Autocrine Secretion of Transforming Growth Factor-β

in Cultured Rat Mesangial Cells

培養ラットメサンギウム細胞は transforming growth factor-β

をオートクリン因子として分泌する

夷　佛　也

学位論文

主論文

論文題目：**Autocrine Secretion of Transforming Growth Factor-β in Cultured Rat Mesangial Cells.**

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氏　　名：要　　伸　也

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a. Introduction

Transforming growth factor-β (TGF-β) is a disulfide-linked homodimer of 25 kilo dalton (kDa) polypeptide [1, 2]. TGF-β is synthesized as an inactive precursor protein which may bind to a 120-130 kDa TGF-β binding protein, and is proteolytically processed into an active mature form [3]. Since its first purification from human platelets [4], TGF-β has been shown to be a multifunctional regulator of cell proliferation and differentiation [1, 2, 5-7]. Inhibition of cell proliferation is generally seen in many cells, in particular, epithelial cells, whereas some mesenchymal cells may be stimulated to proliferate by TGF-β [1, 5, 6]. In addition, TGF-β has been shown to contribute to the accumulation of extracellular matrix proteins by stimulating their synthesis and/or decreasing the activities of some extracellular proteases [7, 8] .

The kidney is one of the targets of TGF-β action. The presence of specific receptors for TGF-β has been demonstrated in both isolated glomeruli and in the individual cell types of the glomerulus, i.e., mesangial, epithelial and endothelial cells [9]. TGF-β has been shown to inhibit mesangial cell growth [9, 10] and to stimulate the synthesis of collagen, fibronectin, laminin and other proteoglycans such as heparan sulfate by mesangial cells as well as glomerular epithelial cells [9, 11].

TGF-β is richest in platelets [4] but is found in almost all normal and transformed cells. Recently isolated glomeruli have been shown to express TGF-β mRNA and produce TGF-β proteins [13, 14]. Furthermore, Border et al. have recently demonstrated in a rat model of experimental

glomerulonephritis that TGF-β mRNA expression and its production in the glomeruli are augmented in parallel to the mesangial matrix accumulation [14], which is prevented by injecting anti-TGF-β neutralizing antibodies [15]. Based on these observations they have suggested that TGF-β may play a crucial role in the pathogenesis of glomerulonephritis, although it is not known what cell types in the glomerulus are responsible for the TGF-β production and what is the mechanism by which the TGF-β secretion is stimulated.

Recently mesangial cells have been shown to have many functions such as contractile properties in response to vasoactive peptides, phagocytosis of macromolecules, and synthesis of matrix proteins, eicosanoids, oxygen radicals, and several autacoids [16]. In addition, they may produce and respond to growth factors such as interleukin-1 [17], platelet-derived growth factor (PDGF) [18], insulin-like growth factor-1 [19], and interleukin-6 [20]. These growth factors may play some important roles for mesangial cell proliferation in vivo as autocrine or paracrine factors in the physiology and pathophysiology affecting the kidney.

The present study was designed, therefore: 1) to elucidate whether mesangial cells express TGF-β mRNA and secrete TGF-β proteins, and if so, 2) to investigate whether the secreted TGF-β is an active or inactive form and 3) what is the mechanism by which the induction of TGF-β is regulated, and finally 4) to examine the role of TGF-β in the mesangial cell growth. The results showed that mesangial cells clearly express TGF-β mRNA, which may be stimulated by serum and 12-O-tetradecanoyl phorbol-13-acetate (TPA), and

that mesangial cells secreted substantial amounts of TGF-β proteins mostly in latent forms. In addition, the secreted TGF-β demonstrated growth-inhibitory action on mesangial cells themselves and thus TGF-β may function as an autocrine factor in mesangial cells.

b. Materials and Methods

a. Mesangial cell culture

Mesangial cells were obtained by culturing glomeruli isolated from kidneys of 150-200 g male Wistar rats by conventional sieving methods [21, 22]. Cells which outgrew in Eagle's minimal essential medium (MEM) containing 17% fetal calf serum (FCS) were identified to be mesangial cells by phase contrast microscopy according to the morphological criteria; i.e., flat polygonal or spindle-shaped cells with multiple processes [22]. All experiments were performed using the cells between the 4th and the 10th passages.

