TGF-β-like Transcriptional Effects of Thyroglobulin (Tg) in Mouse Mesangial Cells

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Abstract. TGF-β-like activities of proteins unrelated to the cytokine could mimic its actions in fibrosis and cell proliferation. Thyroglobulin (Tg) has been identified as having a TGF-β receptor (TGFβR)-binding activity and is deposited in the glomerulus in certain immune-complex diseases. The aim of the present study is to determine whether Tg can reproduce the transcriptional activity of TGF-β1 in the mouse glomerular mesangial cell (MC), and to examine whether such activity is manifested through TGFβR. Real-time RT-PCR was employed to examine the effects of TGF-β1 and bovine Tg on the expression of three genes (TGF-β1, plasminogen activator inhibitor 1 [PAI-1], and Pax-8) regulated by TGF-β1 in other cell types. In addition, a pentacosapeptide TGF-β1 antagonist, β125 (41-65) was employed to determine whether the transcriptional activity of Tg was mediated through the TGF-β binding site on the TGFβR. A 6h exposure to TGF-β1 resulted in increased TGF-β1 and PAI-1 transcript, and a decrease in Pax-8. Similarly, a 6h exposure to Tg resulted in increases of about 5-fold in TGF-β1 and PAI-1 mRNA and a decrease of 53% in Pax-8. In comparison with other proteins, Tg had the greatest positive effect on TGF-β1 transcript levels. β125 (41-65) significantly reduced the TGF-β1-, but not the Tg-induced changes in TGF-β1, PAI-1 and Pax-8 transcript levels. We conclude from these studies that Tg possesses a TGF-β-mimetic transcriptional activity in the MC that is not mediated by its binding to TGFβR. These results suggest that Tg and other proteins could initiate glomerular injury by reproducing the actions of TGF-β1 in the mesangial cell.

Key words: TGF-β1, thyroglobulin, mesangial cells, Pax-8, PAI-1

(INTRACELLULAR signaling pathways leading to the increased mesangial cell expression of TGF-β are thought to play a central role in glomerular disease [1, 2]. Consequently, signaling mechanisms, including TGF-β autoregulation, leading to the transcriptional up-regulation of this cytokine have received considerable attention [3–5]. However, several studies suggest that TGF-β1-like activity may reside not only in TGF-β isoforms, but also in proteins unrelated to TGF-β such as insulin-like growth factor binding proteins (IGFBPs) and thyroglobulin (Tg) [6, 7]. One means by which specific proteins could mimic TGF-β action is through binding to and activation of TGF-β receptors (TGFβR) via TGF-β active-site motifs [6, 7]. Another is through the activation of downstream signaling pathways also utilized by TGF-β, for instance, the receptor-activated Smads [8].

Tg, the major protein product of the thyroid follicular cell and substrate for thyroid hormone formation does not ordinarily circulate at significant levels [9], however Tg accumulation in the glomerular basement membrane has been demonstrated in several cases of immune complex glomerulonephritis occurring with autoimmune thyroid disease [10–12], and chronic im-
munization of experimental animals with Tg has been shown to cause glomerular lesions [13]. The renal damage in these reports has been ascribed to Tg/anti-Tg immune complexes [12]. However, due to the proposed TGFβR binding ability of Tg, it is also possible that glomerular damage could result from intrinsic activity of Tg following its precipitation as an immune complex. Furthermore, Tg has been shown to possess transcriptional activity unrelated to its conversion to thyroid hormone. In the thyroid, Tg regulates the expression of several genes involved in thyroid hormone biosynthesis, including Tg itself [14–19]. This regulation is achieved primarily by a Tg-mediated decrease in the expression of two thyroid-restricted transcription factors [TTF-1 and TTF-2], and a third transcription factor, Pax-8 [14], a paired box factor with a very restricted tissue expression whose principal functions thus far identified include the regulation of thyroid and kidney development, and of mature thyroid function [20–22]. We found using Northern analysis that Pax-8 is also expressed in mouse mesangial cells in culture and that as in the thyroid follicular cell, transcript levels of Pax-8 are suppressed by Tg [23]. In addition, we found that Tg, but not most other proteins tested, significantly increased mesangial cell proliferation, raising the possibility of direct glomerular actions of Tg in vivo [23].

