TIMP-1 Promotes Age-Related Renal Fibrosis Through Upregulating ICAM-1 in Human TIMP-1 Transgenic Mice

Xueguang Zhang, Xiangmei Chen, Quan Hong, Hongli Lin, Hanyu Zhu, Qingxin Liu, Jianzhong Wang, Yuansheng Xie, Xiyao Shang, Suozhu Shi, Yang Lu, and Zhong Yin

Department of Nephrology, Kidney Center & Key Lab of PLA, Chinese General Hospital of PLA, Beijing, China.

Imbalance of matrix metalloproteinases and tissue inhibitors of metalloproteinases (MMPs/TIMPs) takes part in age-related renal fibrosis; so does molecular inflammation. As several inflammatory mediators including intercellular adhesion molecule-1 (ICAM-1) are substrates of MMPs, we speculated that TIMP-1 might affect ICAM-1 through MMPs and subsequently promote age-related renal fibrosis. Then, we observed changes of kidney in human TIMP-1 transgenic mice and wild-type mice of different ages. It was found that the expressions and activities of gelatinases were downregulated; the expressions of ICAM-1, collagen III, collagen IV, and transforming growth factor (TGF)-β1 were upregulated; and the number of infiltrating macrophages was increased in kidneys of 24-month-old TIMP-1 transgenic mice with high expressions of TIMP-1, compared with wild-type mice. Our results indicated that TIMP-1 could promote age-related renal fibrosis, which was partly attributed to enhancing inflammation through upregulation of ICAM-1.

Morphological changes in kidney with aging are characterized by the development of structural changes, including progressive renal sclerosis with glomerulosclerosis and interstitial fibrosis (1,2). Mechanisms involved in the development of the morphological changes associated with aging have not been exactly elucidated. However, it is well-known that extracellular matrix (ECM) accumulation is the ultimately pathway to cause renal fibrosis (3), including renal fibrosis with aging (4,5).

ECM degradation is catalyzed by matrix metalloproteinases (MMPs), which consist of collagenases, gelatinases, stromelysins, and membrane type MMPs. The activities of MMPs are inhibited by tissue inhibitors of metalloproteinases (TIMPs) through formation of noncovalent 1:1 complexes with the MMPs. Four members of the TIMP family have been characterized so far, which have been designated as TIMP-1, TIMP-2, TIMP-3, and TIMP-4. TIMP-1 is capable of inhibiting the activities of all known MMPs, and plays a key role in maintaining the balance between ECM deposition and degradation in different physiological processes. With downregulation of MMPs and/or upregulation of TIMPs in diseased kidneys, ECM degradation was inhibited, which promoted ECM accumulation (6).

It has been shown that ECM accumulation was always accompanied by the changes of MMPs and TIMPs during renal fibrosis of aged kidneys (1,7,8). The abundance of TIMP-1 was low in normal kidneys, but increased significantly in most experimental models of renal diseases (including aged kidney), and the degree of TIMP-1 increase was associated with the extent of fibrosis (4.9–11).

Besides inhibition of ECM degradation induced by downregulation of MMPs by TIMP-1, whether TIMP-1 could promote renal fibrosis through other pathways become noticeable. It has been found that the components of ECM are not the only substrates of MMPs. Some cytokines could also be degraded by MMPs (12–14), especially intercellular adhesion molecule-1 (ICAM-1), which is a cell-surface protein with five immunoglobulinlike domains and is one of the major molecules involved in promoting leukocytes’ firm attachment to the endothelium, transmigration through its expression on the vascular endothelium, and binding to β-2 leukocyte integrins (15). ICAM-1 has been proven to be upregulated in most experimental models and several human renal diseases, which had certain relationships with progress of the lesions (16–18). Renal injury could be attenuated by inhibition of ICAM-1 through different approaches, such as blocking antibody (19), antisense oligonucleotide (20), and gene knockout to ICAM-1 (21,22). It has also been demonstrated that the upregulation of ICAM-1 was involved in the mechanism that promotes senescence (23,24).

Therefore, in present study, we investigate whether upregulation of TIMP-1 might influence the expression of ICAM-1 and subsequently aggravate fibrosis in kidney with aging. To explore the phenomena, we have successfully constructed homozygote human TIMP-1 transgenic mice, and established the model of kidney with aging by using these transgenic mice.