b. Preparation of conditioned media (CM) by mesangial cells and activation of latent TGF-β

The serum-free media conditioned by confluent mesangial cells for 24 hours were collected, centrifuged to remove cell debris, and subjected to bioassay and immunoblot analysis. The activation of latent TGF-β was performed by heating CM at 80 °C for 10 min or by acidifying CM with one-tenth amount of 10 N HCl (final pH 1.5~2) for 30 min at room temperature, followed by neutralization with an equimolar NaOH [23, 24].

c. Bioassay for TGF-β activities

The bioassay for TGF-β was performed based on the observation that TGF-β sensitively inhibits the growth of CCL-64 cells (mink lung epithelial cell line) [25]. In brief, CCL-64 cells were plated into 24-well culture plates in MEM containing 10% FCS. After incubation for 24 hours the medium was discarded and changed to MEM containing 1% FCS. After additional

incubation for 24 hours control or test samples were added, and cells were incubated for another 16-24 hours. Subsequently, 18 kBq/well [^{3}H]-thymidine was pulsed for 3-5 hours and the amounts of [^{3}H]-thymidine incorporated into trichloroacetic acid (TCA)-precipitable materials were measured by a liquid scintillation counter. TGF-β activities were determined as compared with the standard curve which was drawn by using human TGF-β1.

d. Immunoblot analysis of TGF-β proteins

CM were dialyzed against 1 mM acetic acid for 24 hours and lyophilized. The lyophilized products were solubilized by boiling in the presence of sodium dodecyl sulphate (SDS) and 2-mercaptoethanol, size-fractionated by 5-10% SDS-polyacrylamide gel electrophoresis under reducing conditions, and electrophoretically transferred to a polyvinylidene difluoride membrane filter (Immobilon-P, Millipore, Bedford, MA). The filter was incubated in blocking buffer (150 mM NaCl, 20 mM Tris, 3% instant nonfat dry milk, pH 7.5) for 1 hour to block nonspecific antibody binding and treated with specific anti-TGF-β1 antibodies in Tris-NaCl buffer (150 mM NaCl, 20 mM Tris, 0.3% instant nonfat dry milk) for 3 hours at room temperature. After washing four times with Tris-NaCl buffer and 0.05% Tween 20, the filter was incubated in Tris-NaCl buffer with a diluted horseradish peroxidase-anti-rabbit IgG conjugate for 1 hour. The blot was visualized by staining the filter with color reaction substrates (4-chloro-1-naphthol and N, N'-dialkyl-4-aminoaniline derivatives) according to manufacture's instructions (Immunostaining HRP Kit IS-50B, Konica, Tokyo).

e. Northern blot analysis

Total RNA was extracted from cultured mesangial cells by a isothiocyanate/CsCl ultracentrifugation. In some experiments poly $(A)^+$ RNA was prepared using oligo (dT)-cellulose chromatography (type 3, Collaborative Research, Bedford, MA). Aliquots (15-20 μg total RNA or 2 μg poly $(A)^+$ RNA) were size-fractionated in 2.2 M formaldehyde-1% agarose gel and transferred to a nylon membrane by capillary action for 12-18 hours. The blot was prehybridized for 4-6 hours at 42 °C in hybridization solution (5 x SSC, 10 x Denhardt's solution, 0.1% SDS and 50% formamide), and was then hybridized with a nick-translated TGF-β1 cDNA probe at 42 °C in the hybridization solution for 20-24 hours. The filter was washed four times with 2 x SSC, 0.1% SDS at room temperature and once with 0.1 x SSC, 0.1% SDS at 55 °C for 30 min. Autoradiography was performed by a standard method. The filter was rehybridized with $[^{32}P]$-labeled β-actin probe as an internal control after removing the radiolabeled probe in a boiled water for 10 min.

f. $[^3H]$-Thymidine incorporation in mesangial cells and cell number analysis

Mesangial cell growth was analyzed by two methods; $[^3H]$-thymidine incorporation in mesangial cells and cell number analysis. For $[^3H]$-thymidine incorporation study, mesangial cells were plated on 24-well plates, grown to confluence, and growth-arrested by incubation for 48 hours in MEM containing 0.5% FCS. Subsequently, TGF-β1 or anti-TGF-β neutralizing antibodies were added to each well, and the cells were incubated for 48 hours. The amounts of $[^3H]$-thymidine (37 kBq/well) incorporated into TCA-precipitable materials during the last 6 hours or 48 hours of the incubation

time were measured as described above. Additionally, after incubation for 3 or 7 days in MEM containing 10% FCS without or with TGF-β1 at the indicated concentrations or anti-TGF-β neutralizing antibodies (20 μg/ml), mesangial cells were harvested with trypsin-0.05% ethylenediaminetetraaceticacid (EDTA) and then the cell numbers were counted.

