DOCA and TGF- β Induce Early Growth Response Gene-1 (Egr-1) Expression

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Key Words

Egr-1 • DOCA-induced fibrosis • Pro-fibrotic genes • Renal fibroblasts

Abstract

Renal fibrosis is characterized by excessive accumulation of extracellular matrix proteins. Recent findings show that transforming growth factor- β (TGF- β) induces a rapid but transient expression of early growth response gene-1 (Egr-1) by skin fibroblasts. The present study aims to define the role of Egr-1 in mineralocorticoid-induced renal fibrosis. Therefore, we transiently transfected immortalized human renal fibroblasts (TK188) with recombinant Egr-1 and analysed the transcription of several pro-fibrotic genes (Coll1A1, Coll1A2, osteopontin, TIMP-1, and CTGF). We also examined Egr-1 expression and the regulation of pro-fibrotic genes in DOCA- (deoxycorticosterone acetate) and TGF- β -treated renal fibroblasts. Finally, we compared Egr-1 gene expression in DOCA/high salt-induced fibrotic kidneys and untreated mice. Egr-1 transfection of TK188 fibroblasts induced the expression of TIMP-1 and osteopontin mRNA. Similar results were obtained after DOCA-activation of TK188 cells. Stimulation of TK188 with TGF- β , but not with DOCA, resulted in elevated

Coll1A1/Coll1A2 and CTGF levels. Co-stimulation with DOCA and TGF- β was followed by enhanced Egr-1, Coll1A1, TIMP-1, and CTGF transcription. In conclusion, both DOCA and TGF- β alone or in combination synergistically induced Egr-1 expression by human renal fibroblasts. DOCA induction of TIMP-1/osteopontin is Egr-1 dependent, whereas TGF- β appears to induce Coll1A1 and CTGF by an Egr-1 independent pathway. *In vivo* analyses revealed significantly higher Egr-1 transcript levels in DOCA/high salt-induced fibrotic kidneys compared to untreated mice. Thus, we show for the first time that Egr-1 might participate in DOCA-induced renal fibrosis.

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Introduction

Glomerulosclerosis and tubulointerstitial fibrosis are final manifestations of several chronic kidney diseases (CKD) [1]. The pathogenesis of renal fibrosis is a progressive process that leads to complete destruction of kidney microstructure and finally to end-stage renal failure. Renal fibrosis is characterized by excessive

synthesis and accumulation of collagen and extracellular matrix proteins [1]. The underlying signaling pathways comprise mesangial and fibroblast activation as well as tubular epithelial-mesenchymal transition [1]. Stimulators of renal fibrosis include the mineralocorticoids aldosterone and deoxycorticosterone acetate (DOCA), hormones that decrease renal salt excretion [2], increase salt appetite [3, 4], increase blood pressure [5], and lead to remodelling of the heart [6, 7]. Activation of the renin-angiotensin-aldosterone system (RAAS) stimulates transforming growth factor- β (TGF- β) expression in the kidney through various mechanisms and upregulates receptors for TGF- β . TGF- β is one of the key factors stimulating synthesis of collagen and other extracellular matrix components [8, 9]. Recent findings show that TGF- β induces a rapid but transient accumulation of Egr-1 transcripts and protein in human skin fibroblasts [10].

Early growth response gene-1 (Egr-1) is a zinc finger transcription factor encoding a 80-82 kDa protein which binds to a GCGGGGGCG consensus binding site to regulate transcription of several genes. Members of the early growth response gene family have been implicated in the activation of cells, inducing proliferation and differentiation, and altering gene expression [11-14]. Egr-1 is thought to play an important role during fibrosis [15-18]. Growth factors such as PDGF-A chain, PDGF-B chain, b-FGF, and TGF- β may also be induced by Egr-1 [10, 19-22]. Han et al demonstrated that high glucose stimulates proliferation and collagen type I synthesis by renal cortical fibroblasts and increases Egr-1 mRNA [23]. There is also crosstalk between the renin-angiotensin-aldosterone system and Egr-1. In a pulmonary fibrosis model, bleomycin upregulated the expression of angiotensin-converting enzyme via the transcription factor Egr-1 [24]. Targeting of Egr-1 expression results in the reduction of interstitial fibrosis in unilateral ureteral obstructed rat kidneys [25]. In immortalized synovial fibroblasts, Egr-1 transfection induces the expression of type I collagen and tissue inhibitor of metalloproteinases 1 (TIMP-1) [15, 26].

