Gene transfer of Smad7 using electroporation of adenovirus prevents renal fibrosis in post-obstructed kidney

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Background. Unilateral ureteral obstruction (UUO) leads to interstitial fibrosis of the obstructed kidney, and TGF-β is considered to play an important role in this fibrotic process. Smad7 has been recently identified as an antagonist of TGF-β signaling. To investigate whether this novel molecule can be exploited for therapy of renal fibrosis, we determined the effect of exogenous Smad7, introduced by a recombinant adenovirus vector combined with in vivo electroporation (EP), on UUO-induced renal fibrosis in rats.

Methods. A model of UUO was made in SD rats by ligating their left ureters. The next day, the rats were divided into four groups and adenovirus was injected into the extended pelvic space (two groups received AdCMV-LacZ and two groups received AdCMV-Smad7). Then, EP was performed in one group of AdCMV-LacZ-injected rats and one group of AdCMV-Smad7-injected rats. The renal tissues were obtained 3, 5, 10, and 14 days after the UUO operation. We detected the efficiency of transgene by immunoblots of renal cortical and medullary tissues and immunohistochemical studies for Smad7 and FLAG (the FLAG gene was introduced in the AdCMV-Smad7 as a marker). The renal fibrosis was monitored by histological scoring of Masson stainings.

Results. In immunoblotting, both Smad7 and FLAG were clearly detected in the renal medullary tissue of the rats given AdCMV-Smad7 with EP. In contrast, immunoblots of renal cortical tissue did not demonstrate positive bands. In immunohistochemical study, Smad7 was stained in the renal medulla in the rats given AdCMV-Smad7 with EP. In the rats given AdCMV-Smad7 without EP, only a weak signal was detected in renal medullary tissue. The rats given AdCMV-Smad7 with EP demonstrated significantly more suppression of renal fibrosis than rats treated with AdCMV-LacZ. The rats treated with AdCMV-Smad7 without EP did not demonstrate significant suppression of renal fibrosis.

Conclusion. These data indicate that gene transfer of Smad7 prevents UUO-induced renal fibrosis, suggesting that Smad7 may be applicable for the treatment of renal fibrosis. In vivo electroporation of adenovirus may be a powerful tool for gene delivery in renal tissue.

Tubulointerstitial fibrosis is the final common pathway in the process of human progressive renal diseases. Persistent inflammation and repeated injury to the renal interstitium progressively destroy an extensive amount of kidney tissue and, as a consequence, usually produce a considerable decrease in renal function [1–3]. The mechanisms underlying the progression of renal disease to end-stage renal failure are not well understood. The process of tubulointerstitial fibrosis involves many changes in the renal expression of a number of genes associated with cell proliferation, differentiation, and survival. These include marked stimulation of transforming growth factor-β (TGF-β) [4–6]. Thus, the development of a gene transfer technique to regulate TGF-β signaling in renal tubules and interstitial fibroblasts is an appropriate strategy for the treatment of tubulointerstitial fibrosis. A gene transfer technique of this type also may help to clarify our understanding of the pathophysiological role of interstitial fibroblasts and to further investigate the process of renal fibrosis.

In the early 1990s Klahr and colleagues pioneered a model of chronic unilateral ureteral obstruction (UO), a well characterized experimental model of renal injury leading to tubulointerstitial fibrosis [7]. Chronic UO results in the stimulation of the renin-angiotensin system and TGF-β system [5, 6]. TGF-β is up-regulated and plays an important role in the pathogenesis of renal interstitial fibrosis in the UO model [8]. The prolonged overproduction of TGF-β induced by chronic ureteral obstruction is believed to lead to an accumulation of pathological amounts of extracellular matrix in the renal tissue, ultimately resulting in functional deterioration [5, 7].

Recent studies have revealed mechanisms of intracellular transduction of TGF-β (Fig. 1). TGF-β transmits signals from the membrane to nucleus using Smad proteins [9, 10]. The activated TGF-β receptors induce phosphorylation of Smad2 and Smad3. Smad2 and Smad3 make hetero-oligomeric complexes with Smad4. The complexes translocate to the nucleus and regulate transcriptional
responses [9–12]. Recently, Smad7 has been reported as an intracellular antagonist of TGF-β signaling [13]. Smad7 associates with and blocks the activated type I TGF-β receptor and interferes with the activation of Smad2 and Smad3 by preventing their receptor interaction and phosphorylation. These basic reports indicate the therapeutic potential of Smad7 to prevent TGF-β-mediated renal tubulointerstitial fibrosis via the blockade of TGF-β signaling.

To test the hypothesis, we determined the effect of exogenous Smad7 introduced by a recombinant human type 5 adenovirus vector [14, 15] into the kidney in a UUO rat model that required TGF-β for the development of the tissue fibrosis [5, 6]. To investigate whether this novel molecule can be exploited for therapy of renal fibrosis, we determined the effect of exogenous Smad7, introduced by a recombinant adenovirus vector combined with in vivo electroporation (EP), on UUO-induced renal fibrosis in rats.