g. Materials

TGF-β1 cDNA from rat platelets was kindly provided by Dr. Toshikazu Nakamura (Kyushu University Faculty of Science, Fukuoka). About 500-base pair Bgl I/Eco RI fragment from plasmid clone was used for Northern blot analysis. Human platelet TGF-β1 and human epidermal growth factor (EGF) were purchased from R & D Systems (Minneapolis, MN), and human PDGF from Collaborative Research Incorp. (Bedford, MA). Rabbit anti-TGF-β neutralizing antibody was obtained from R & D Systems, and polyclonal anti-TGF-β antibody for immunoblot analysis was a gift of Dr. Kohei Miyazono (Ludwig Institute for Cancer Research, Uppsala, Sweden). [α-^{32}P] dCTP (111 TBq/mmol) and [^{3}H]-thymidine (185 GBq/mmol) were the products of Amersham Corp. (Arlington Heights, IL). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

c. Results

a. TGF-β mRNA expression in mesangial cells and upregulation by serum and TPA

Mesangial cells growing in MEM containing 17% FCS expressed 2.5 kb mRNA (Fig. 1), the size compatible with TGF-β mRNA [4]. TGF-β mRNA levels did not vary with cell density (data not shown), thus mesangial cells which were grown to confluence were used in the subsequent experiments.

To elucidate the mechanism by which TGF-β mRNA is expressed, we examined which factor(s) could modulate the mRNA level in mesangial cells. Removal of FCS for two days decreased the TGF-β mRNA levels, while β-actin mRNA remained constant (Fig. 1). The TGF-β mRNA level was then stimulated by re-addition of 17% FCS reaching the maximum at 9 hours (Fig. 2A). The effect of FCS to induce TGF-β mRNA levels was dose-dependent between 0.5 and 8 %. The maximum effect was achieved by addition of 8 % FCS (Fig. 2B).

It has been reported in other cell types that TGF-β mRNA can be induced by TPA, one of the phorbol esters [27] and by growth factors such as PDGF, epidermal growth factor (EGF) in normal rat kidney fibroblasts, and by TGF-β itself in rat osteosarcoma cells [28]. Our results show that TPA also markedly increased TGF-β mRNA expression in mesangial cells (Fig. 3). Time course of the induction by TPA showed the stimulation was seen as early as 1 hour and reached the maximum at 6 or 9 hours, which was almost the same as the induction by FCS (Fig. 4). However, upregulations of TGF-β mRNA were not observed by addition of either PDGF, TGF-β, EGF (data not

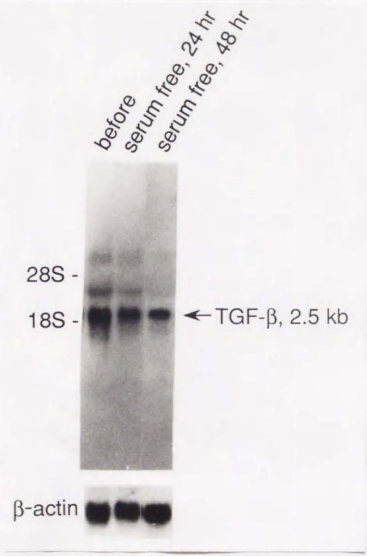

Figure 1. Effect of FCS on TGF-β mRNA expression in cultured mesangial cells. Total RNA was isolated from cultured mesangial cells growing in MEM containing 17% FCS or following incubation in serum-free MEM for 24 hours and for 48 hours. Samples were size-fractionated in 1% agarose gel, transferred to a nylon membrane and the northern blot was hybridized with nick-translated TGF-β1 and β-actin cDNA probes.

