Biochemical and Biophysical Research Communications 424 (2012) 801–806

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Oncostatin M inhibits TGF-β1-induced CTGF expression via STAT3 in human proximal tubular cells

Rita Sarközi *, Kathrin Flucher, Viktoria Maria Haller, Markus Pirklbauer, Gert Mayer, Herbert Schramek

*Department of Internal Medicine IV, Nephrology and Hypertension, Innsbruck Medical University, Innsbruck, Austria*

ARTICLE INFO

*Article history:*
Received 9 July 2012
Available online 16 July 2012

*Keywords:*
Oncostatin M
TGF-β1
CTGF
STAT3
SMAD3
Proximal tubular epithelial cells

ABSTRACT

Matricellular proteins play a critical role in the development of tubulointerstitial fibrosis and renal disease progression. Connective tissue growth factor (CTGF/CCN2), a CCN family member of matricellular proteins, represents an important mediator during development of glomerular and tubulointerstitial fibrosis in progressive kidney disease. We have recently reported that oncostatin M (OSM) is a potent inhibitor of TGF-β1-induced CTGF expression in human proximal tubular cells (PTC). In the present study we examined the role of TGF-β1- and OSM-induced signaling mechanisms in the regulation of CTGF mRNA expression in human proximal tubular HK-2 cells. Utilizing siRNA-mediated gene silencing we found that TGF-β1-induced expression of CTGF mRNA after 2 h of stimulation at least partially depends on SMAD3 but not on SMAD2. In contrast to TGF-β1, OSM seems to exert a time-dependent dual effect on CTGF mRNA expression in these cells. While OSM led to a rapid and transient induction of CTGF mRNA expression between 15 min and 1 h of stimulation it markedly suppressed basal and TGF-β1-induced CTGF mRNA levels thereafter. Silencing of STAT1 or STAT3 attenuated basal CTGF mRNA levels indicating that both STAT isoforms may be involved in the regulation of basal CTGF mRNA expression. However, knockdown of STAT3 but not STAT1 prevented OSM-mediated suppression of basal and TGF-β1-induced upregulation of CTGF mRNA expression. Together these results suggest that the inhibitory effect of OSM on TGF-β1-induced CTGF mRNA expression is mainly driven by STAT3, thereby providing a signaling mechanism whereby OSM may contribute to tubulointerstitial protection.



## 1. Introduction

Matricellular proteins are known to modulate the activity and bioavailability of other extracellular matrix (ECM) components such as growth factors, cytokines and extracellular enzymes and to regulate cell function through direct binding to cell surface receptors [1,2]. Their expression is highly sensitive to environmental perturbations including mechanical stress, inflammation, tissue injury and repair. Connective tissue growth factor (CTGF/CCN2) represents a member of the CCN family of matricellular proteins, modulates adhesion, migration, ECM production and plays a critical role in many fibrotic conditions including kidney fibrosis [2,3]. Several studies have reported increased CTGF expression in renal disorders associated with kidney fibrosis such as diabetic nephropathy [2,4,5]. Glomerular podocytes, mesangial cells and proximal tubular epithelial cells have been shown to express elevated levels of CTGF in human and experimental models of diabetic nephropathy [6–11]. Antisense oligonucleotides targeting CTGF in mouse models of type 1 and type 2 diabetes, for example, attenuated progression of nephropathy [11], while CTGF overexpression in podocytes of mice worsened proteinuria and mesangial expansion [12]. Furthermore, increased urinary excretion as well as elevated plasma levels of CTGF have been observed in type 1 diabetic patients [13–17]. In a large cross-sectional study of patients with type 1 diabetes, increased urinary CTGF levels were associated with urinary albumin excretion and correlated with the rate of decline in glomerular filtration rate (GFR) [16].

In diabetic nephropathy renal CTGF expression is likely to be induced by high levels of glucose, mechanical strain, advanced glycation end product proteins, reactive oxygen species and by different soluble mediators such as transforming growth factor-β1 (TGF-β1) [18]. Indeed, CTGF was shown to mediate many of the profibrotic actions of TGF-β1 [19–21] and both CTGF and TGF-β1, act synergistically to promote tissue fibrosis [4]. In the remnant kidney of subtotal nephrectomized, TGF-β1 transgenic mice intravenous administration of CTGF antisense oligonucleotide inhibited both CTGF expression in proximal tubular cells (PTC) and interstitial fibrogenesis despite sustained levels of TGF-β1 mRNA [22]. In this

* Corresponding author. Address: Department of Internal Medicine IV, Nephrology and Hypertension, Innsbruck Medical University, Anichstrasse 35, A-6020 Innsbruck, Austria. Fax: +43 512 504 23309.

*E-mail addresses:* rita.sarkozi@i-med.ac.at (R. Sarközi), kathrin.flucher@uki.at (K. Flucher), viktoriamaria.haller@student.uibk.ac.at (V.M. Haller), markus.pirklbauer@i-med.ac.at (M. Pirklbauer), gert.mayer@i-med.ac.at (G. Mayer), herbert.schramek@i-med.ac.at (H. Schramek).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.042

context, we have recently reported that in human PTC TGF-β1 leads to a strong induction of the expression of four matricellular proteins including CTGF, secreted protein acidic and rich in cysteine (SPARC), tenascin C (TNC) and thrombospondin-1 (TSP-1) [23]. In addition, we were able to show that oncostatin M (OSM), an IL-6 type cytokine, acts as a potent inhibitor of basal and TGF-β1-induced expression of CTGF regardless of the sequence of ligand administration [23]. Our results utilizing receptor-blocking molecules suggested that the inhibitory effect of OSM on TGF-β1-induced CTGF mRNA expression occurs independently of SMAD2/3 signaling and may be driven by OSM receptor-activated STAT1 and/or STAT3 signaling pathways [23]. In the present study we thus investigated the specific contribution of these two STAT isoforms to OSM's inhibitory effect on basal and TGF-β1-driven CTGF mRNA expression. Furthermore, we studied time course and early signaling mechanisms of TGF-β1-induced CTGF mRNA expression in human PTC.