The reported TGF-β receptor binding activity of Tg suggests that it could mimic the transcriptional effects of TGF-β in the glomerulus, in particular in up-regulation of TGF-β itself [3] and in the increased expression of genes involved in fibrosis, such as plasminogen activator inhibitor (PAI-1) [7]. Such actions of TGF-β on the mesangial cell are thought to play a role in the glomerular scarring occurring in diabetic nephropathy, IgA glomerulonephritis, and several other kidney diseases [24].

In the present report we employ real-time PCR to test the hypothesis that unmodified Tg is capable of replicating the transcriptional effects of TGF-β1 in the mesangial cell, specifically the upregulation of TGF-β1 and PAI-1. We also compare the effects of TGF-β1 and Tg on Pax-8, a transcription factor that has been shown to mediate the effects of TGF-β in the thyroid, and which we have previously identified in the mesangial cell. Finally, using a pentacosapeptide TGF-β antagonist we test the hypothesis that Tg mediates those effects via binding to the putative TGF-β binding site on TGFβR.

**Materials and Methods**

**Materials**

Bovine Tg, bovine lactoferrin, α-1 glycoprotein, albumin, γ-globulins and human TGF-β1 were purchased from Sigma, St. Louis, MO.

**Cells**

Mesangial cells, a gift of the late Dr. L.J. Striker, University of Miami Medical School, Miami, Fla., USA derived from glomeruli microdissected from normal mice (SJL × C57BL/6) were routinely grown in medium (3 : 1 DMEM/F-12) containing 6 mM glucose, 1 mM glutamine, 0.075% NaHCO₃, penicillin (100 U/ml)/streptomycin (100 ug/ml), and 20% fetal bovine serum (FBS) in a humidified incubator at 37° with 5% CO₂ [23, 25]. Experimental treatments were performed on cells plated into culture dishes and equilibrated in the above medium modified to contain 1 : 1 DMEM/F-12 medium and 5% FBS.

**Preparation of TGF-β Peptide Antagonist, β125 [41-65]**

As previously described by Huang et al. [26], a synthetic pentacosapeptide, (β125 [41-65]; ANFCLG-PCPYIWSLDTQYSQVLALY) identical in sequence to residues 41 to 65 of human TGF-β1, and enclosing the putative TGF-β1 active-site motif [27] was synthesized by the Biomedical Instrumentation Center at Uniformed Services University of the Health Sciences, Bethesda, MD. This peptide has been shown previously to inhibit TGF-β1 binding to TGFβR-I, TGFβR-II, TGFβR-III, and TGFβR-V, and to suppress the inhibitory action of TGF-β on the proliferation of cultured mink lung cells [26].

**RT-PCR**

Total RNA was prepared from mesangial cells grown in 6-well plastic culture dishes using Rneasy (Qiagen, Alameda, CA) according to the manufacturer’s protocol. Following reverse transcription using the display-Thermo-RT kit (Display Systems Biotech, Vista, CA), cDNA (2 ul) was used in each PCR reaction in a total volume of 50 ul, with specific primers for the target molecules (mouse Pax-8, mouse TGF-β1, PAI-1, mouse β-actin and glyceraldehyde-3-phosphate
dehydrogenase [G3PDH]).

Primers used for PCR amplification were as follows: 5’ to 3’; mouse Pax-8 (GenBank accession no. X57487) GCCTCCATCAACAGAATCATCCG (sense, positions 532 to 554) and AGGTGTATTGGAAGATGCAGG (antisense, positions 1010–1032); mouse TGF-β1 (GenBank accession no. NM 13177) ATACAGGGCTTTCGATTCAGC (sense) and GTCCAGGCTCCAAATATAGG (antisense); G3PDH (Clontech Laboratories, Palo Alto, CA), ACCACAGTCATGCACTCAC (sense) and TCCACCACCTGTTGCTGTA (antisense); PAI-1 (GenBank accession no. M33960) AGTGATGGAGCCTTGACAGTGG (sense) and GAGAAGGCTCGCTATTGG (antisense); and mouse β-actin (GenBank accession no. M12481) GCATTGACCACTCCG (sense) and ATCCTGTCAGCAATGCCTGG (antisense). The PCR cycling parameters for Pax-8 were as follows: initial denaturation at 94°C for 5 min (one cycle), annealing at 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min (35 cycles), and final extension at 72°C for 7 min (one cycle). For amplification of the other molecules, these parameters were kept constant except for the annealing temperature and the cycle number, which were optimized for each pair of primers. The specific annealing temperatures and cycle numbers were as follows: for TGF-β1, 55°C, 30 cycles; for G3PDH, 60°C, 25 cycles; for β-actin, 60°C, 30 cycles; and for PAI-1, 57°C, 29 cycles.