To define the contribution of Egr-1 to renal fibrosis following stimulation with mineralocorticoids, we examined the influence of DOCA and TGF- β on Egr-1 expression and the regulation of pro-fibrotic genes.

Materials and Methods

Cell cultures and induction experiments

Immortalized human renal fibroblasts (TK 188, [27]) were cultured in DMEM (Life Technologies GmbH, Karlsruhe,

Germany) supplemented with 10% FCS and 100 U/ml penicillin/streptomycin.

For long-term induction experiments with deoxycorticosterone acetate (DOCA), TK188 cells were cultured in serum-containing medium (10%) in the presence of 10 μ M DOCA for 24 h, 48 h, 3 d, 5 d, and 10 d ($n = 3$ for each time point).

For short-term induction experiments with transforming growth factor- β (TGF- β , 12.5 ng/ml) and DOCA (10 μ M), alone or in combination, TK188 cells were cultured in serum-free medium for 6, 20, 30, and 48 h ($n = 4$ for each time point).

Transient transfection experiments

TK188 cells were plated in 6-well culture dishes at a density of 1×10^5 cells/well. After an incubation period of 16 h, cells were transfected with the circular pcDNA3.1(+)-Neo-Egr-1 plasmid DNA using the FugeneTM 6 transfection reagent. Control cells were transfected with the same vector excluding Egr-1 (mock controls). After a 5 h incubation, the transfection medium was replaced and cells were cultured for an additional 48 h. Gene and protein expression analysis in Egr-1 transfected cells and mock controls were performed 6, 20, 30, and 48 h ($n = 3$ for each time point) after transfection.

Western blot analysis

For western blot analysis, 2×10^6 cells were washed with ice-cold PBS and detached from flasks on ice using a cell scraper. After centrifugation (1400 rpm at 4°C), pelleted cells were dissolved in 60 μ l lysis buffer (1% NP40; 150 mM NaCl; 50 mM Tris/HCL, pH 8 plus protease inhibitors Complete, EDTA-free, Roche, Germany) and incubated overnight at -70°C. Cell debris were removed by centrifugation (AvantiTM 30 Beckman centrifuge, 20627g, 4°C, 20 min.) and protein concentrations in the supernatants were measured by a colorimetric assay (RD DC Protein Assay, Bio Rad, Germany) according to the manufacturer's instructions. After addition of Laemmli sample buffer and denaturation at 95°C, 100 μ g of cellular extract from each sample was separated by SDS-PAGE (10%) and transferred onto nitrocellulose membranes. The membranes were analyzed by immunoblotting using anti-Egr-1 (rabbit polyclonal, Santa Cruz Biotechnology, USA) and GAPDH specific primary antibody (mouse monoclonal [6C5], Abcam, UK) overnight at 4°C. Binding of the primary antibodies was detected with peroxidase conjugated goat-anti rabbit (Jackson ImmunoResearch, Germany) and goat-anti mouse (Santa Cruz Biotechnology, USA) secondary antibodies and visualized by the enhanced chemiluminescence method (Amersham Pharmacia Biotech, Germany).

RNA extraction and quantitative real-time PCR

Total cellular RNA was extracted from transfected and activated cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. First-strand cDNA for the real-time PCR experiments was synthesized by reverse transcription at 42°C in a 20 μ l reaction mixture containing 1 μ g of total RNA. After heating the samples at 95°C for 10 min for denaturation and cooling to 4°C, cDNA was used for PCR amplification. Reactions were diluted to 100 μ l with DEPC-treated water.

Fig. 1. Egr-1 protein expression in TK188 cells transiently transfected with Egr-1. After transient transfection of TK188 cells, lysates were analyzed for protein expression of recombinant Egr-1 (80 KDa) by western blotting. Analyses were performed 6, 20, 30, and 48 hours after transfection. Lane 1: untransfected TK188; lane 2: transfected TK188 with vector only; lane 3: transfected TK188 with Egr-1. GAPDH (36 KDa) shows similar loading of samples.