## 2. Materials and methods

### 2.1. Cell culture

Proximal tubular human kidney 2 (HK-2) cells were cultured in Keratinocyte-Serum Free Medium (KSFM) containing 10% fetal bovine serum (FBS), 5 ng/ml recombinant epidermal growth factor (rEGF), 0.05 mg/ml bovine pituitary extract (BPE), 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were grown at 37 °C in a humidified 5% $CO_2$ atmosphere and split at a 1:5 ratio once a week. After growth to subconfluent state, cells were washed once, made quiescent by incubation in serum- and supplement-free medium for 48 h, and then used for experiments. Stimulations with oncostatin M (OSM) or with transforming growth factor-β1 (TGF-β1) were performed in the absence of serum and any other growth supplements. Cell culture reagents were obtained from Gibco (Life Technologies, Lofer, Austria). OSM was purchased from Sigma (St. Louis, MO), TGF-β1 from PeproTech (PeproTech Austria, Vienna) all other reagents were obtained from Sigma (St. Louis, MO).

### 2.2. siRNA transfection

HK-2 cells were cultured under serum- and supplement-free conditions at 60–70% confluency and transfected with SMAD2, SMAD3, STAT1, STAT3 ON-TARGETplus SMART pool siRNA constructs (Thermo Scientific, Lafayette, CO) or negative control siRNA (ON-TARGETplus non-targeting pool siRNA, Thermo Scientific, Lafayette, CO) at a concentration of 50 nM utilizing DharmaFect 1 Transfection Reagent (Thermo Scientific, Lafayette, CO) according to the manufacturer's instructions. 72 h post-transfection cells were left untreated or stimulated with 10 ng/ml OSM or with 10 ng/ml TGF-β1 and utilized for Western blot or real-time PCR analysis.

### 2.3. Western blot analysis

Cells were washed with ice-cold phosphate buffered saline (PBS) and lysed in RIPA lysis buffer as previously described [23]. The protein content of the samples was determined using a microbicinchoninic acid assay (Pierce, Fisher Scientific, Vienna). Cell lysates were matched for protein, separated on 10% SDS–PAGE, and transferred to a polyvinylidene difluoride microporous membrane. Subsequently, membranes were probed with one of the following antibodies: Smad2 (L16D3), P-SMAD2 (Ser465/467), SMAD3 (C67H9), P-SMAD3 (Ser423/425), STAT1, P-STAT1 (Tyr701), STAT3, P-STAT3 (Tyr705) (Cell Signaling Technology, Danvers, MA), ERK2 (C-14) (Santa Cruz Biotechnology, Santa Cruz, CA). After extensive washing of the sheets in TBS, 0.1% Tween-20, the primary antibodies were detected using horseradish peroxidase conjugated goat anti-rabbit IgG or rabbit anti-goat IgG (Santa Cruz Biotechnology, Santa Cruz, CA) and visualized by LumiGLO Western Blot Detection system (Cell Signaling Technology, Danvers, MA).

### 2.4. RNA isolation and real-time PCR analysis

Total RNA was isolated using the RNeasyx Mini Kit (Qiagen, Valencia CA). For each sample 1 μg RNA was reverse transcribed into cDNA with the High Capacity cDNA reverse Transcription kit (Applied Biosystems, Foster City, CA). Samples were analyzed by using one of the following TaqMan® Gene Expression Assays:GAPDH (Hs99999905_m1), CTGF (Hs00170014_m1), SMAD2 (Hs00183425_m1), SMAD3 (Hs00969210_m1), STAT1 (Hs01013996_m1), STAT3 (Hs01047580_m1). Reactions were prepared in duplicate for each sample and analyzed on the 7500 Fast Real-Time PCR System (Applied Biosystems) under the following conditions: 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Relative quantification method was used to analyze real-time PCR data. The fractional cycle number at which the amount of amplified target reached a threshold is the CT number, the mean CT for each gene was determined and the relative amounts of transcripts for each gene was normalized to the reference gene GAPDH as follows: $\Delta CT = CT\ (\text{gene of interest}) - CT\ (\text{GAPDH})$. The ΔCT was linearized according to the formula $2^{-dCT}$ to determine the relative expression of each gene of interest.

### 2.5. Statistical analysis of quantitative real-time PCR

All values are expressed as mean ± SE. Comparison of two groups was made by *t*-test. Multiple group comparisons were made by analysis of variance (ANOVA). *P* values <0.05 were considered significant.