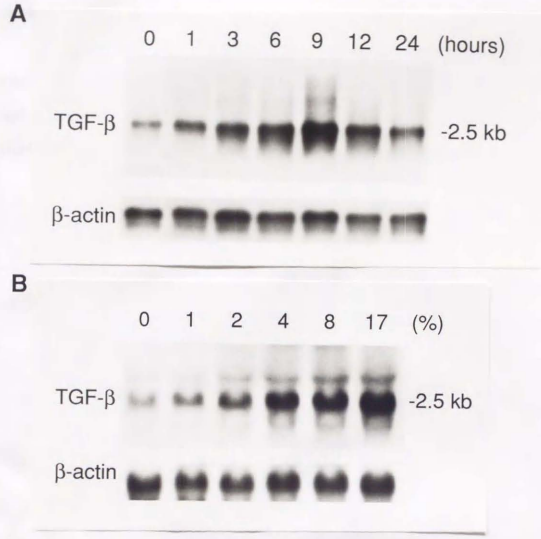

Figure 2. Time course and dose-response relationship of FCS-induced TGF-β mRNA expression.

A. Mesangial cells were serum-starved for 48 hours, and subsequently exposed to MEM containing 17% FCS. At the indicated time point following treatment with FCS, RNA was isolated from the cells and subjected to Northern blot analysis (2 μg poly(A)$^+$ /lane) using nick-translated TGF-β1 and β-actin cDNA.

B. Serum-starved mesangial cells were incubated for 6 hours in MEM containing FCS at the indicated concentrations. At the end of incubation period, total RNA was isolated from the cells and subjected to Northern blot analysis (10 μg/lane) using nick-translated TGF-β1 and β-actin cDNA probes.

shown) in mesangial cells.

Next we examined the effect of H-7, an inhibitor of protein kinase C, on the FCS-induced TGF-β mRNA expression. However, the TGF-β mRNA level did not seem to change by addition of H-7 (data not shown), suggesting that the induction of TGF-β mRNA by FCS was C-kinase independent.

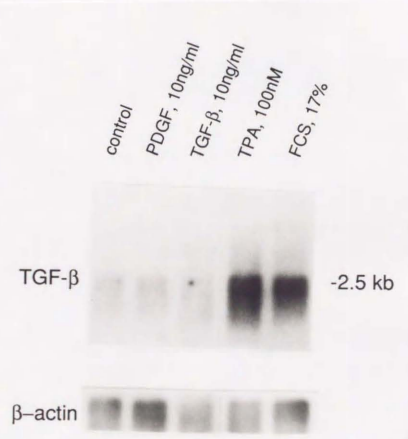

Figure 3. Effects of growth factors and TPA on TGF-β mRNA expression. Mesangial cells were serum-starved for 48 hours in MEM containing 0.5% FCS, and incubated for 6 hours in serum-free MEM with no addition, PDGF (10 ng/ml), TGF-β (10 ng/ml), TPA (100 nM), or in MEM containing 17% FCS. At the end of incubation period, RNA was isolated from the cells and subjected to Northern blot analysis (2 μg poly $(A)^+$ RNA/lane).

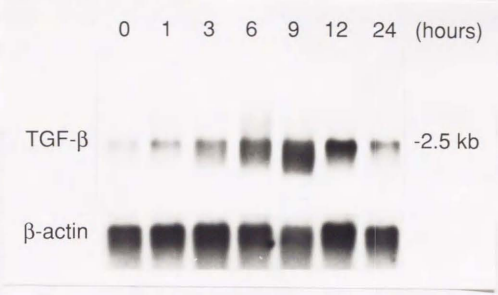

Figure 4. Time course of TPA-induced TGF-β mRNA expression. Mesangial cells were serum-starved for 48 hours, and exposed to TPA (100 nM) in MEM containing 0.5% FCS. At the indicated time point following stimulation with TPA, RNA was isolated from the cells and subjected to Northern blot analysis (2 μg poly(A)$^+$ /lane) using nick-translated TGF-β1 and β-actin cDNA probes.

b. Bioassay for TGF-β activities in the conditioned media by mesangial cells

To examine whether cultured mesangial cells secrete biologically active TGF-β, TGF-β-like activities in the conditioned media were measured by use of CCL-64 cell growth-inhibition assay. As shown in Fig. 5A, TGF-β inhibited [^{3}H]-thymidine incorporation in CCL-64 cells dose-dependently (ED_{50} =10-15 pM). CM with heat or acid treatment inhibited [^{3}H]-thymidine incorporation in CCL-64 cells dose-dependently, although CM without treatment had less effect. Anti-TGF-β neutralizing antibodies completely abrogated the inhibitory effect of CM on [^{3}H]-thymidine incorporation in CCL-64 cells (Fig.