Methods

Construction of Homozygote Human TIMP-1 Transgenic Mice

Plasmid containing human TIMP-1 (namely, pcDNA3-TIMP-1) had been constructed by Lin and colleagues (25).
Figure 1. Construction of human tissue inhibitor of metalloproteinases (TIMP)-1 transgenic mouse. A, Schema of the transgene construct of hTIMP-1 transgenic mouse. hTIMP-1 cDNA was driven by the cytomegalovirus (CMV) promoter. A bovine growth hormone polyadenylation (BGHPA) sequence was inserted into the transgene to stabilize the expression. The 2.1 Kb construct was released from the expression vector by restriction digestion with BglII and DraIII. B, Integration ratio target genes were tested by polymerase chain reaction (PCR). M: 1 kb DNA marker; 1: positive control; 2: negative control; 3~8: generation F1~F6. C, Integration ratio target genes were tested by Southern blot. 1: Negative control; 2: Positive control; 3~8: Generation F1~F6. D and E, Fluorescence in situ hybridization (FISH) and inverse PCR (IPCR) were respectively used to examine the integrated site and copy of transgene in mouse chromosomes. F, Expression of transgene in tissue of kidney, liver, and spleen of the transgenic mice examined by Northern blot. 1~4: Kidney, liver, spleen, and brain of NIH mice; 5~8: Kidney, liver, spleen, and brain of transgenic mice.
The entire 2.1 kb nucleic acid sequence containing human TIMP-1 cDNA and cytomegalovirus (CMV) promoter was deleted from the plasmid by restriction enzyme digestion with BglII and DraIII and purified by cesium chloride density gradient centrifugation (Figure 1A). The gene was microinjected into the male pronuclei of one-cell NIH hybrid mouse embryos with standard transgenic technology (26). The offspring generated from the founders were mated with other NIH mice. The integration ratios of the target gene were tested by polymerase chain reaction (PCR) (Figure 1B) and Southern-blot hybridization (Figure 1C). When the integration ratio was almost 50%, the positive mice were mated in inbreeding to generate homozygote mice. It was proven by Southern blot that each gene were tested by polymerase chain reaction (PCR) (Figure 1B) and Southern-blot hybridization (Figure 1C). The expressions of transgene in tissue of the 5th generation mice without transgene were considered as wild-type mice in this study.

For construction of the model of kidney with aging, we bred newborns of human TIMP-1 transgenic and wild-type mice for 3, 12, and 24 months (each group, n = 8). Freshly voided morning urine samples of all age groups were collected. After that, all animals were killed by exsanguination under general anesthesia. At the same time, blood samples were collected, then the body weights were recorded. Bilateral kidneys were harvested, immediately weighed, and equally divided into several parts: one part was used for paraffin section, and the others were snap frozen in liquid nitrogen and stored at −80°C for frozen section, zymography, reverse zymography, mRNA, and protein extraction. The piece to be embedded in paraffin section was fixed in 10% buffered formalin. Paraffin sections (3 μm thick) were stained with periodic acid–Schiff (PAS).

### Metabolic Data

Serum and urine creatinine were measured by enzymatic method. Freshly voided morning urine samples were measured by enzyme-linked immunosorbent assay (ELISA) for detecting albumin levels, using a kit from Bethyl Laboratories, Inc. (Houston, TX). The urine albumin excretion rate was expressed as the ratio of albumin to creatinine in the same urine sample.

### Immunofluorescence

Frozen sections (4 μm thick) of kidney were used to detect the presence of F4/80 (a specific marker of mouse macrophages), collagen III, and collagen IV. Endogenous peroxidase was quenched with 4% hydrogen peroxide in methanol. The nonspecific binding was blocked by using 10% goat serum. The primary antibodies incubated on sections overnight at 4°C were 1:200 diluted rat antimouse F4/80 monoclonal antibody (Serotec Ltd., Oxford, U.K.),

![Figure 2. Urine albumin excretion and serum creatinine level.](https://academic.oup.com/biomedgerontology/article-abstract/61/11/1130/63033419)

**Table 1. Bilateral Kidney and Body Weights in Two Genotypes of Mice at Different Ages**

<table>
<thead>
<tr>
<th>Group</th>
<th>Bilateral Kidney Weight (g)</th>
<th>Body Weight (g)</th>
<th>Weight Ratio of Kidney to Body (g/g × 100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>Transgenic</td>
<td>Wild type</td>
</tr>
<tr>
<td>3-month-old</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>22.1 ± 1.4</td>
</tr>
<tr>
<td>12-month-old</td>
<td>0.7 ± 0.2*</td>
<td>0.8 ± 0.1*</td>
<td>29.7 ± 2.8*</td>
</tr>
<tr>
<td>24-month-old</td>
<td>1.0 ± 0.1*</td>
<td>1.2 ± 0.2*</td>
<td>39.2 ± 3.1*</td>
</tr>
</tbody>
</table>