## 3. Results

### 3.1. Time-dependent effects of TGF-β1 and OSM on CTGF mRNA expression

We have recently reported that OSM, a member of the IL-6 type cytokine family, represents a novel and potent inhibitor of basal and TGF-β1-stimulated CTGF expression in human proximal tubular cells (PTC) [23]. This inhibitory action of OSM was effective after 2 h and not affected by inhibition of DNA methylation [23]. To be able to study the underlying early mechanisms of these ligand-dependent effects on CTGF mRNA expression, we first investigated the time course of CTGF mRNA expression in the presence of TGF-β1 alone and OSM alone when compared with a combination of these two cytokines in human proximal tubular HK-2 cells (Fig. 1). CTGF mRNA levels in the absence of any of these ligands remained unchanged during the experimental period of 4 h (data not shown). Treatment of the cells with 10 ng/ml TGF-β1 alone led to a time-dependent induction of CTGF mRNA expression, which started as early as 15 min after stimulation (Fig. 1), was 5.1-, 4.6-, and 5.3-fold after 2, 3 and 4 h, respectively (Fig. 1), and lasted for at least 24 h [23]. Interestingly, when administered alone, 10 ng/ml OSM initially led to a similar time-dependent upregulation of CTGF mRNA levels between 15 min and 1 h (Fig. 1). However, after 2 h of incubation OSM inhibited basal and TGF-β1-induced CTGF mRNA expression (Fig. 1). These results suggest that in contrast to TGF-β1, which represents a strong stimulator of CTGF mRNA expression in human PTC, OSM seems to have a dual effect. Whereas it stimulates CTGF mRNA expression

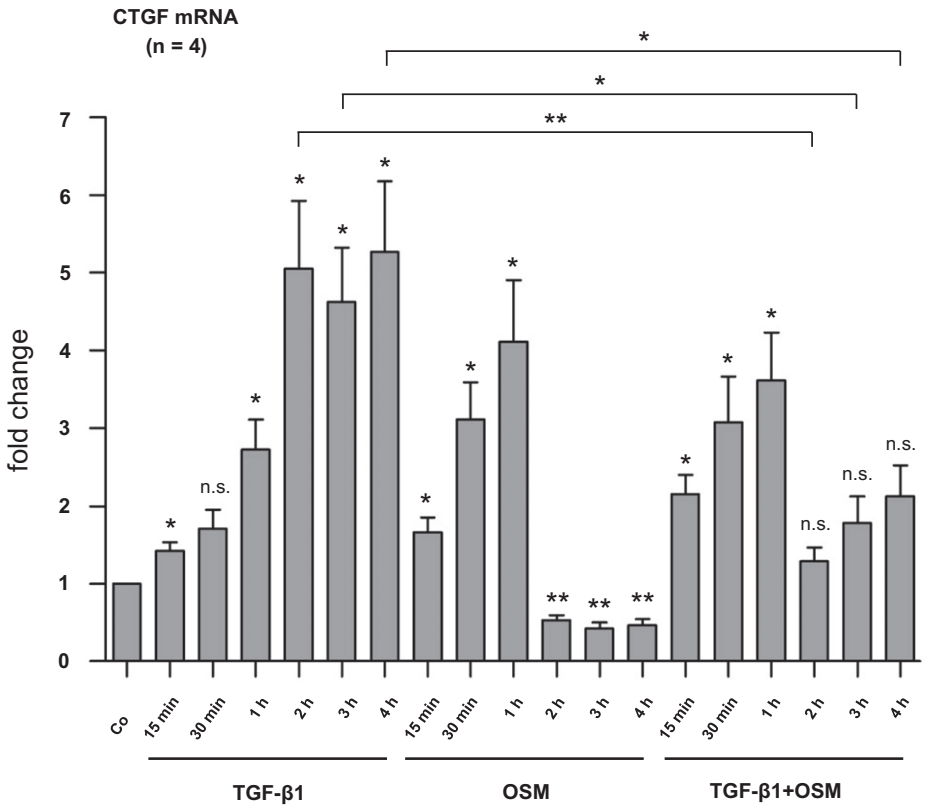


**Fig. 1.** Time-dependent differential effects of TGF-β1 and OSM on CTGF expression in human PTCs. HK-2 cells were serum- and supplement-starved for 48 h, and were then stimulated with 10 ng/ml TGF-β1 alone, 10 ng/ml OSM alone or with the combination of the two cytokines for the indicated periods of time. Data are presented as fold induction above unstimulated CTGF mRNA control levels after normalizing to GAPDH mRNA expression. CTGF mRNA expression in the absence of any ligand remained unchanged during the experimental period of 4 h (1.03-fold after 4 h; $n = 4$). Each data point indicates the average of 4 independent experiments with error bars corresponding to SE ($^{*}P < 0.05$, $^{**}P < 0.01$ vs. control).

at very early time points (in between 15 min and 1 h), it leads to a strong and long-lasting inhibition of basal and TGF-β1-induced CTGF mRNA levels thereafter (Fig. 1) [23].