5A, 5B), whereas non-immune IgG had no effect (data not shown), indicating that the inhibitory effect of CM was specific for TGF-β. The specificity of the anti-TGF-β neutralizing antibodies was confirmed by the observation that the

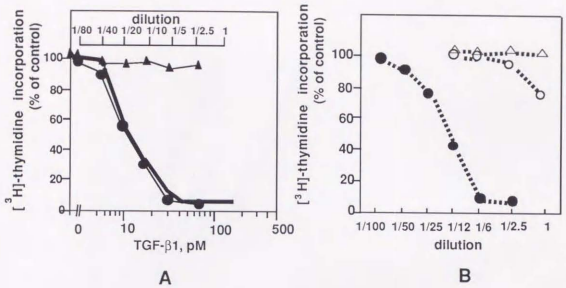

Figure 5. Measurement of TGF-β like activities in the conditioned medium by mesangial cells before and after activation of latent TGF-β using CCL-64 cell growth-inhibition assay.

A. CCL-64 cells plated in 24-well culture plates were incubated for 24 hours in MEM containing 1% FCS, and then control or test samples were added and incubated for another 16-24 h. Subsequently [^{3}H]-thymidine was pulsed for 3-5 hours and [^{3}H]-thymidine incorporation was measured. Samples are : TGF-β at the indicated concentration (solid line), CM with heat or acid activation (●), CM with heat or acid activation in the presence of anti-TGF-β neutralizing antibodies (▲). CM were added at the indicated dilutions.

B. [^{3}H]-thymidine incorporation in CCL-64 cells were measured as described above. Samples are: CM with heat or acid activation (●), CM without heat or acid activation (○), CM without heat or acid activation in the presence of anti-TGF-β neutralizing antibodies (△).

CCL-64 cell growth-inhibitory effect of TGF-β was abrogated by the antibodies, but not by non-immune rabbit IgG (data not shown).

From these bioassay studies, the concentrations of TGF-β in the conditioned media for 24 hours without activation were estimated to be 10~

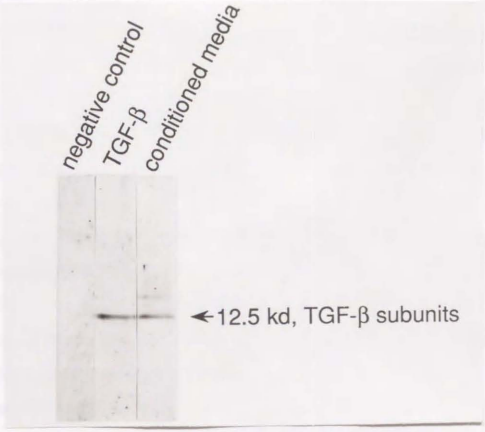

Figure 6. Immunoblot analysis of TGF-β secreted by mesangial cells. Serum-free MEM conditioned by mesangial cells for 24-48 hours was dialyzed against 1 mM acetic acid, lyophilized, and subjected to SDS-polyacrylamide electrophoresis and transfer to a polyvinylidene difluoride filter followed by immunoblot analysis using specific anti-TGF-β1 antibodies as described under "Materials and methods". Left (negative control), serum-free MEM without conditioning; Middle (TGF-β), TGF-β proteins (100 ng); Right (conditioned media), serum-free MEM conditioned by mesangial cells for 24-48 hours.

20 pM (14.8± 3.6 pM: mean±SD) but markedly increased to 150~450 pM (282.5± 119.3 pM) after heat or acid activation. Based on these results, the amounts of TGF-β produced by mesangial cells were calculated to be 22.1± 6.5 ng/10^6 cells/24 hours including inactive and active ones, but 96% of TGF-β in the conditioned media was in inactive or latent forms.

c. Immunoblot analysis for TGF-β proteins

To confirm the existence of TGF-β protein in CM, we further performed immunoblot analysis. The results revealed single 12.5 kDa protein, the size compatible with that of a subunit of mature TGF-β1 peptides (Fig. 6). Together with the results of bioassay shown above, it is indicated that cultured mesangial cells secrete biologically active TGF-β proteins but mostly in inactive or latent forms.

d. Inhibition of [^{3}H]-thymidine incorporation in mesangial cells by TGF-β and stimulation by anti-TGF-β neutralizing antibodies.