Amplification of β-actin or G3PDH was used to indicate the initial amount of RNA in each sample. Reaction products were analyzed by electrophoresis on ethidium bromide-stained, 4% agarose gels and were observed with ultraviolet transillumination. Negative images of the gels were analyzed by densitometry using NIH Image software (version 1.6; National Institutes of Health, Bethesda, MD).

Real-time PCR

For real-time PCR, 100 ng of total RNA was used for reverse transcription (RT) and amplification of target cDNA in the ABI Prism 7700 Sequence Detection System (Applied Biosystems). TaqMan® One-Step RT-PCR master mix reagents were purchased from Applied Biosystems. Primers and 5’FAM-labeled probe for mouse TGF-β1, PAI-1, Pax-8 and mouse β-actin were designed from NCBI GenBank sequences using Primer Express, version 1.5 software (Applied Biosystems), and synthesized by Epoch Biosciences, San Diego, CA. Primer and probe sequences for mouse TGF-β1 are as follows: sense, ATCGACATGGAGCTGGTGAAC; antisense, CGAGGCTTTGGTGGACAGGAT; Taqman® probe, AAGCGCATCGACAGGAGCCTCCG. Primers and probe for mouse β-actin are: sense, ACGGCGACATGCACTACTATATG; antisense, CAAAGAAGGCGCTGGAAAGAG, and TaqMan® probe, CAACGGACGTCCGATGCC. PAI-1 primers are: sense, GCCCTGGCCGACCTTGCAAA; antisense, ACCTCGATCTCGACATTTG; and Taqman® probe, CAAGAGCAGCTCTCGTGAGCAGCAG. Pax-8 primers are: sense, CGAGGAGCTCGTTGCAGAGT; antisense, TGCACTTTGGTCCGGATGAT; and Taqman® probe CTGTCCCGATGTCGACAGCTGCAACACTCAACA. For mouse β-actin, reaction mix contained 0.25 U/μL MultiScribe enzyme, 0.4 U/μL RNAse inhibitor, 300 nM sense and antisense primer, 150 nM probe and 10 ng of RNA in a total volume of 50 μL. Reaction mix for TGF-β1, PAI-1 and Pax-8 was identical except for a TaqMan probe concentration of 250 nm. Thermal cycling parameters were as follows: 48°C, 30 min; 95°C, 10 min; and 40 cycles of denaturing at 95°C for 15 sec, and annealing/extension for 1 min at 60°C. Threshold cycle (Ct), the cycle at which emission rises above baseline, was determined for both target gene and β-actin for each sample, and relative quantitation of TGF-β1, PAI-1 or Pax-8 transcript was determined by a modification of the comparative Ct method as described in the ABI Prism 7700 Sequence Detection System Manual, User Bulletin #2 (Applied Biosystems). In this procedure, the β-actin Ct value for each sample (values in triplicate for each treatment) was subtracted from the target gene Ct value for each sample to produce a ΔCt value that was then averaged. Mean ΔCt values for each treatment were compared by ANOVA to determine significant differences, and relative quantitation of transcript levels (compared to control) was determined by evaluating the expression $2^{-\Delta\Delta\text{Ct}}$, where ΔΔCt represents the subtraction of the ΔCt determined for control from the ΔCt determined for each treatment group.

Cell proliferation assay

The effects of Tg and TGF-β1 on cell proliferation were measured by determination of cell DNA content in mesangial cells seeded in 24-well plates and grown for either 4 (TGF-β1) or 5 (Tg) days before assay. Following aspiration of medium, cells were washed twice
with Hank’s balanced salt solution and fixed with 10% trichloroacetic acid (0.5 ml/well) for 10 min. DNA content was determined by the addition of 0.5 ml/well of a working diphenylamine (DPA) reagent [20 volumes of concentrated DPA solution (1 g of DPA, 90 ml of glacial acetic acid and 2 ml of sulfuric acid), 8 vol H₂O, 0.1 volume 1% acetaldehyde] for 24 h and compared with a calf thymus DNA standard (0–25 ug/well). Absorbance was measured at OD 580 nm.