### 3.2. SMAD3 but not SMAD2 is a mediator of early TGF-β1-induced CTGF mRNA expression

In human PTC, rapid TGF-β1-induced expression of CTGF mRNA between 15 min and 1 h of stimulation is associated with rapid phosphorylation of both SMAD2/3 and SMAD1/5/8 [23]. In addition, our studies utilizing small-molecule inhibitors of TGF-β1 type I and BMP type I receptors suggested that TGF-β1-induced rapid and long-lasting phosphorylation of SMAD2/3 but not of SMAD1/5/8 plays an important role in the long-term induction of CTGF mRNA in these cells [23]. Thus, we next investigated the specific role of SMAD2 and SMAD3 for TGF-β1-induced rapid upregulation of CTGF. To accomplish selective inhibition of these two SMAD isoforms, siRNA-mediated gene silencing was performed. As depicted in Fig. 2, SMAD2-specific siRNA led to a strong and selective downregulation of both SMAD2 mRNA (Fig. 2A) and SMAD2 protein (Fig. 2C) when compared with non-targeting (NT siRNA) controls. Downregulation of SMAD2 was associated with a nearly complete suppression of TGF-β1-induced SMAD2 phosphorylation after 2 h of incubation (Fig. 2C) but did not impair TGF-β1-stimulated CTGF mRNA expression at this time point (Fig. 2D). Exposure of the cells to SMAD3 siRNA resulted in selective downregulation of SMAD3 mRNA but not SMAD2 mRNA (Fig. 2B) and diminished SMAD3 protein expression (Fig. 2C). In addition, knockdown of SMAD3 almost completely abolished TGF-β1-induced phosphorylation of SMAD3 but not SMAD2 (Fig. 2C). In contrast to SMAD2, selective knockdown of SMAD3 was associated with partial inhibition of TGF-β1-stimulated CTGF mRNA expression at the 2 h time point (Fig. 2D). These results suggest that in human proximal tubular HK-2 cells, SMAD3 but not SMAD2 is involved in TGF-β1-mediated early upregulation of CTGF mRNA levels.

### 3.3. OSM-mediated inhibition of TGF-β1-induced CTGF mRNA expression depends on STAT3 but not on STAT1

Our previously published results utilizing a function-blocking antibody against OSM receptor subunit gp130 revealed evidence that the inhibitory effect of OSM on TGF-β1-induced CTGF mRNA expression may be driven by OSM receptor-mediated STAT1 and/or STAT3 signaling pathways [23]. In order to investigate the specific role of these two STAT isoforms in OSM's inhibitory action on CTGF expression, we performed siRNA-mediated selective knockdown of STAT1 and STAT3. As shown in Fig. 3, knockdown of the respective STAT isoforms was specific and selective resulting in almost complete repression of STAT1 mRNA (Fig. 3A) and STAT3 mRNA (Fig. 3B) under all experimental conditions tested. Western blot analyses confirmed these results for STAT1 and STAT3 protein expression (Fig. 3C). Furthermore, after 1 h of stimulation STAT1 siRNA led to specific inhibition of OSM-mediated phosphorylation of STAT1 but not STAT3, while STAT3 siRNA selectively diminished OSM-induced phosphorylation of STAT3 but not STAT1 (Fig. 3C). Interestingly, silencing of STAT1 expression or STAT3 expression significantly attenuated basal CTGF mRNA expression ($P < 0.01$; $n = 6$) (Fig. 3D), suggesting that both STAT1 as well as STAT3 play a role in the regulation of basal CTGF mRNA expression in HK-2 cells. More importantly, siRNA-mediated silencing of STAT3 abolished the inhibitory effect of OSM on basal and TGF-β1-induced CTGF mRNA expression