METHODS
Gene transfer into renal tubules using adenovirus

Several recent reports have been published on in vivo adenoviral gene transfer to the kidney. When the adenovirus was simply injected into the renal artery, very weak and patchy expression of the reporter gene was observed in the cortex [16]. The expression lasted for two weeks. The same group also reported that the retrograde transfection from the renal pelvis caused expression of the reporter gene in the papilla and medulla. The expression lasted for one to two weeks. In contrast, Heikkila et al demonstrated that no tubular expression was observed by perfusing the kidney with the adenovirus [17]. In addition, Zhu et al reported that there was no significant transduction of adenovirus to the tubular cells, while there was transduction to the interstitial vasculature [18]. Further studies will be needed to clarify the reasons for these discrepant results in spite of the use of similar adenoviral vectors. In an investigation of the ex vivo gene transfer into the isolated human kidney under conditions of organ preservation using an adenovirus polylysine DNA complex and pulsatile perfusion for two hours at 4°C, Zeigler et al found that the gene expression was localized to a significant fraction in the proximal tubular epithelial cells. Adenoviruses can transduce many terminally differentiated cell types, including hepatocytes, skeletal muscle, endothelium, glia, neuron, pulmonary epithelium, and synovial cells [19, 20]. For efficient transduction with adenovirus, prolonged exposure of the tissue to adenovirus is believed to be necessary [21]. For this reason, injection of adenovirus into closed pelvic space of the UUO model may be an efficient condition for gene transfer by adenovirus to renal tissue.

Gene transfer by in vivo electroporation

Electroporation has been widely used to introduce DNA into various types of cells in vitro. Recently, gene transfer by electroporation in vivo (DNA injection followed by application of electric fields) has been effective for introducing DNA into mouse muscle [22] and rat liver [23]. Reports have demonstrated that in vivo electroporation provides an efficient approach for muscle- and liver-targeted gene transfer compared to DNA injection without electroporation. Thus, we hypothesized that adenovirus-mediated gene transfer with in vivo electroporation would increase the efficiency of gene transfer by adenovirus to renal tissue.

Expression of Smad7 transgene in rat cortex and medulla of UUO model

Male Sprague-Dawley rats (200–250 g of body weight at the start of the experiments) were used in this study. Under light anesthesia with pentobarbital, complete ureteral obstruction of the left kidney was produced by ligating the ureter with 4-0 silk suture through a small abdominal incision. The next day, the rats were divided into four groups, and adenovirus was injected into the extended pelvic space (two groups injected with AdCMV-LacZ and two groups injected with AdCMV-Smad7). Then, EP (electroporation) was performed in one AdCMV-LacZ-injected group and one AdCMV-Smad7-injected group. Electric pulses were generated with an Electro Square Porator (T820:BTX, San Diego, CA). Pulses were administered to the kidney by a pair of electrode disks (1 cm diameter) rigged on the tips of tweezers (Pinsettes-Type electrode 449-10PRG: Meiwa Syoji, Tokyo, Japan) [23]. Ten electric pulses were admin-
Fig. 2. Expression of Smad7 transgene in rat renal cortex and medulla UUO-treatment. Complete ureteral obstruction of the left kidney was produced by ligating the ureter. The next day, the rats were divided into four groups and adenovirus was injected into the extended pelvic space (two groups received AdCMV-LacZ and two groups received AdCMV-Smad7). Then, EP (electroporation) was performed in one group of AdCMV-LacZ-injected rats and one group of AdCMV-Smad7-injected rats. Expression of Smad7 transgene in rat cortex (C) and medulla (M) was detected by Western blotting using anti-FLAG antibody.

Fig. 3. Quantitative analysis of expression of Smad7 transgene in the renal tissue of rats after UUO-treatment. The rats were divided into four groups and adenovirus was injected into the extended pelvic space (two groups received AdCMV-LacZ and two groups received AdCMV-Smad7). Then, EP (electroporation) was performed in one group of AdCMV-LacZ-injected rats and one group of AdCMV-Smad7-injected rats. Expression of Smad7 transgene was detected by Western blotting using anti-FLAG antibody, and the intensity of the bands detected at days 3 was measured using NIH Image software. Data are mean ± SEM (N = 4).