Statistical analyses

In semi-quantitative RT-PCR studies, differences in transcript levels of TGF-β1, PAI-1 and Pax-8 between control and treated groups were determined by normalizing the density of each band for target transcript to the density of the corresponding band for β-actin resulting from PCR of the same cDNA sample. In the studies of long-term treatment of mesangial cells with Tg, the house-keeping gene G3PDH was used in place of β-actin for normalization. These ratios (target transcript/β-actin or target transcript/G3PDH in arbitrary units; 3 per group) were then compared using Instat software (GraphPad Software, Inc., San Diego, CA).

In real-time PCR studies, significant differences between treatment groups were evaluated following the determination of ΔCt and comparison of the mean ΔCt values among treatment groups by ANOVA followed by the Tukey-Kramer test. Subsequent determination of the relative amount of target transcript compared to control was performed as described above and values are graphed showing the +error and –error of the calculated (2^ΔΔCt) value.

Results

TGF-β1 and Tg have similar effects on mesangial cell proliferation and gene transcription

We had earlier observed that Tg induced a significant dose-related increase in mesangial cell proliferation. Since the hypothesized TGF-β-like agonist activity of Tg suggests that its pro-mitotic activity might be due to the activation of TGF-β1 signaling pathways, we evaluated the effects of TGF-β1 on cell growth in our mouse mesangial cell model. We found that like Tg, TGF-β1 produces a dose-dependent increase in DNA content, reflecting a stimulation of cell proliferation (Fig. 1A and B). On a weight basis, TGF-β1 (Fig. 1A) is the more effective mitogen, requiring only 0.11 ng/ml to achieve the 50% elevation in DNA content (proliferation) obtained with 0.11 mg/ml Tg in this study. Real-time PCR and conventional RT-PCR were used to evaluate the dose-related effects of human TGF-β1 on the mouse mesangial cell expression of three mRNA transcripts, TGF-β1, PAI-1 and Pax-8 that have been previously identified as TGF-β1 target genes in either mesangial cells or other cell systems (Fig. 2). Results of real-time PCR (Fig. 2A–C) closely matched those of conventional RT-PCR (Fig. 2A–C, insets) and support the autoinduction of TGF-β1 in this mouse mesangial cell culture, with transcript levels attaining a maximal level of 6.7-fold control value following a 6h treatment with 10 ng/mL TGF-β1 (Fig. 2A). Similarly, a 6h exposure to TGF-β1 significantly increased transcript levels of PAI-1 to a maximal level of approximately 4-fold control (Fig. 2B). On the other hand, Pax-8 mRNA showed a dose-related decrease in response to TGF-β1 (Fig. 2C). Maximal suppression of Pax-8 transcript to approximately 40% of control value was achieved at a dose of 1 ng/mL TGF-β1.

Figure 3 illustrates the dose-related effects of a 6 h and 24 h incubation with bovine Tg on mouse mesan-
gial cell expression of the same three genes shown to be regulated by TGF-β1. A 6h incubation with Tg resulted in a significant increase in TGF-β1 transcript at a concentration of 1 mg/mL Tg, and a maximum level (4.8-fold control value) at a concentration of 10 mg/mL. Continuous exposure of mesangial cells to these doses of Tg over a 24 h period resulted in an overall reduction in the Tg effect on TGF-β1 transcript levels (Fig. 3A). PAI-1 transcript levels exhibited a similar dose-related elevation with increasing Tg, with significant increases above control persisting through 24 h of Tg treatment (Fig. 3B). In contrast to TGF-β1 and PAI-1, transcript levels of Pax-8 were suppressed by increasing concentrations of Tg following both a 6 h and 24 h exposure (Fig. 3C).

The long-term consequences of Tg exposure on TGF-β1 and Pax-8 mRNA levels were tested using conventional RT-PCR and gel electrophoresis (Fig. 4). The results show that the enhancing effect of Tg on TGF-β1 transcript (Fig. 4A), and the inhibitory effect of Tg on Pax-8 transcript (Fig. 4B) are maintained at least a 10-day period in the presence of high levels (10 mg/ml) of Tg.