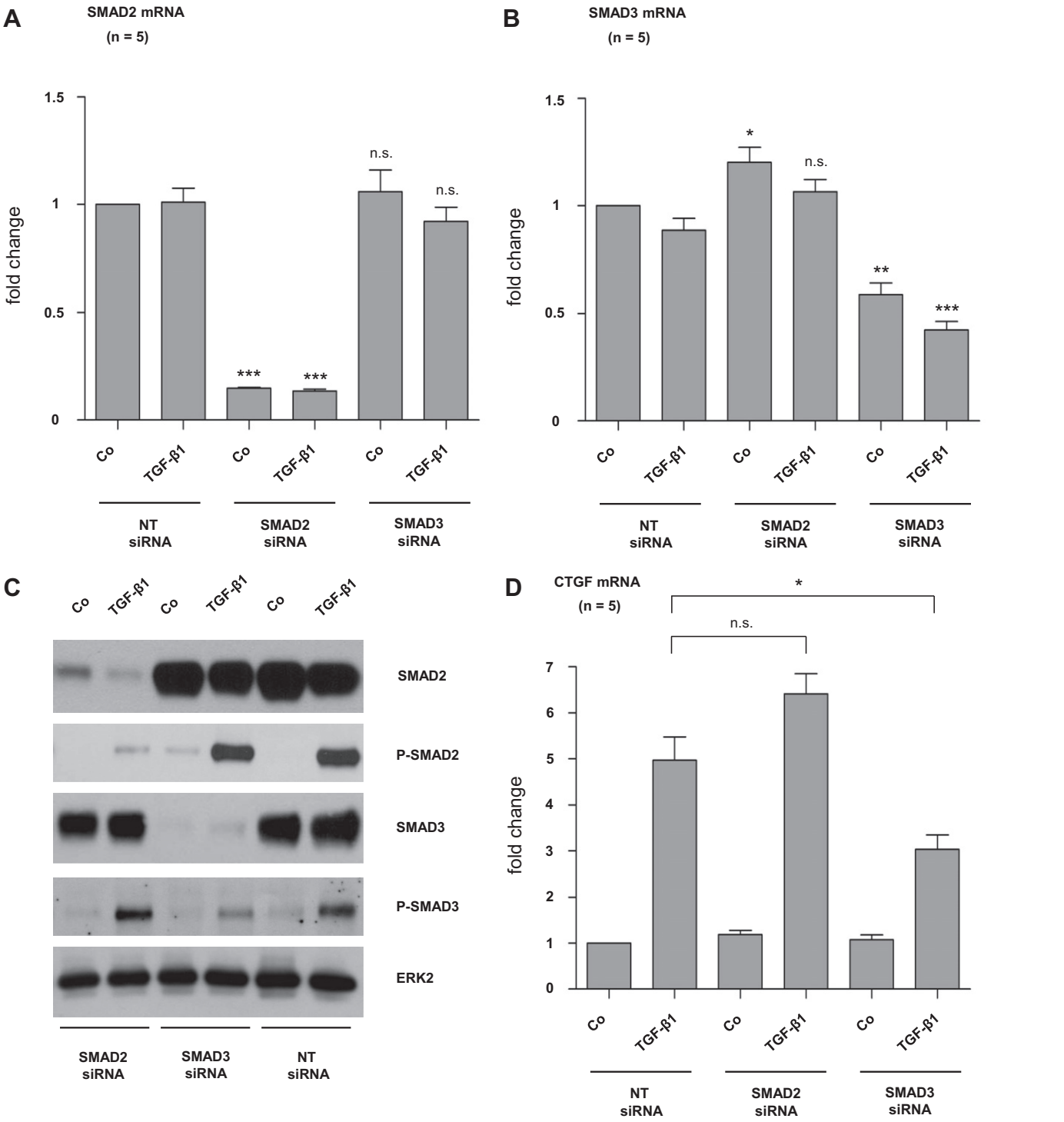


**Fig. 2.** SMAD3 but not SMAD2 is a mediator of early TGF-β1-induced CTGF mRNA expression in human PTCs. HK-2 cells were transfected with non-targeting siRNA (NT siRNA), SMAD2 siRNA or SMAD3 siRNA for 72 h, followed by stimulation with 10 ng/ml TGF-β1 for additional 2 h. The efficiency and selectivity of siRNA treatments were assessed by real-time PCR analysis as well as by Western immunoblotting. The effect of SMAD2 and SMAD3 knockdown on CTGF mRNA expression was analyzed by real-time PCR analysis. (A) Real-time PCR analysis of SMAD2 mRNA expression in cells exposed to SMAD2 siRNA when compared with SMAD3 siRNA and NT siRNA. Each data point indicates the average of 5 independent experiments with error bars corresponding to SE (***$P < 0.001$ vs. NT siRNA-treated controls). (B) Real-time PCR analysis of SMAD3 mRNA expression in cells exposed to SMAD3 siRNA when compared with SMAD2 siRNA and NT siRNA. Each data point indicates the average of 5 independent experiments with error bars corresponding to SE (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. NT siRNA-treated controls). (C) Western blot analysis of protein expression and phosphorylation (P-) of both SMAD2 and SMAD3 in unstimulated and TGF-β1-stimulated cells exposed to SMAD3 siRNA or SMAD2 siRNA when compared with NT siRNA controls. Protein expression of ERK2 shows equal protein loading. The results from one representative Western blot of $n = 5$ separate experiments are depicted. (D) Real-time PCR analysis of CTGF mRNA expression in unstimulated and TGF-β1-stimulated cells exposed to SMAD2 siRNA or SMAD3 siRNA when compared with NT siRNA controls. Each data point indicates the average of 5 independent experiments with error bars corresponding to SE (*$P < 0.05$).

(Fig. 3D). In contrast, STAT1 knockdown did neither affect OSM-mediated suppression of basal nor of TGF-β1-stimulated CTGF mRNA expression (Fig. 3D). Taken together, these results provide evidence that in human PTC the inhibitory action of OSM on basal and TGF-β1-induced CTGF mRNA expression is primarily mediated by STAT3.