To quantify mRNA expression, real-time PCR with the LightCycler System (Roche Diagnostics, Mannheim, Germany) was used. Real-time PCR analyses of all genes were performed in a final volume of 20 μ l containing 2 μ l cDNA, 2 μ l primer mix (Search LC, Heidelberg, Germany), 2 μ l DNA Master Sybr Green I mix (Roche Molecular Biochemicals, Mannheim, Germany), and 14 μ l DEPC treated water. Amplification of the target DNA was performed for 35-38 cycles of 95°C for 10s, 68°C for 10s and 72°C for 16s, each with a temperature transition rate of 20°C/s and a secondary target temperature of 58°C with a step size of 0.5°C. Melting curve analysis was performed at 95°C 0s, 58°C 10s, 95°C 0s to allow discrimination between unspecific primer dimers and specific PCR products. The transcript levels of the housekeeping gene GAPDH as well as Coll1A1, Coll1A2, CTGF, osteopontin, and TIMP-1 were determined for each sample using commercial primer kits (Search LC, Heidelberg, Germany). Finally, results were calculated as the ratio of the target to housekeeping gene transcripts.

Mouse model for DOCA/high salt-induced fibrosis

To explore the role of Egr-1 in DOCA/high salt-induced renal fibrosis *in vivo*, we treated wild type mice (129S1/SvImJ background with minor genetic contamination from C57Bl/6 after backcrossing over 2-3 generations, $n = 4$; for details, see [28]) with deoxycorticosterone acetate (DOCA) and high salt (1% NaCl = 171 mM as drinking water) [29]. The drug was applied by repeated implantation of 21-day sustained release DOCA pellets (Innovative Research of America, Sarasota, FL, USA) corresponding to 2.4 mg/day. Pellets were reimplanted every 3 weeks to achieve a treatment period of 12 weeks. For control experiments, untreated mice were used ($n = 4$). After implantation of DOCA pellets, mice were switched to 1% saline in drinking water (= 171 mM NaCl). At the end of DOCA treatment, animals were sacrificed with 100 mg/kg Ketamin and 5 mg/kg Xylazin i.p., and kidneys were harvested by nephrectomy. After snap-freezing, whole kidneys from treated and untreated mice were examined for Egr-1 gene expression. The study was approved by local authorities and were in accordance with German laws of animal welfare.

Statistical analysis

Data are provided as means $\pm$ SEM, n represents the number of independent experiments. All data were tested for significance using ANOVA or t-tests, as appropriate, and only results with $p < 0.05$ were considered statistically significant.

Results

Egr-1 protein expression in TK188 cells transiently transfected with Egr-1 exhibited a strong 80 kDa Egr-1 band by western blotting (Fig. 1). Twenty hours after transfection, high Egr-1 protein levels were detected in transfected TK188 which further increased after 30 and 48 h of incubation in comparison to untreated and mock controls. GAPDH protein signals (36 kDa) were uniform in all analysed samples.

Induction of fibrotic genes in TK188 cells transiently transfected with Egr-1

In Egr-1 transfected TK188 cells, gene expression of pro-fibrotic genes was analysed ($n = 3$) and compared to mock controls. Coll1A1 gene expression was slightly induced in Egr-1 transfected kidney fibroblasts (1.37 ± 0.15 -fold), an effect reaching statistical significance at 30 h after transfection (Fig. 2A). No relevant changes in Coll1A2 were detected in the same cells (Fig. 2B). TIMP-1 transcript levels were significantly increased 20, 30, and 48 h after Egr-1 transfection (1.65 ± 0.13 , 1.9 ± 0.09 , and 2.4 ± 0.27 -fold, respectively; Fig. 2C). Transcript levels of CTGF, a known pro-fibrotic factor, were slightly but significantly increased 30 h after Egr-1 transfection (Fig. 2D). Analysis of osteopontin gene expression revealed significantly increased levels in TK188 cells at 6, 20, and 30 h after transfection (1.22 ± 0.05 , 2.0 ± 0.24 , and 2.74 ± 0.41 -fold increases, respectively). After 48 h, osteopontin transcripts tended to be enhanced up to 3.13-fold on average, however, this value did not reach statistical significance (Fig. 2E).

Egr-1 gene and protein expression in long-term DOCA induction experiments

In long-term DOCA induction experiments ($n = 3$), we found enhanced Egr-1 transcript and protein levels in TK188 cells. In comparison to untreated controls, DOCA-treated TK188 cells showed higher Egr-1 transcript

Fig. 2. Induction of fibrotic genes in TK188 cells transiently transfected with Egr-1. TK188 were transiently transfected with recombinant Egr-1 or mock vector. Gene expression of Coll1A1 (A), Coll1A2 (B), TIMP-1 (C), CTGF (D), and osteopontin (E) were determined after 6, 20, 30, and 48h compared to mock controls by quantitative real-time PCR (n = 3 for each time point). Expression in mock controls was set as one, * p < 0.05.

levels (Fig. 3A). After one day of induction with DOCA, a significant increase (3.68 ± 1.16 -fold) in Egr-1 gene expression was observed. Following stimulation with DOCA for 3 days and 10 days, Egr-1 transcript levels remained significantly enhanced compared to untreated TK188 (2.52 ± 0.67 and 2.06 ± 0.55 -fold, respectively).

