelial-mesenchymal transition and the expression of connective tissue growth factor via inhibiting TGF-beta/Smads signaling in human proximal tubular epithelial cells. Pharmazie 2011; 66: 961-967.

[8] Margetts PJ, Bonniaud P, Liu L, Hoff CM, Holmes CJ, West-Mays JA and Kelly MM. Transient overexpression of TGF-{beta}1 induces epithelial mesenchymal transition in the rodent peritoneum. J Am Soc Nephrol 2005; 16: 425-436.

[9] Urakami C, Kurosaka D, Tamada K, Kishimoto S, Tezuka Y and Nishigori H. Lovastatin Alters TGF-beta-Induced Epithelial-Mesenchymal Transition in Porcine Lens Epithelial Cells. Curr Eye Res 2012; 37: 479-485.

[10] Pignatelli J, Tumbarello DA, Schmidt RP and Turner CE. Hic-5 promotes invadopodia formation and invasion during TGF-beta-induced epithelial-mesenchymal transition. J Cell Biol 2012; 197: 421-437.

[11] Kalluri R and Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009; 119: 1420-1428.

[12] Eddy AA. Can renal fibrosis be reversed? Pediatr Nephrol 2005; 20: 1369-1375.

[13] Tailleux A, Wouters K and Staels B. Roles of PPARs in NAFLD: Potential therapeutic targets. Biochim Biophys Acta 2012; 1821: 809-818.

[14] Dobrian AD. The complex role of PPARgamma in renal dysfunction in obesity: managing a Janus-faced receptor. Vascul Pharmacol 2006; 45: 36-45.

[15] Guan Y, Zhang Y and Breyer MD. The Role of PPARs in the Transcriptional Control of Cellular Processes. Drug News Perspect 2002; 15: 147-154.

[16] Michalik L and Wahli W. PPARs Mediate Lipid Signaling in Inflammation and Cancer. PPAR Res 2008; 2008: 134059.

[17] Simpson-Haidaris PJ, Pollock SJ, Ramon S, Guo N, Woeller CF, Feldon SE and Phipps RP. Anticancer Role of PPARgamma Agonists in Hematological Malignancies Found in the Vasculature, Marrow, and Eyes. PPAR Res 2010; 2010: 814609.

[18] Tan X, Dagher H, Hutton CA and Bourke JE. Effects of PPAR gamma ligands on TGF-beta1-induced epithelial-mesenchymal transition in alveolar epithelial cells. Respir Res 2010; 11: 21.

[19] Reka AK, Kurapati H, Narala VR, Bommer G, Chen J, Standiford TJ and Keshamouni VG. Peroxisome proliferator-activated receptor-gamma activation inhibits tumor metastasis by antagonizing Smad3-mediated epithelial-mesenchymal transition. Mol Cancer Ther 2010; 9: 3221-3232.

[20] Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M, Qi N, Wang J, Avery MA and Kurtz TW. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. Hypertension 2004; 43: 993-1002.

[21] Schupp M, Janke J, Clasen R, Unger T and Kintscher U. Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-gamma activity. Circulation 2004; 109: 2054-2057.

[22] Cianchetti S, Del FA, Colognato R, Di Stefano R, Franzoni F and Pedrinelli R. Anti-inflammatory and anti-oxidant properties of telmisartan in cultured human umbilical vein endothelial cells. Atherosclerosis 2008; 198: 22-28.

[23] Tian Q, Miyazaki R, Ichiki T, Imayama I, Inanaga K, Ohtsubo H, Yano K, Takeda K and Sunagawa K. Inhibition of tumor necrosis factor-alpha-induced interleukin-6 expression by telmisartan through cross-talk of peroxisome proliferator-activated receptor-gamma with nuclear factor kappaB and CCAAT/enhancer-binding protein-beta. Hypertension 2009; 53: 798-804.