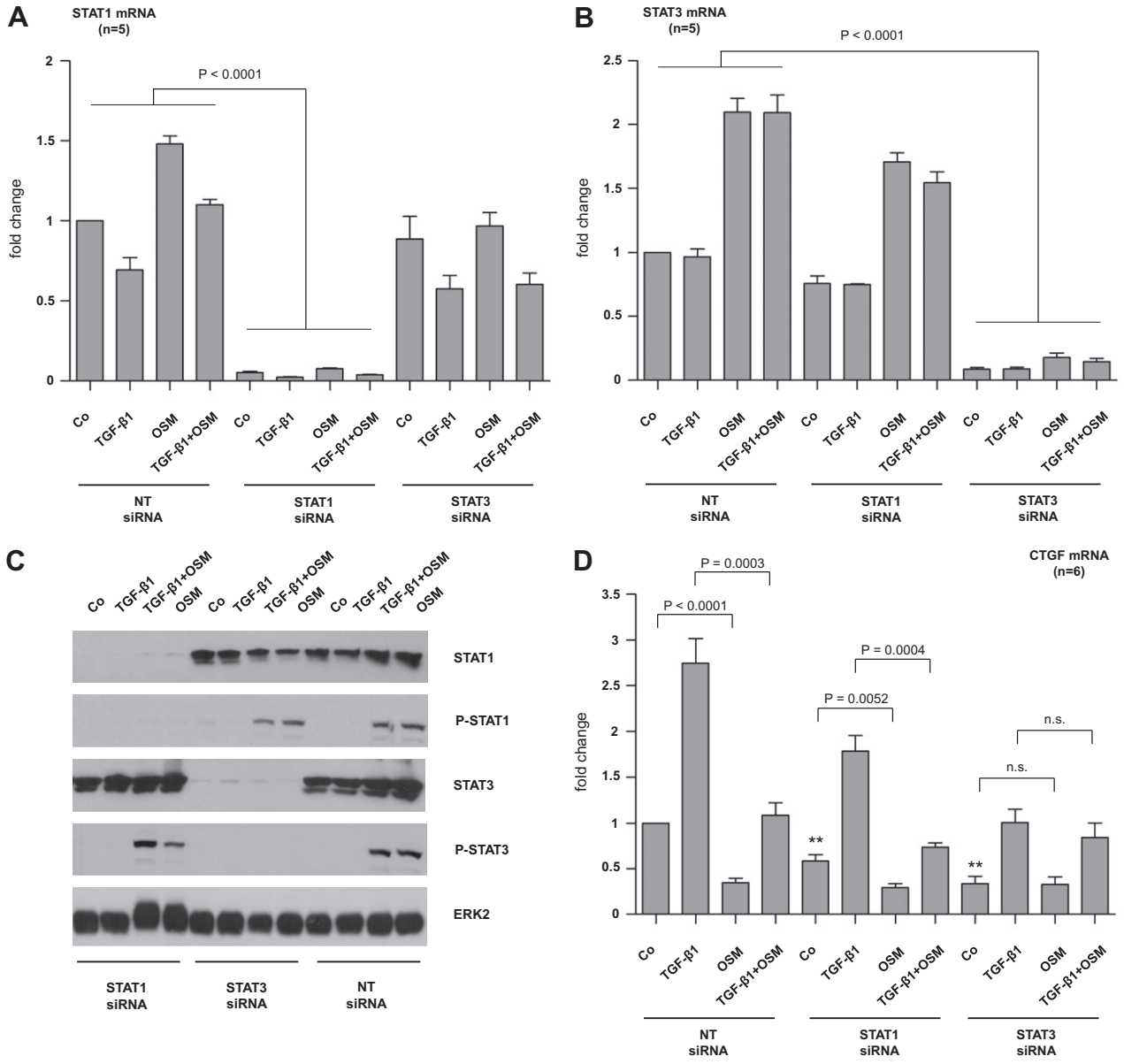


**Fig. 3.** The inhibitory effect of OSM on TGF-β1-induced expression of CTGF mRNA is STAT3 dependent. HK-2 cells were transfected with non-targeting siRNA (NT siRNA), STAT1 siRNA or STAT3 siRNA. 72 h post-transfection cells were treated with 10 ng/ml TGF-β1 alone, 10 ng/ml OSM alone or with a combination of the two cytokines (TGF-β1 + OSM) either for 3 h (real-time PCR analysis in A, B and D) or for 1 h (Western blot analysis in C). The efficiency and selectivity of siRNA treatments were assessed by real-time PCR analysis as well as by Western immunoblotting. The effect of STAT1 and STAT3 knockdown on CTGF mRNA expression was analyzed by real-time PCR analysis. (A) Real-time PCR analysis of STAT1 mRNA expression in cells exposed to STAT1 siRNA when compared with NT siRNA and STAT3 siRNA. Each data point indicates the average of 5 independent experiments with error bars corresponding to SE ($P < 0.0001$ is valid for every separate treatment in STAT1 siRNA expressing cells when compared with NT siRNA expressing controls). (B) Real-time PCR analysis of STAT3 mRNA expression in cells exposed to STAT3 siRNA when compared with NT siRNA and STAT1 siRNA. Each data point indicates the average of 5 independent experiments with error bars corresponding to SE ($P < 0.0001$ is valid for every separate treatment in STAT3 siRNA expressing cells when compared with NT siRNA expressing controls). (C) Western blot analysis of protein expression and phosphorylation (P-) of both STAT1 and STAT3 in unstimulated and ligand stimulated cells exposed to STAT1 siRNA or STAT3 siRNA when compared with NT siRNA controls. Protein expression of ERK2 shows equal protein loading. The results from one representative Western blot of $n = 3$ separate experiments are depicted. (D) Real-time PCR analysis of CTGF mRNA expression in unstimulated and ligand-stimulated cells exposed to STAT1 siRNA or STAT3 siRNA when compared with NT siRNA controls. Each data point indicates the average of 6 independent experiments with error bars corresponding to SE (**$P < 0.01$ vs. NT siRNA control or $P$ values as depicted).

## 4. Discussion

Excessive deposition of extracellular matrix (ECM) components within the glomerulus and tubular interstitium is a characteristic structural alteration observed during progression of different renal diseases such as chronic glomerulonephritis, obstructive nephropathy and diabetic nephropathy. Especially in diabetic nephropathy, a growing number of studies exists showing that CTGF plays an important role in the regulation of ECM remodeling [2]. Furthermore, CTGF has been recognized to have its own profibrotic effects as well as to amplify some of the fibrotic actions of TGF-β1, and thus has been proposed as an alternative therapeutic target in diabetic nephropathy [4,5,18]. TGF-β1 is generally accepted as a central mediator of pathological events related to renal fibrosis as it is involved in a number of fibrogenic events including the induction of fibroblast proliferation, epithelial-to-mesenchymal transition (EMT) and ECM production by tubular epithelial cells and fibroblasts [24,25]. Interestingly, we have recently found that oncostatin M (OSM), a pleiotropic cytokine of the interleukin-6 (IL-6) family, is a strong inhibitor of TGF-β1-induced expression