Analysis of protein expression revealed similar results at the examined time points (1, 3, 5, and 10 days, Fig. 3B). In contrast to gene analysis results, induced protein levels were observed even after stimulation for 5 days. GAPDH protein signals (36 kDa) were uniform across all analysed samples.

Expression of pro-fibrotic genes in long-term DOCA induction experiments

Coll1A1 and Coll1A2 gene expression was slightly, but not significantly elevated after long-term DOCA induction (n = 3, Fig. 4A-B). Following long-term treatment with DOCA, persistently and significantly higher TIMP-1 transcript levels were measured in DOCA treated vs. untreated TK188 after d1 (1.50 ± 0.17 -fold), d3 (2.07 ± 0.14 -fold), and d10 (2.10 ± 0.38 -fold). Data from d5 showed the same tendency, but did not reach statistical significance (Fig. 4C). CTGF transcripts were significantly higher after DOCA induction from d1

Fig. 3. Egr-1 gene and protein expression in long-term DOCA induction experiments. TK188 were treated with 10 μ M DOCA for 24 hours, 3, 5, and 10 days (n = 3 for each time point, * p < 0.05). Cell lysates were analyzed for Egr-1 gene expression by quantitative real-time PCR (A) and for protein expression by western blotting; GAPDH shows similar loading of samples (B).

Fig. 4. Expression of pro-fibrotic genes in long-term DOCA induction experiments. TK188 gene expression of Coll1A1 (A), Coll1A2 (B), TIMP-1 (C), CTGF (D), and osteopontin (E) were determined after 1, 3, 5, and 10 days (n = 3 for each time point, * p < 0.05) of DOCA treatment by quantitative real-time PCR.

(1.89 $\pm$ 0.29-fold) until d10 (1.52 $\pm$ 0.12-fold) (Fig. 4D). Stimulation of kidney fibroblasts with DOCA for three

and five days led to a significant increase in osteopontin levels (d3: 2.2 $\pm$ 0.23-fold; d5: 1.63 $\pm$ 0.01 fold, Fig. 4E).

Fig. 5. Egr-1 expression in short-term DOCA and/or TGF- β induction experiments. TK188 were activated with 10 μ M DOCA and/or 12.5 ng/ml TGF- β for 6, 20, 30, and 48 hours ($n = 4$ for each time point, $* p < 0.05$), and cells were analyzed for Egr-1 gene expression by quantitative real-time PCR (A). Furthermore, Egr-1 protein expression was detected in cell lysates by western blotting (B). Lane 1: untreated TK188; lane 2: TGF- β ; lane 3: DOCA; lane 4: DOCA/TGF- β induced TK188; Po = positive control. GAPDH expression was used as a loading control.

Egr-1 gene and protein expression in short-term DOCA and/or TGF- β induction experiments

TK188 cells were activated with DOCA and TGF- β alone or in combination then were analyzed for Egr-1 expression in the short-term (6, 20, 30 and 48 h, $n = 4$ for each time point). Maximal Egr-1 expression occurred 30 h after induction (Fig. 5A). After 6 h of induction, no significant changes in Egr-1 transcript levels were detected. However, at 20, 30, and 48 h after treatment, Egr-1 gene expression was significantly increased by TGF- β or DOCA alone, with a clear synergistic effect when both stimulators were applied (20 h: 4.49 ± 0.94 -fold; 30 h: 5.77 ± 1.17 -fold; 48 h: 2.59 ± 0.73 -fold). The combined treatment yielded significantly higher levels after 20 and 30 h compared to treatment with TGF- β alone, but not DOCA alone.