**A TGF-β1 pentacosapeptide antagonist suppresses TGF-β1- but not Tg-induced changes in gene transcription**

Transcriptional effects of TGF-β1 and Tg on TGF-β1, PAI-1 and Pax-8 were assessed in the presence of β125 (41-65), a pentacosapeptide homologous to residues 41-65 of TGF-β1 which acts as an antagonist to TGF-β receptor binding. As shown in Fig. 5A, an 8.2-fold autoinduction of TGF-β1 transcript by 1 ng/ml TGF-β1 was significantly reduced to less than half by pre-incubation with 50 µM β125 (41-65). PAI-1 up-regulation (Fig. 5C) and Pax-8 suppression (Fig. 5E) by TGF-β1 were also significantly curtailed in the presence of β125 (41-65), suggesting that all three transcriptional activities of TGF-β1 require an active-site motif contained within residues 41-65 of the cytokine. In contrast to TGF-β1, significant Tg-induced changes in TGF-β1, PAI-1, and Pax-8 transcript levels were unaffected by pre-treatment with 50 µM β125 (41-65) (Fig. 5B, D, and F, respectively). These results suggest that the up-regulation of TGF-β1 by Tg is unlikely to occur via direct activation of TGFβR by Tg mimicry of the putative active site of TGF-β1.
Tg, in comparison with other proteins, has the greatest stimulatory effect on TGF-β1 transcript levels.

Figure 6 compares the effects of Tg (1 and 10 mg/ml) with those of lactoferrin, α1-glycoprotein, γ-globulins, and serum albumin, all of bovine origin, on the transcript levels of TGF-β1 after a 6 h incubation. Like Tg, α1-glycoprotein and γ-globulin are both glycosylated proteins, and lactoferrin has been shown to bind to two proteins, an asialoglycoprotein receptor, and megalin, that have also been shown to bind Tg. At 1 mg/ml, only Tg and γ-globulin significantly elevated
TGF-β1 transcript above control in this experiment, and at 10 mg/ml Tg showed a significantly greater ability than γ-globulin to elevate TGF-β1 mRNA levels. None of the other proteins tested increased TGF-β1 transcript levels, and lactoferrin on the contrary suppressed transcript levels at the lower of the two doses.

Fig. 5. Effect of the TGF-β1 antagonist, β₁₂₅ (41-65) on the TGF-β1- and Tg-induced changes in gene expression. Confluent cultures of mouse mesangial cells in 1 : 1 DMEM/F-12 medium containing 5% FBS received either 50 uM β₁₂₅ (41-65) or control medium (containing DMSO vehicle) for 18 h at 37° in a 5% CO₂ incubator. One hour prior to adding TGF-β1 (1 ng/ml) or Tg (1 mg/ml), medium was aspirated and replaced with fresh medium containing either 50 uM β₁₂₅ (41-65) or an equivalent volume of DMSO vehicle (control). TGF-β1 and Tg at twice their final concentrations were then added directly to the antagonist or control media and incubated for an additional 6 h at 37°, 5% CO₂. Wells were run in triplicate, except for Tg and Tg + β₁₂₅ (41-65) which contained 6 wells in each group. Cells were then homogenized for total RNA collection and real-time RT-PCR was performed for TGF-β and normalized to β-actin as described in Methods. Statistical significance was determined by ANOVA on ΔCt values for each group, followed by a Tukey-Kramer test.
Discussion

The results of the present study show that at sufficient concentration Tg mimics the transcriptional effects of TGF-β in the mouse mesangial cell, including the up-regulation of TGF-β itself. These findings extend the concept of gene regulation by Tg outside of the thyroid, where it has already been determined that Tg is an important regulator of several genes via down-regulation of three transcription factors, TTF-1, TTF-2, and Pax-8 [14–19]. The mechanisms underlying the transcriptional activities of Tg are still under investigation, and at least in the thyroid gland have been theorized to result from Tg binding to an asialoglycoprotein receptor (ASGPR), one of several transmembrane proteins known to bind Tg [28]. Of equivalent interest as possible transducers of Tg activity are the TGFβR since Tg has been shown to compete with I125 TGF-β1 binding to cell-surface TGFβR in mink lung cells, including the TGFβR-I and TGFβR-II receptors involved in Smad-signaling [7]. Furthermore, it has been suggested that a Tg/TGFβR interaction could occur through the repeated WCVD motif of Tg which has been theorized to act as the functional equivalent of the putative TGFβ-1 active site, WSILD, in binding TGFβR and altering gene transcription [7].