of CTGF and three additional matricellular proteins, namely of SPARC, tenascin C and thrombospondin-1 in human PTC. Moreover, this inhibitory effect of OSM on the TGF-β1-induced expression of CTGF started after 2 h of cytokine administration and lasted for at least 24 h [23]. We now show that OSM exerts a time-dependent dual effect on the expression of CTGF mRNA in human proximal tubular HK-2 cells. At early time-points (between 15 min and 1 h of ligand administration) OSM leads to a significant but transient induction of CTGF mRNA expression followed by a strong and long-lasting inhibition of basal and TGF-β1-mediated upregulation of CTGF mRNA levels. This is in contrast to TGF-β1, which mediates rapid and long-lasting mRNA expression of CTGF [23]. Of the canonical signaling pathways induced by TGF-β1, activation of SMAD2/3 plays a central role. Although SMAD2 and SMAD3 are highly similar in their structure and although TGF-β1 activates both SMAD2 and SMAD3 in most cell types, it seems likely that these SMAD isoforms mediate different transcriptional responses and thus exert distinct (patho)physiological functions [26–28]. In this context, SMAD3 has been suggested to be pathogenic and a mediator of fibrotic responses while SMAD2 might possess a protective role [24,26–28]. Our present results demonstrate that TGF-β1-mediated early induction of CTGF mRNA (2 h after cytokine administration) occurs independently of SMAD2. In contrast, ablation of SMAD3 significantly attenuated TGF-β1-induced upregulation of CTGF mRNA at this time point, suggesting that SMAD3 is involved in TGF-β1-dependent rapid induction of CTGF mRNA levels. Interestingly, SMAD3 siRNA treatment resulted in approximately 42% reduction of basal SMAD3 mRNA but in complete suppression of SMAD3 protein expression, which was associated with an approximate 40% inhibition of TGF-β1-stimulated CTGF mRNA expression. The fact that TGF-β1 is still able to partially stimulate CTGF mRNA expression in the absence of SMAD3 protein suggests that additional signaling pathways activated by this cytokine may play a role in early TGF-β1-driven induction of CTGF mRNA. Selective downregulation of the two STAT isoforms revealed that both STAT1 and STAT3 are likely to play a role in the regulation of basal CTGF mRNA expression in human PTC. Most importantly and in contrast to STAT1 knockdown, siRNA-mediated knockdown of STAT3 prevented OSM-dependent inhibition of basal and TGF-β1-stimulated CTGF mRNA expression. All together, our results suggest that early TGF-β1-stimulated expression of CTGF mRNA in human PTC is at least partially mediated by SMAD3, while OSM's inhibitory effect on basal and TGF-β1-induced CTGF expression is mainly driven by STAT3.

## Acknowledgments

This study was supported by European Union's FP7 Grant "Syskid" (Grant Agreement No. HEALTH-F2-2009-241544) to Gert Mayer and Herbert Schramek.