TGF-β has been shown to modulate mesangial cell growth [9, 10]. Our results showed that exogenously added TGF-β dose-dependently inhibited [^{3}H]-thymidine incorporation in mesangial cells (Fig. 7). The effect of TGF-β was seen at concentrations higher than 10 pM, reaching the maximum at 40 pM. To determine whether the growth of cultured mesangial cells were under influence of TGF-β endogenously secreted by mesangial cells into the media, we next examined the effect of anti-TGF-β neutralizing antibodies on mesangial cell growth. As shown in Fig. 8, addition of anti-TGF-β neutralizing antibodies significantly augmented [^{3}H]-thymidine incorporation in mesangial cells incubated both in the MEM containing either 0.5 % FCS or

10% FCS, whereas control non-immune IgG had no effect (data not shown).

e. Cell number analysis

To confirm the effects of TGF-β and anti-TGF-β neutralizing antibodies on the mesangial cell proliferation, we performed the cell number analysis. As shown in table 1, significant reduction of cell number was observed as

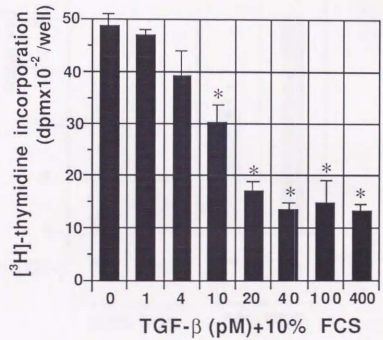

Figure 7. Effect of TGF-β on [^{3}H]-thymidine incorporation in mesangial cells. Mesangial cells plated in 24-well plates were serum-starved for 2 days in MEM containing 0.5% FCS and subsequently the medium was changed to MEM containing 10% FCS with addition of various concentrations of TGF-β, and incubated for 2 days. [^{3}H]-thymidine incorporation in mesangial cells during the last 6 hours was measured. Bars are mean ± SD of triplicate determinations from a representative experiments. Similar results were obtained in three additional experiments.

compared to control values 3 days after addition of TGF-β at 100 pM, and at 10 pM or higher 7 days later. Moreover, proliferative effect was seen at 3 or 7 days after addition of anti-TGF-β neutralizing antibodies. These results were almost consistent with the [^{3}H]-thymidine incorporation data shown above.

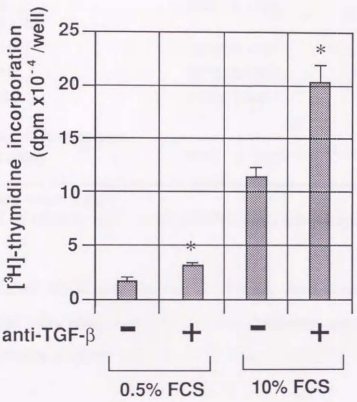

Figure 8. Effect of anti-TGF-β neutralizing antibodies on [^{3}H]-thymidine incorporation in mesangial cells. Mesangial cells were incubated in MEM containing 0.5% or 10% FCS in the absence or the presence of anti-TGF-β neutralizing antibodies (20 μg/ml), and [^{3}H]-thymidine incorporation in mesangial cells for 48 h was measured. - or + indicates the absence or the presence of anti-TGF-β neutralizing antibodies. Bar is the mean ± SD of triplicate determinations from a representative experiment. * significantly different ($p<0.01$, by Student's t -test) from the value in the absence of anti-TGF-β neutralizing antibodies.