Egr-1 protein expression reflected the gradual induction of Egr-1 mRNA from treatment with TGF- β or DOCA (Fig. 5B). One representative of three replicate experiments is shown in Fig. 5B (upper panel Egr-1, lower panel GAPDH as loading control). Clear differences were observed 20 h after induction, when DOCA proved to be the more potent stimulator of Egr-1 protein. Nearly the same pattern was observed after 30 h of treatment. The combination of both stimulators yielded the highest

Egr-1 protein levels. After 48 h of DOCA or DOCA/TGF- β treatment, enhanced Egr-1 protein levels persisted. GAPDH protein signals (36 kDa) were uniform in all analysed samples.

Expression of pro-fibrotic genes in short-term DOCA and/or TGF- β induction experiments

In short-term experiments, mRNA expression of Coll1A1 was significantly increased by TGF- β at all timepoints (6 h: 1.60 ± 0.01 -fold; 20 h: 2.56 ± 0.12 -fold; 30 h: 3.37 ± 0.18 -fold; 48 h: 2.7 ± 1.08 -fold, $n = 4$ for each time point, Fig. 6A). DOCA alone showed no measurable effect on Coll1A1 transcript levels. When TK188 were activated with both DOCA and TGF- β , nearly the same induction levels were obtained, indicating that DOCA does not augment TGF- β effects on Coll1A1 gene expression (Fig. 6A). TGF- β significantly induced Coll1A2 mRNA levels after 20, 30, and 48 h of stimulation (20 h: 1.59 ± 0.20 -fold; 30 h: 1.59 ± 0.06 -fold; 48 h: 1.92 ± 0.11 -fold; Fig. 6B). TGF- β as well as DOCA alone slightly enhanced TIMP-1 transcript levels after 20, 30, and 48 h of treatment (Fig. 6C). However, the combined DOCA/TGF- β activation led to a further increase in TIMP-1 transcript levels to values significantly higher than those following 20, 30, and 48 h of stimulation with DOCA

Fig. 6. Expression of pro-fibrotic genes in short-term DOCA and/or TGF- β induction experiments. TK188 were activated with 10 μ M DOCA and/or 12.5ng/ml TGF- β for 6, 20, 30, and 48 hours (n = 4 for each time point, * p < 0.05), and cells were analyzed for expression of Coll1A1 (A), Coll1A2 (B), TIMP-1 (C), CTGF (D), and osteopontin (E) by quantitative real-time PCR.

or TGF- β alone (20 h: 2.53 ± 0.22 -fold; 30 h: 3.24 ± 0.22 -fold; 48 h: 3.26 ± 0.06 -fold; Fig. 6C). Expression of CTGF was significantly increased by TGF- β at all timepoints (6 h: 2.12 ± 0.16 -fold; 20 h: 2.33 ± 0.16 -fold; 30 h: 2.34 ± 0.17 -fold; 48 h: 1.83 ± 0.18 -fold; Fig. 6D). DOCA alone induced a measurable increase in CTGF expression only after 48 h (1.63 ± 0.07 -fold). When TK188 were activated with both DOCA and TGF- β , nearly the same CTGF induction levels were obtained, indicating that DOCA does not augment TGF- β effects on CTGF gene expression (Fig. 6D). In contrast to Egr-1 transfected cells, only small and variable effects on osteopontin expression were detected in DOCA $\pm$ TGF- β induced TK188 cells (Fig. 6E).

Egr-1 gene expression in a mouse model for DOCA/high salt-induced fibrosis

Analysis of Egr-1 gene expression in DOCA/high salt-induced fibrotic kidneys by quantitative real-time PCR showed significantly higher Egr-1 transcript levels (2.17 ± 0.4 -fold, Fig. 7) than the respective untreated controls (n = 4 for each group).

Discussion

In our study, we demonstrate for the first time that sustained DOCA treatment induces Egr-1 gene and protein expression in immortalized human renal fibroblasts (TK188) and in a mouse model for DOCA/high salt-in-

Fig. 7. Egr-1 gene expression in a mouse model for DOCA/high salt-induced fibrosis. Analysis of Egr-1 gene expression in fibrotic kidneys of DOCA/high salt treated mice vs. controls was performed by quantitative real-time PCR (n = 4 for each group, * p < 0.05).

duced fibrosis. Not only in short-term, but also in long-term *in vitro* experiments and independent of culture conditions, DOCA alone leads to increased Egr-1 levels (Fig. 3,5). The apparently transient effect of DOCA on Egr-1 expression *in vitro* (Fig. 3A, 5A) could be due to positive/negative feedback loops within the Egr-1 promoter. In FCS-containing medium (long-term induction), the serum-response factor (SRF) is constantly activated and binds to serum-response elements (SRE) within the Egr-1 promoter initiating its transcription [30]. On the other hand, Egr-1 induces the expression of its co-repressor NAB2 in a negative feedback loop [31], providing an explanation for Egr-1 repression in short-term experiments after 48 h and for its repression in long-term experiments after day 5. *In vivo* observations revealed significantly increased Egr-1 levels after long-term DOCA-treatment over 12 weeks (Fig. 7). However, we analysed only this time point, and for this reason, we can only speculate that a wavelike effect similar to that observed *in vitro* occurs *in vivo*.