## References

[1] P. Bornstein, E.H. Sage, Matricellular proteins: extracellular modulators of cell function, Curr. Opin. Cell Biol. 14 (2002) 608–616.
[2] J.I. Jun, L.F. Lau, Taking aim at the extracellular matrix: CCN proteins as emerging therapeutic targets, Nat. Rev. Drug Discovery 10 (2011) 945–963.
[3] A.W. Rachfal, D.R. Brigstock, Structural and functional properties of CCN proteins, Vitam. Horm. 70 (2005) 69–103.
[4] M.K. Phanish, S.K. Winn, M.E.C. Dockrell, Connective tissue growth factor-(CTGF, CCN2)-a marker, mediator and therapeutic target for renal fibrosis, Nephron Exp. Nephrol. 114 (2010) e83–e92.
[5] F.A. van Nieuwenhoven, L.J.N. Jensen, A. Flyvbjerg, R. Goldschmeding, Imbalance of growth factor signalling in diabetic kidney disease: is connective tissue growth factor (CTGF, CCN2) the perfect intervention point?, Nephrol Dial. Transplant. 20 (2005) 6–10.
[6] P. Roestenberg, F.A. van Nieuwenhoven, J.A. Joles, C. Trischberger, P.P. Martens, N. Oliver, J. Aten, J.W. Höppener, R. Goldschmeding, Temporal expression profile and distribution pattern indicate a role of connective tissue growth factor (CTGF/CCN-2) in diabetic nephropathy in mice, Am. J. Physiol. Renal Physiol. 290 (2006) F1344–F1354.
[7] N.A. Wahab, L. Schaefer, B.S. Weston, O. Yiannikouris, A. Wright, A. Babelova, R. Schaefer, R.M. Mason, Glomerular expression of thrombospondin-1, transforming growth factor beta and connective tissue growth factor at different stages of diabetic nephropathy and their interdependent roles in mesangial response to diabetic stimuli, Diabetologia 48 (2005) 2650–2660.
[8] B.L. Riser, M. Denichilo, P. Cortes, C. Baker, J.M. Grondin, J. Yee, R.G. Narins, Regulation of connective tissue growth factor activity in cultured rat mesangial cells and its expression in experimental diabetic glomerulosclerosis, J. Am. Soc. Nephrol. 11 (2000) 25–38.
[9] S.G. Adler, S.W. Kang, S. Feld, D.R. Cha, L. Barba, L. Striker, G. Striker, B.L. Riser, J. LaPage, C.C. Nast, Glomerular mRNAs in human type 1 diabetes: biochemical evidence for microalbuminuria as a manifestation of diabetic nephropathy, Kidney Int. 60 (2001) 2330–2336.
[10] S. Wang, M. Denichilo, C. Brubaker, R. Hirschberg, Connective tissue growth factor in tubulointerstitial injury of diabetic nephropathy, Kidney Int. 60 (2001) 96–105.
[11] M. Guha, Z.G. Xu, D. Tung, L. Lanting, R. Natarajan, Specific down-regulation of connective tissue growth factor attenuates progression of nephropathy in mouse models of type 1 and type 2 diabetes, FASEB J. 21 (2007) 3355–3368.
[12] H. Yokoi, M. Mukoyama, K. Mori, M. Kasahara, T. Suganami, K. Sawai, T. Yoshioka, Y. Saito, Y. Ogawa, T. Kuwabara, A. Sugawara, K. Nakao, Overexpression of connective tissue growth factor in podocytes worsens diabetic nephropathy in mice, Kidney Int. 73 (2008) 446–455.
[13] B.L. Riser, P. Cortes, M. DeNichilo, P.V. Deshmukh, P.S. Chahal, A.K. Mohammed, J. Yee, D. Kahkonen, Urinary CCN2 (CTGF) as a possible predictor of diabetic nephropathy: preliminary report, Kidney Int. 64 (2003) 451–458.
[14] R.E. Gilbert, A. Akdeniz, S. Weitz, W.R. Usinge, C. Molineaux, S.E. Jones, R.G. Langham, G. Jerums, Urinary connective tissue growth factor excretion in patients with type 1 diabetes and nephropathy, Diabetes Care 26 (2003) 2632–2636.
[15] A.A. Jaffa, W.R. Usinger, M.B. McHenry, M.A. Jaffa, S.R. Lipstiz, D. Lackland, M. Lopes-Virella, L.M. Luttrell, P.W. Wilson, Connective tissue growth factor and susceptibility to renal and vascular disease risk in type 1 diabetes, J. Clin. Endocrinol. Metab. 93 (2008) 1893–1900.
[16] T.Q. Nguyen, L. Tarnow, S. Andersen, P. Hovind, H.H. Parving, R. Goldschmeding, F.A. van Nieuwenhoven, Urinary connective tissue growth factor excretion correlates with clinical markers of renal disease in a large population of type 1 diabetic patients with diabetic nephropathy, Diabetes Care 29 (2006) 83–88.
[17] T.Q. Nguyen, L. Tarnow, A. Jorsal, N. Oliver, P. Roestenberg, Y. Ito, H.H. Parving, P. Rossing, F.A. van Nieuwenhoven, R. Goldschmeding, Plasma connective tissue growth factor is an independent predictor of end-stage renal disease and mortality in type 1 diabetic nephropathy, Diabetes Care 31 (2008) 1177–1182.
[18] R.M. Mason, Connective tissue growth factor (CCN2), a pathogenic factor in diabetic nephropathy. What does it do? How does it do it?, J Cell. Commun. Signal 3 (2009) 95–104.
[19] A. Igarashi, H. Okochi, D.M. Bradham, G.R. Grotendorst, Regulation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair, Mol. Biol. Cell 4 (1993) 637–645.
[20] S. Gupta, M.R. Clarkson, J. Duggan, H.R. Brady, Connective tissue growth factor: potential role in glomerulosclerosis and tubulointerstitial fibrosis, Kidney Int. 58 (2000) 1389–1399.
[21] G.R. Grotendorst, Connective tissue growth factor: a mediator of TGF-beta action on fibroblasts, Cytokine Growth Factor Rev. 8 (1997) 171–179.
[22] H. Okada, T. Kikuta, T. Kobayashi, T. Inoue, Y. Kanno, M. Takigawa, T. Sugaya, J.B. Kopp, H.J. Suzuki, Connective tissue growth factor expressed in tubular epithelium plays a pivotal role in renal fibrogenesis, Am. Soc. Nephrol. 16 (2005) 133–143.
[23] R. Sarközi, C. Hauser, S.J. Noppert, A. Kronbichler, M. Pirklbauer, V.M. Haller, J. Grillari, R. Grillari-Voglauer, G. Mayer, H. Schramek, Oncostatin M is a novel inhibitor of TGF-β1-induced matricellular protein expression, Am. J. Physiol. Renal Physiol. 301 (2011) F1014–F1025.
[24] H.Y. Lan, Diverse roles of TGF-β/Smads in renal fibrosis and inflammation, Int. J. Biol. Sci. 7 (2011) 1056–1067.
[25] F.J. López-Hernández, J.M. López-Novoa, Role of TGF-β in chronic kidney disease: an integration of tubular, glomerular and vascular effects, Cell Tissue Res. 347 (2012) 141–154.
[26] K.C. Flanders, Smad3 as a mediator of the fibrotic response, Int. J. Exp. Pathol. 85 (2004) 47–64.
[27] M. Sato, Y. Muragaki, S. Saika, A.B. Roberts, A. Ooshima, Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction, J. Clin. Invest. 112 (2003) 1486–1494.
[28] H.Y. Lan, TGF-β/Smad signaling in diabetic nephropathy, Clin. Exp. Pharmacol. Physiol. (2011 Dec 28), http://dx.doi.org/10.1111/j.1440-1681.2011.05663.x.