[24] Funao K, Matsuyama M, Kawahito Y, Sano H, Chargui J, Touraine JL, Nakatani T and Yoshimura R. Telmisartan is a potent target for prevention and treatment in human prostate cancer. Oncol Rep 2008; 20: 295-300.

[25] Funao K, Matsuyama M, Kawahito Y, Sano H, Chargui J, Touraine JL, Nakatani T and Yoshimura R. Telmisartan as a peroxisome proliferator-activated receptor-gamma ligand is a new target in the treatment of human renal

cell carcinoma. Mol Med Report 2009; 2: 193-198.

[26] Hamano Y, Okude T, Yokosuka O and Ogawa M. Attenuation of Immune-Mediated Renal Injury by Telmisartan, an Angiotensin Receptor Blocker and a Selective PPAR-gamma Activator. Nephron Extra 2011; 1: 78-90.

[27] Lakshmanan AP, Watanabe K, Thandavarayan RA, Sari FR, Harima M, Giridharan VV, Soetikno V, Kodama M and Aizawa Y. Telmisartan attenuates oxidative stress and renal fibrosis in streptozotocin induced diabetic mice with the alteration of angiotensin-(1-7) mas receptor expression associated with its PPAR-gamma agonist action. Free Radic Res 2011; 45: 575-584.

[28] Zou R, Xu G, Liu XC, Han M, Jiang JJ, Huang Q, He Y and Yao Y. PPARgamma agonists inhibit TGF-beta-PKA signaling in glomerulosclerosis. Acta Pharmacol Sin 2010; 31: 43-50.

[29] Shao D, Rangwala SM, Bailey ST, Krakow SL, Reginato MJ and Lazar MA. Interdomain communication regulating ligand binding by PPAR-gamma. Nature 1998; 396: 377-380.

[30] Amano Y, Yamaguchi T, Ohno K, Niimi T, Orita M, Sakashita H and Takeuchi M. Structural basis for telmisartan-mediated partial activation of PPAR gamma. Hypertens Res 2012;

[31] Goyal S, Arora S, Mittal R, Joshi S, Nag TC, Ray R, Kumari S and Arya DS. Myocardial salvaging effect of telmisartan in experimental model of myocardial infarction. Eur J Pharmacol 2009; 619: 75-84.

[32] Kasahara Y, Taguchi A, Uno H, Nakano A, Nakagomi T, Hirose H, Stern DM and Matsuyama T. Telmisartan suppresses cerebral injury in a murine model of transient focal ischemia. Brain Res 2010; 1340: 70-80.

[33] Takai S, Jin D, Kimura M, Kirimura K, Sakonjo H, Tanaka K and Miyazaki M. Inhibition of vascular angiotensin-converting enzyme by telmisartan via the peroxisome proliferator-activated receptor gamma agonistic property in rats. Hypertens Res 2007; 30: 1231-1237.

[34] Arozal W, Watanabe K, Veeraveedu PT, Ma M, Thandavarayan RA, Sukumaran V, Suzuki K, Kodama M and Aizawa Y. Telmisartan prevents the progression of renal injury in daunorubicin rats with the alteration of angiotensin II and endothelin-1 receptor expression associated with its PPAR-gamma agonist actions. Toxicology 2011; 279: 91-99.

[35] Ihm CG, Jeong KW, Lee SH, Lee TW and Park JK. Effects of therapeutic agents on the inflammatory and fibrogenic factors in IgA nephropathy. Nephrology (Carlton) 2007; 12 Suppl 3: S25-26.

[36] Kanaguchi Y, Suzuki Y, Osaki K, Sugaya T, Horikoshi S and Tomino Y. Protective effects of L-type fatty acid-binding protein (L-FABP) in proximal tubular cells against glomerular injury in anti-GBM antibody-mediated glomerulonephritis. Nephrol Dial Transplant 2011; 26: 3465-3473.

[37] Ladino M and Hernandez SI. Renovascular and renoprotective properties of telmisartan: clinical utility. Int J Nephrol Renovasc Dis 2010; 3: 33-38.