Notes: *p < .05, compared with 3-month-old mice of the same genotype.  
1p < .05, compared with 12-month-old mice of the same genotype.
1:50 diluted rabbit antimouse collagen III and collagen IV polyclonal antibody (Rockland Immunochemicals, Inc., Gilbertsville, PA), respectively. Negative control materials used were normal rat and rabbit immunoglobulin G (IgG; Sigma Chemical Co., St. Louis, MO) in place of the primary antibody. All sections were incubated with 1:300 diluted FITC-conjugated goat antirat IgG or goat antirabbit IgG for 60 minutes at room temperature (Santa Cruz Biotechnology, Santa Cruz, CA), then observed by confocal laser scanning microscope (Bio-Rad Laboratories, Richmond, CA). F4/80 positive cells were counted at a magnification of ×400 and were quantitated as cells per field. The area (expressed as percentage of total tubulointerstitial area) of the glomerulus or tubulointerstitium occupied by collagen IV-positive staining were measured by computerized image analysis using the Image-Pro Plus version 5.0 (Media Cybernetics, Silver Spring, MD). All of these data were expressed as the mean values of 10 fields in each section.

**Gene Expression Analysis**

Reverse transcription–PCR and preparation of the mouse cDNA probe—PCR was performed with a DNA Thermal Cycler (MJ Inc., Watertown, MA). The PCR products were isolated from low-melting-point agarose, and were purified using a GENECLEAN kit (Cambridge Biosystems,
Cambridge, U.K.). Among them, h/mTIMP-1 primer was designed for detecting total TIMP-1 mRNA, including human and mouse, the sequence as follows: upper: 5'-CAG ACC ACC TTA TAC CAG CGT-3', lower: 5'-GAT AAA CAG GGA AAC ACT GTG C-3', but mTIMP-1 primers was designed for detecting the mouse TIMP-1 mRNA. The h/mTIMP-1, hTIMP-1, mTIMP-1, TIMP-2, MMP-2, MMP-9, ICAM-1, and transforming growth factor (TGF)-β1 primers were designed from the sequence of the human and mouse genebank.

Northern blot.—Total RNA was isolated using TRIZOL reagent (Invitrogen, Carlsbad, CA) and quantitated with ultraviolet spectrophotometry. RNA (20 µg) from each sample was loaded into a 1.0% agarose formaldehyde gel and separated by electrophoresis. A photomicrograph of the ethidium bromide–stained gel was obtained to evaluate RNA loading equality, then the RNA was transferred to a hybridization membrane (GeneScreen Plus; New England Nuclear Life Science Products, Boston, MA) and fixed by ultraviolet cross-linking (UV Crosslinker; Hoefer Scientific Instruments, San Francisco, CA). Complementary DNA probes were radiolabeled with 32P-dCTP (3000 Ci/mmol) by using a random primer labeling kit (Stratagene, La Jolla, CA). The membranes were hybridized with the radiolabeled cDNA probes for 24 hours. Autoradiographs were obtained, and the density of each band was quantified using the Alphalmager 2200 analysis program. The densities of the 28 s ribosomal bands in the formaldehyde gels were also quantified, and the results were used to adjust for any RNA-loading inequality.

Western blot.—Tissue pieces were homogenized in 1 mL of lysis buffer (20 mM HEPES-KOH, 250 mM sucrose, 10 mM KCl, 1.5 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, digitonin at 500 µg/mL, 0.1 mM phenylmethylsulphonylfluoride, aprotinin at 2 mg/mL, leupeptin at 10 mg/mL, and pepstatin at 5 mg/mL) with a handheld homogenizer. All samples were spun (10,000 g for 30 minutes at 4°C), and the protein concentration in each lysate was determined spectrophotometrically. The extracted proteins were solubilized by boiling in sodium dodecyl sulfate (SDS) loading buffer, and then subjected to electrophoresis on 10% (for detecting TIMP-1, TIMP-2, MMP-2, MMP-9, and ICAM-1), 7.5% (for detecting collagen III and collagen IV), and 12% (for detecting TGF-β1) polyacrylamide gels (50 µg/lane). The proteins of the gel were transferred to a 0.45 μm-pore nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) by the semidry method (Bio-Rad Laboratories). The nitrocellulose membranes were blocked with 10 mL of Tris-buffered saline (TBS)/T buffer (10 mM Tris-HCl, 0.15 M NaCl, 8 mM sodium azide, 0.05% Tween-20) with 3% bovine serum albumin overnight at 4°C. The membranes were incubated with the primary antibody, rabbit polyclonal anti-MMP-9 (1:200; Santa Cruz Biotechnology), rabbit polyclonal anti-MMP-2 (1:200; Santa