Suppression of renal fibrosis by exogenous expression of Smad7

The area of interstitium with fibrotic lesions was determined on the sections by staining the collagen fibers in the tubular basement membrane, glomeruli, and interstitial space by Masson’s trichrome method. Renal tissues obtained from control or UUO rats were fixed in formalin overnight and then dehydrated and embedded in paraffin. Thin sections were examined by Masson’s trichrome staining as described previously [24]. The fibrotic areas stained in light blue in the interstitium and tubular basement membrane were picked up on the digital images using a computer-aided manipulator (microscope, Leitz, DM IRB; software, Quantimet 500+, Leica, Tokyo, Japan), and the percentage of the fibrotic area relative to the whole area of the field was calculated (percent fibrotic area) as described previously [24].

In histological examination of renal specimens, renal tubulointerstitial fibrosis was most evident in rat kidney 14 days after UUO-treatment. Treatment of rats with AdCMV-LacZ plus EP did not significantly affect the renal interstitial fibrotic changes induced by UUO treatment. However, when rats were treated with AdCMV-Smad7 plus EP following UUO-treatment, fibrotic lesions were significantly reduced. Quantitative evaluation of the histological findings by scoring of fibrotic lesions showed significant suppression of UUO-induced fibrotic changes in the rats treated with AdCMV-Smad7 (AdCMV-LacZ treatment = 97 ± 12% mean ± SD; AdCMV-Smad7 treatment = 52 ± 11% mean ± SD; N = 4, P < 0.01).

DISCUSSION

In this study, we showed that transient gene transfer and expression of Smad7, introduced by recombinant
human type 5 adenovirus vector with in vivo electroporation into the kidney, prevented UUO-induced renal tubulointerstitial fibrosis in rats. We propose that the expression of exogenous Smad7 might exert its antifibrotic effect in rat kidney via the blockade TGF-β signal transduction.

Although the precise mechanisms of the tubulointerstitial fibrosis process remain unclear, recent studies have demonstrated the importance of TGF-β and the renin-angiotensin system in the pathogenesis of renal tubulointerstitial fibrosis [5, 25]. Shimizu et al. reported that TGF-β1 mRNA increased from day 3 after the UUO procedure, and expression of type IV collagen and type I collagen mRNA also increased in this model [5]. Prolonged overexpression of TGF-β is one of the major pathological factors responsible for the fibrotic changes in renal tissue in the UUO model.

Smad7 has recently been identified [12, 13], and the role of Smad7 as an intracellular inhibitor of TGF-β signaling is well accepted [26]. However, the in vivo biological activity of the inhibitory Smad7 has not yet been defined. Our findings suggested that Smad7 functioned as an antagonist of UUO-induced fibrosis in vivo. Furthermore, recent studies showed that Smad2/Smad3 stimulated transcription of collagen genes [27], and overexpression of Smad7 prevented the transcriptional activation of type I procollagen in human fibroblasts in vitro [27]. Taken together, it is likely that expression of exogenous Smad7 prevented UUO-induced renal tubulointerstitial fibrosis by exerting direct effect on collagen expression in the rat kidney. However, more studies will clearly be needed to define the exact site of Smad7 action. Smad7 might also act via influencing fibroblast chemotaxis, a process important in developing tissue fibrosis in rat kidney [4].

We found that the expression of exogenous Smad7 did not completely inhibit UUO-induced renal tubulointerstitial fibrosis. In light of the finding of Itoh et al [26], who showed that the inhibitory effect of Smad7 on TGF-β signaling was correlated with the level of Smad7 protein expression, one explanation could be that the level of Smad7 transgene expression was not sufficient to completely suppress the TGF-β–induced pathways that lead to collagen production in vivo. In addition, previous studies suggested the role of various cytokines in the development of renal tubulointerstitial fibrosis, including renin-angiotensin systems [5, 7, 25]. Thus, it may be necessary to block the action of other hormonal systems, as well as TGF-β, to achieve complete inhibition of renal interstitial fibrosis.

In this study, we successfully transferred the Smad7 gene and induced its exogenous expression in the kidney by adenoviral vectors with in vivo electroporation in a UUO-model. We demonstrated that in vivo electroporation dramatically increased the efficiency of gene transfer using adenovirus. However, the exact mechanisms of in vivo electroporation of adenovirus are not known. For now we should examine the effects of different electrode voltages, pulse lengths, numbers of pulses, and DNA concentrations in our experimental model. The use of Smad7 as an antifibrotic agent should be investigated as a potentially novel therapy for several kinds of renal fibrotic disorders. However, in its present form, the adenovector-mediated, in vivo electroporation approach has potential problems for application to human renal disorders. In light of this problem, we clearly need to develop strategies to promote the efficiency gene transfer before initiating such an investigation. Furthermore, unfavorable effects that might develop as a result of the long-term elimination of the TGF-β action, such as malignancy, must be investigated carefully.

In conclusion, our data indicate that the gene transfer of Smad7 prevents UUO-induced renal fibrosis, suggesting that Smad7 may be applicable in the treatment of renal fibrosis. In vivo electroporation of adenovirus may be a powerful tool for gene delivery in renal tissue.

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REFERENCES