Fu and co-authors [32] examined Egr-1 mediated gene expression and identified more than 300 genes regulated by Egr-1 overexpression in human endothelial cells (HUVEC). Based on our previous work, we decided to analyse four pro-fibrotic key regulators: type I collagen, TIMP-1, CTGF, and osteopontin. Recently, Chen et al. identified Egr-1 as an intracellular TGF- β target

that is required for maximal stimulation of the α 2-chain of type I collagen (Coll1A2) in human dermal fibroblasts [10]. In that study, TGF- β stimulated both Egr-1 promoter activity and its interaction with the consensus binding site within the Coll1A2 promoter. We demonstrate in our experiments that TGF- β induces Egr-1 gene and protein expression in human renal fibroblasts (TK188). Our results indicate that induction of type I collagen transcript levels is stimulated by TGF- β , but not by DOCA (Fig. 4, 6). In both short- and long-term experiments, DOCA alone did not upregulate Coll1A1 and Coll1A2 expression in TK188 and the combination of both stimulators revealed no further effect. This is consistent with our transient Egr-1 transfection experiments, where only a slight induction of Coll1A1 after 30 h (Fig. 2) was observed in comparison to the stronger induction obtained by TGF- β treatment. These data confirm an additional Egr-1 independent mechanism mediating the stimulation of collagen expression by TGF- β in TK188 fibroblasts.

Both DOCA and TGF- β alone enhanced mRNA levels of TIMP-1, an effect that was significantly enhanced by combined treatment with both stimulators of fibrosis. As shown by Aicher et al., Egr-1 up-regulates TIMP-1 transcription via binding to a GC-rich Egr-1 binding site within the TIMP-1 promoter [26]. Thus, DOCA and TGF- β mediated TIMP-1 activation may be Egr-1 dependent with an additive effect of both activators. Increases in TIMP-1 could explain renal fibrosis *in vivo* by disrupting the collagenolytic balance, even without an increase of collagen production.

TGF- β induces fibroblasts to synthesize components of the extracellular matrix. CTGF, which is induced by TGF- β , is considered a critical downstream mediator of TGF- β in fibroblasts [33]. Qi et al. [34] demonstrated the requirement of TGF- β for the pro-fibrotic response of CTGF, such as up-regulation of fibronectin and type IV collagen gene expression. In the present study, we confirmed previous observations [35] that TGF- β induces CTGF gene expression. However, in TK188 cells, DOCA does not further enhance CTGF transcription, indicating TGF- β dependent activation.

In our experiments, DOCA induced significant osteopontin gene expression in long-term experiments (d3 and d5). However, this effect was not detected in short-term experiments, indicating that the accumulation of Egr-1 protein is required. Osteopontin mediates bone remodelling, tumor invasion, cell survival, and wound repair [36] and is produced in bone by epithelial cells, endothelial cells, and by macrophages [37]. Chronic hydronephrosis in adult rats results in an early and persistent

up-regulation of renal osteopontin, which is associated with the accumulation of interstitial macrophages [38]. Studies in OPN knockout and wild-type mice with hydronephrotic kidneys showed that osteopontin suppresses apoptosis, but promotes interstitial fibrosis by recruitment and activation of interstitial fibroblasts to myofibroblasts [39]. In conclusion, both DOCA and TGF- β , alone or in combination, synergistically induce Egr-1 gene and

protein expression in human renal fibroblasts. DOCA induction of TIMP-1/osteopontin appears to be Egr-1 dependent, whereas TGF- β appears to induce Coll1A1 and CTGF by an Egr-1 independent pathway. *In vivo* data from DOCA-treated mice revealed upregulated Egr-1 levels. Even without increases in collagen, Egr-1 could implement DOCA-induced renal fibrosis by TIMP-1 upregulation and associated disruption of the collagenolytic balance.

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