Figure 4. Photomicrographs of F4/80 positive cells in kidney with aging detected by indirect immunofluorescence. Number of positive cells per ×400 field examined by computer image analysis system was shown in Table 2. A–C, 3-month-old, 12-month-old, and 24-month-old wild-type mice, respectively. D–F, 3-month-old, 12-month-old, and 24-month-old transgenic mice, respectively. Magnification, ×400.
Cruz Biotechnology), goat polyclonal anti-TIMP-1 (1:200; Santa Cruz Biotechnology), rabbit polyclonal anti-TIMP-2 (1:500; US Biological, Swampscott, MA), or rat monoclonal anti-ICAM-1 (1:200; Research Diagnostics, Inc., Concord, MA), rat monoclonal anti-TGF-β1 (1:200; Research Diagnostics, Inc.), rabbit polyclonal anticolonagen III and anticolonagen IV antibody (1:5000, Rockland Immunochemicals, Inc.), diluted in the same buffer for 2 hours at room temperature. Thereafter, the nitrocellulose membrane was washed three times with TBST with 3% bovine serum albumin, and incubated for 90 minutes at room temperature with peroxidase-conjugated AffiniPure goat antirabbit IgG, rabbit antigoat IgG, and goat antirat IgG (1:1000; Santa Cruz Biotechnology). After washing with TBST, blots were developed with enhanced chemiluminescence (ECL) reagents (Santa Cruz Biotechnology). Rabbit polyclonal anti-β-actin antibody (1:100; Santa Cruz Biotechnology) was used as the control for each sample.

Gelatin Zymography

Gelatin zymography was performed following the method described by Kenagy and colleagues (27). In brief, kidney protein was extracted by using extraction buffer (0.05 M Tris, 0.01 M CaCl₂, 2.0 M guanidine HCl, 0.2% Triton X-100 [pH 7.5]). The samples were centrifuged for 5 minutes (14,000 g), and the supernatant was aliquoted after protein concentration was measured using the Bradford protein assay (Bio-Rad, Hercules, CA). The aliquoted samples were stored at −70°C until analyzed. Samples (10 mg/well) were loaded without heating onto a 7% acryl/Bis, 10% SDS polyacrylamide gel containing porcine skin gelatin at 1 mg/mL (Sigma Chemical Co.) as substrate. Molecular markers and human MMP-2 and MMP-9 standards (Chemicon International Inc., Temecula, CA) were also loaded into the outer wells. After protein separation by electrophoresis, the gel was rinsed in 2.5% Triton X-100 at room temperature with gentle shaking for 30 minutes. After incubation for 17–20 hours at 37°C in a solution containing 50 mM Tris and 10 mM CaCl₂ (pH 7.8), the gel was stained with 0.002% Coomassie blue, then was photographed, and the optical value of each lytic band was measured using the Alphalmager 2200 analysis program.

Reverse Zymography

Samples were analyzed for the activity of TIMP-1 essentially as described by Oliver and colleagues (28). Briefly, samples prepared as described above were subjected to electrophoresis on reducing 0.1% SDS, 12% polyacrylamide gels containing gelatin at 1 mg/mL, and conditioned medium from MDA-MB-468 cell line, which was added up to 8.0% (vol/vol) as a source of gelatin-degrading enzyme. Electrophoresis was carried out at 4°C after which the gel was washed at room temperature in buffer containing 2.5% Triton X-100, 50 mM Tris-Cl (pH 7.5), and 5 mM CaCl₂ once for 15 minutes, then again overnight. The next day, the gel was rinsed once in water and incubated in 50 mM Tris-Cl (pH 7.5)
and 5 mM CaCl₂ for 24 hours at 37°C, then stained with Coomassie blue. The optical value of each lytic band was measured using the AlphaImager 2200 analysis program.

**Statistical Analysis**

All values are presented as means ± 1 standard deviation. The Kruskal–Wallis test, followed by the Mann–Whitney U test, was used for statistical analysis. Values of $p < .05$ were considered significant.