Table 1. Effect of TGF-β and anti-TGF-β neutralizing antibodies on mesangial cell number.

samples	incubation time	
	3 days	7days
control	6220 ± 1750	30830 ± 1410
TGF-β (pM)		
10	4970 ± 410	24170 ± 1730 [a]
40	3770 ± 1350	16440 ± 4090 [a]
100	3050 ± 640 [a]	13000 ± 2190 [b]
anti-TGF-β neutralizing antibodies	9970 ± 950 [a]	42000 ± 4300 [a]

Values are mean ± SD
a < 0.05, b <0.005, significantly different from control by Student's t -test

Based on these results it is suggested that TGF-β secreted by mesangial cells indeed exerts a growth-inhibitory action on mesangial cells themselves in culture.

d. Discussion

It has been recently reported that in experimental glomerulonephritis the expression of TGF-β was augmented in parallel to the increased mesangial matrix accumulation [14], which could be inhibited by anti-TGF-β neutralizing antibodies, observations suggesting a crucial role of TGF-β in the pathogenesis of glomerulonephritis, although what cell types in the glomerulus were responsible for the production of TGF-β and the mechanism by which the TGF-β mRNA expression was stimulated remained to be defined [15].

Here we first demonstrated that rat glomerular mesangial cells synthesize and release into the culture media a polypeptide that shares immunological and biological features consistent with TGF-β. Bioassay for TGF-β using CCL-64 cells is a reliable and well established method to quantify TGF-β [15, 25, 26-28]. The observation that the CCL-64 cell growth-inhibitory effects of the conditioned media were totally recovered by anti-TGF-β neutralizing antibodies at all data points clearly showed the activities we measured were TGF-β specific (Fig. 5). Moreover, immunoblot analysis using specific polyclonal anti-TGF-β antibodies directly demonstrated the presence of TGF-β proteins in the conditioned media (Fig. 6).

Our study showed that the amounts of TGF-β produced by mesangial cells were estimated to be 22.1±6.5 ng/10^6 cells/24 hours and the concentrations were 150~450 pM (282.5±119.3 pM) , but 96% of TGF-β in the conditioned media was in inactive forms. The amounts of TGF-β production were nearly comparable or larger than those reported in other cell types [26,

29], suggesting a potential role of mesangial cells as a source of TGF-β in the glomerulus. We demonstrated that mesangial cells also expressed TGF-β binding protein mRNA (data not shown), suggesting that TGF-β is secreted by mesangial cells as an inactive form probably bound to TGF-β binding protein. By our biassay study about 4% of TGF-β in the conditioned media was in active or mature forms. The existence of biologically active TGF-β in the conditioned media was further supported by the observation that addition of anti-TGF-β neutralizing antibodies significantly increased [^{3}H]-thymidine incorporation in mesangial cells. Although TGF-β may be activated from its precursor by heat or acid activation in vitro, the mechanism of the activation in our culture conditions remains to be elucidated. It is possible that some proteases such as plasmin generated by mesangial cells or inflammatory cells might be involved in the activation of TGF-β in vitro [1, 23].

Next we investigated whether there are any factor(s) which could modulate TGF-β mRNA expression by mesangial cells. It has been reported that TGF-β mRNA expression is stimulated by TPA in NIH3T3 cells [30], by EGF or PDGF in normal rat kidney fibroblasts [31], and by TGF-β itself in rat osteosarcoma cells [31]. Our results demonstrated that removal of FCS for two days decreased the TGF-β mRNA level, which was then stimulated by re-addition of 17% FCS reaching the maximum at 9 hours (Fig. 1, 2). TPA also stimulated TGF-β mRNA levels of mesangial cells reaching the maximum at 6 or 9 hours (Fig. 4). PDGF, TGF-β, or EGF (data not shown) had no effect. Induction of TGF-β mRNA by TPA is compatible with the report which demonstrated the presence of TPA responsive element at the 5' region of the

TGF-β gene [32], and suggests that the TGF-β mRNA expression was mediated, at least in part, by protein kinase C. However, induction of TGF-β by FCS was only partially inhibited by H-7, an inhibitor of protein kinase C (data not shown), suggesting that a protein kinase C independent pathway might also be present in the induction of TGF-β.