Our finding that Tg alters the expression of three genes (TGF-β1, PAI-1, and Pax-8) in the mouse mesangial cell in the same dose-related manner as TGF-β1 lends support to the hypothesis that Tg can directly activate TGF-β signaling pathways in the cell. However, the inability of β25 (41-65) to block Tg-induced changes in TGF-β1, PAI-1 and Pax-8 mRNA levels in contrast to its effect on TGF-β-induced changes in expression levels of these three genes suggests that the transcriptional effects of Tg in the mouse mesangial cell are not mediated through Tg interaction with TGF-β binding sites on TGFβR. Nonetheless, it remains possible that Tg action in the mesangial cell is mediated via direct interaction with cell surface TGFβR at a site other than the putative binding site for TGF-β1 [29]. It is also possible that Tg could activate intracellular TGF-β signaling pathways without binding to TGFβR. Tg binds to a number of other cell surface receptors [28], and cross-talk with TGF-β signal pathways downstream of these receptors could lead to Tg mimicry of TGF-β action in the mesangial cell. The nature of these putative Tg-activated pathways have yet to be resolved, but based on the similar transcriptional transcriptional effects of Tg and TGF-β (in both the thyroid [14, 16, 22, 30] and mesangial cell), interaction of Tg with Smad transduction pathways is one likely possibility [31].

In the thyroid, down-regulation of the transcription factor Pax-8 by either Tg or TGF-β is important in the regulation of thyroid function by each of these two proteins [14, 22]. We have shown for the first time that this transcription factor is expressed in cultured mouse mesangial cells [23] and that in these cells too it is down-regulated by both Tg and TGF-β. The genes regulated by Pax-8 in the adult mouse kidney have not yet been identified, but expression of this factor in mesangial cells suggests that Pax-8 suppression by TGF-β represents a signaling pathway for TGF-β (and for TGF-β-mimetic protein) function in the glomerulus.

In regard to the clinical implications for TGF-β-like actions of Tg in mesangial cells, the release of Tg into the circulation occurring with autoimmune thyroid disease results in increased glomerular deposition of Tg.
either as unbound Tg or as part of a Tg-anti-Tg circulating immune complex [10–13]. Tg deposition in the glomerular basement membrane and mesangium could conceivably raise local concentrations of Tg to the levels required to produce elevation of TGF-β1 expression, suppression of Pax-8, and increased proliferation in mesangial cells, thereby augmenting the glomerular damage mediated by immune complexes. Clinical [32] and experimental [13] studies showing a mesangio-proliferative glomerulnephritis occurring with thyroid autoimmunity or thyroglobulin immunization lend support to this hypothesis. Moreover, since autoimmune thyroid disease is often asymptomatic [33], it is possible that elevated Tg antigen and anti-Tg may play an even greater role in renal damage than suggested by correlations of glomerular and autoimmune thyroid disease. In addition, truncated isoform(s) of Tg mRNA detected by us [23] and others [34] in the mouse kidney could reflect the presence of an endogenous renal Tg-like protein with a signaling role similar to thyroidal Tg. Further speculation on the role of renal Tg mRNA in kidney function however will require the identification of a protein product encoded by this sequence.

In a study comparing several proteins in their ability to increase TGF-β1 transcript (Fig. 6), only γ-globulins in addition Tg induced a significant increase in mRNA levels (although still of lesser effect than Tg). These results suggest that other circulating proteins if deposited at high enough concentration in the glomerulus could also elicit TGF-β-like effects on transcription.

In summary, the present study demonstrates a TGF-β-mimetic activity of bovine Tg on transcription in the mouse mesangial cell, in particular an up-regulation of TGF-β1 mRNA. However, we were unable to demonstrate in this report that the transcriptional activities of Tg are due to direct activation of TGFβR. These TGF-β-like activities of Tg in the mesangial cell may therefore involve other mechanisms, including signaling through a non-TGFβR. The present results underscore the importance of identifying intracellular signaling mechanisms whereby Tg and other circulating proteins can mimic the transcriptional activities and damaging effects of TGF-β in the renal glomerulus.

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