**RESULTS**

**Kidney and Body Weights**

The body weight, kidney weights, and the ratio of kidney to body weights were increased with aging in each genotype ($p < .05$, Table 1). At the age of 24 months, the kidney weights of transgenic mice were higher than the kidney weights of wild-type mice ($p < .05$, Table 1). With respect to the body weights and the ratio of kidney to body weight at any time points, there was no difference between the two genotypes.

**Metabolic Data**

At the age of 24 months, compared other time points, urine albumin excretion and serum creatinine level were elevated in each genotype ($p < .05$, Figure 2); these elevations were much more significant in transgenic mice than in wild-type mice ($p < .05$, Figure 2).

**Histological and Immunostaining Changes in Kidney With Aging**

No pathological change was found between two genotypes at the age of 3 and 12 months. In 24-month-old mice of two genotypes, glomerular hypertrophy, focal segmental glomerular sclerosis, focal tubular atrophy and interstitial fibrosis, and infiltrated inflammatory cells could be found in kidney (Figure 3).

The number of macrophages infiltrated in kidney and the expressions of collagen III and collagen IV increased with aging in each genotype. The values were greater in 24-month-old transgenic mice than in 24-month-old wild-type mice ($p < .05$, Figures 4–6; Tables 2 and 3), but no difference was found between the two genotypes at the age of 3 and 12 months.

**Gene Expressions**

At the age of 3 months, compared with wild-type mice, h/mTIMP-1 mRNA expression in transgenic mice was much higher, whereas MMP-2 and TIMP-2 mRNA expressions were lower ($p < .05$, Figure 7A, C, and D). No differences in the expressions of mTIMP-1, MMP-9, ICAM-1, and TGF-β1 mRNA were found between the two genotypes ($p > .05$, Figure 7B, E–G).

The expression of h/mTIMP-1 mRNA was significantly increased with aging in the two genotypes, and was higher at the age of 24 months in transgenic than in wild-type mice.
TIMP-1 PROMOTES AGE-RELATED FIBROSIS

Table 2. Quantitative Analysis of the Number of F4/80-Positive Cells in Kidneys of Two Genotypes at Different Ages

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Number of F4/80-Positive Cells (Cells/×400 Field)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type, 3-month-old</td>
<td>8</td>
<td>5.9 ± 1.8</td>
</tr>
<tr>
<td>Wild type, 12-month-old</td>
<td>8</td>
<td>8.5 ± 2.2*</td>
</tr>
<tr>
<td>Wild type, 24-month-old</td>
<td>8</td>
<td>12.7 ± 3.6*</td>
</tr>
<tr>
<td>Transgenic, 3-month-old</td>
<td>8</td>
<td>5.2 ± 2.5</td>
</tr>
<tr>
<td>Transgenic, 12-month-old</td>
<td>8</td>
<td>9.0 ± 2.4*</td>
</tr>
<tr>
<td>Transgenic, 24-month-old</td>
<td>8</td>
<td>18.8 ± 4.4*</td>
</tr>
</tbody>
</table>

Notes: *p < .05, compared with 3-month-old group of the same genotype.  
1p < .05, compared with 12-month-old group of the same genotype.  
*p < .05, compared with wild type at the same age.

(p < .05, Figure 7A). The expressions of mTIMP-1 and TIMP-2 mRNA also increased with aging within the same genotype (p < .05), with no difference between the two genotypes (p > .05, Figure 7B and C). The expressions of MMP-2 and MMP-9 mRNA were decreased with aging in the same genotype (p < .05), and were lower in 24-month-old transgenic mice than in 24-month-old wild-type mice (p < .05, Figure 3D and E). The expressions of ICAM-1 and TGF-β1 mRNA were increased with aging within the same genotype, and were higher in 24-month-old transgenic mice than in wild-type mice (p < .05, Figure 7F and G).

Protein Expressions

TIMP-1 protein expression in 3-month-old transgenic mice was much higher than in wild-type mice, whereas TIMP-2 and MMP-2 protein expressions were lower (p < .05, Figure 8A–C). No differences in the expressions of MMP-9, ICAM-1, collagen III, collagen IV, and TGF-β1 protein were found between the two genotypes (p > .05; Figure 8D and E; Figure 9A–C).