TGF-β inhibited [^{3}H]-thymidine incorporation in mesangial cells at 10 pM or higher (Fig. 7), the data consistent with the report that Kd of TGF-β receptors of mesangial cells was 5 pM [9, 10]. Addition of anti-TGF-β neutralizing antibodies significantly increased [^{3}H]-thymidine incorporation in mesangial cells incubated both in MEM containing 10% FCS and serum-free MEM, indicating that TGF-β secreted by mesangial cells exerted a growth-inhibitory action on mesangial cells themselves. The results of cell number analysis were almost consistent with the [^{3}H]-thymidine incorporation data, although the reduction of cell number at 3 days after addition of 10 or 40 pM TGF-β was not statistically significant. Our study showed the concentration of active forms of TGF-β in the media was less than 20 pM. However, the concentration might be sufficient to inhibit mesangial cell growth. It is also possible that the local concentration of active TGF-β would be higher than that of the conditioned media estimated above. From these observations we conclude that TGF-β may play a role as an autocrine factor in the regulation of mesangial cell functions. In addition to growth-inhibitory effect, TGF-β secreted by mesangial cells might also play a role on the accumulation of the mesangial matrix components, since less than 10 pM TGF-β could stimulate the the synthesis of collagen by mesangial cells [9].

In summary, mesangial cells produce and secrete substantial amounts of TGF-β but mostly in latent forms, and the secreted TGF-β may regulate mesangial cell growth and differentiation, thus functions as an autocrine factor in mesangial cells. From these observations it is speculated that mesangial cells could be a source of TGF-β in *vivo*, and the secreted TGF-β might play an important role for the accumulation of the mesangial matrix not only in normal glomeruli, but also in the affected glomeruli of glomerulonephritis, while the growth-inhibitory effect of TGF-β may be overcome by the various growth factors and cytokines such as PDGF, interleukin 1, and interleukin 6. Moreover, the TGF-β secreted by mesangial cells may also play a role as an autocrine factor in the pathogenesis of glomerulosclerosis due to diabetic nephropathy or other chronically progressive renal diseases.

e. Summary

TGF-β recently has been shown to modulate mesangial cell growth and to stimulate mesangial matrix synthesis by mesangial cells. Here we examined whether mesangial cells expressed TGF-β mRNA and secreted mature TGF-β, and we investigated the role of TGF-β in mesangial cell growth. Cultured rat mesangial cells expressed 2.5 kb TGF-β mRNA, and removal of FCS for two days decreased the TGF-β mRNA level, which was then stimulated by re-addition of 17% FCS reaching the maximum at 9 hours. TPA, one of the phorbol esters, markedly increased the mRNA level reaching the maximum at 6 or 9 hours. Immunoblot analysis of the conditioned media using specific anti-TGF-β1 antibodies revealed single 12.5 kDa proteins, the size compatible with mature TGF-β subunits. By means of bioassay using CCL-64 cell line, TGF-β production rate by mesangial cells was estimated to be 22.1±6.5 (mean±SD) ng/10^6 cells/24 hours, 96 % of which was in latent forms. Exogenously added TGF-β inhibited mesangial cell growth at 10 pM or higher. Moreover, addition of anti-TGF-β neutralizing antibodies augmented mesangial cell growth, indicating that the secreted TGF-β actually exerted a growth-inhibitory action.

In summary, mesangial cells produce and secrete substantial amounts of TGF-β but mostly in latent forms, and the secreted TGF-β may regulate mesangial cell growth and differentiation. We conclude that TGF-β may function as an autocrine factor in mesangial cells.

f. References

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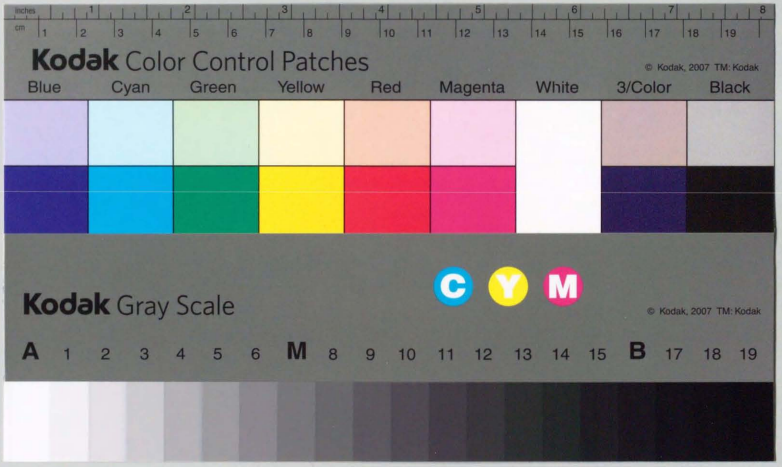
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