The expression of TIMP-1 protein was significantly increased with aging in the two genotypes, and was higher at the age of 24 months in transgenic than in wild-type mice (p < .05, Figure 8A). The expression of TIMP-2 protein also increased with aging in the same genotype (p < .05), with no difference between the two genotypes (p > .05, Figure 8B). The expressions of MMP-2 and MMP-9 protein were decreased with aging within the same genotype (p < .05), and were lower in 24-month-old transgenic mice than in 24-month-old wild-type mice (p < .05, Figure 8C and D). The expressions of ICAM-1, collagen III, collagen IV, and TGF-β1 protein were increased with aging within the same genotype, which were higher in 24-month-old transgenic mice than in wild-type mice (p < .05; Figure 8E; Figure 9A–C).

Activities of Gelatinases and TIMP-1

In 3-month-old transgenic mice, compared with wild-type mice, the activity of MMP-2 was downregulated, but the activity of MMP-9 was upregulated. The activities of gelatinases were decreased with aging in the same genotype, and were lower in 24-month-old transgenic mice than in 24-month-old wild-type mice (p < .05, Figure 10A–C). The activity of TIMP-1 was higher in wild-type mice than in transgenic mice at each time point, and was higher in 24-month-old transgenic type mice than in 24-month-old wild-type mice (p < .05, Figure 11A and B).

Discussion

The progression of senescence is involved in various complex mechanisms, such as DNA damage (29), oxidative stress (30,31), molecular inflammation (23,24), and disturbance of endocrine (32,33), but the definite pathway is unknown. In our model, it could be observed that certain renal fibrosis and macrophages infiltration occurred in kidney with aging, which implied that ECM accumulation and inflammation had taken part in the progression of senescence in kidney. The imbalance of MMPs/TIMPs is the primary cause of the induction of ECM accumulation, so whether there is an interaction between an imbalance of MMPs/TIMPs and inflammation to accelerate age-related renal fibrosis is the main objective of the present study.

As described above, TIMP-1 played main roles in ECM accumulation. To explore the function of TIMP-1 in vivo further, we successfully constructed human TIMP-1 transgenic mice to elucidate the effect of imbalance between MMPs and TIMPs induced by high TIMP-1 expression in kidney with aging.

The renal expression and activity of TIMP-1 in 3-month-old transgenic mice were much higher than that in the corresponding wild-type mice, whereas no significant histological changes were found between them, implying that TIMP-1 may not be the direct cause of renal fibrosis. Further results showing decreased expressions of TIMP-2 and...
Figure 7. Gene expressions of h/m tissue inhibitors of metalloproteinases (TIMP)-1, mTIMP-1, TIMP-2, matrix metalloproteinase (MMP)-2, MMP-9, intercellular adhesion molecule (ICAM)-1, and transforming growth factor (TGF)-β1 in 3-month-old (Lanes 1 and 2), 12-month-old (Lanes 3 and 4), and 24-month-old (Lanes 5 and 6) groups in wild-type (Lanes 1, 3, and 5) and transgenic mice (Lanes 2, 4, and 6) were detected by Northern blot. Densitometric quantifications of relative band intensities are shown in A–G. Relative band intensity calculated by the intensity ratio of each band to 28S was expressed as mean ± 1 standard deviation. *p < .05, compared with 3-month-old group of the same genotype. †p < .05, compared with 12-month-old group of the same genotype. ‡p < .05, compared with wild type at the same age.
Figure 8. Protein expressions of tissue inhibitor of metalloproteinases (TIMP)-1, TIMP-2, matrix metalloproteinase (MMP)-2, MMP-9, and intercellular adhesion molecule (ICAM)-1 in 3-month-old (Lanes 1 and 2), 12-month-old (Lanes 3 and 4), and 24-month-old (Lanes 5 and 6) groups of wild-type (Lanes 1, 3, and 5) and transgenic mice (Lanes 2, 4, and 6) were detected by Western blot. Densitometric quantifications of relative band intensities are shown in A–E. Relative band intensity calculated by the intensity ratio of each band to β-actin was expressed as mean ± 1 standard deviation. *p < .05, compared with 3-month-old group of the same genotype. **p < .05, compared with 12-month-old group of the same genotype. #p < .05, compared with wild type at the same age.
Figure 9. Protein expressions of collagen III, collagen IV, and transforming growth factor (TGF)-β1 in 3-month-old (Lanes 1 and 2), 12-month-old (Lanes 3 and 4), and 24-month-old (Lanes 5 and 6) groups of wild-type (Lanes 1, 3, and 5) and transgenic mice (Lanes 2, 4, and 6) were detected by Western blot. Densitometric quantifications of relative band intensities are shown. Relative band intensity calculated by the intensity ratio of each band to β-actin was expressed as mean ± 1 standard deviation. *p < .05, compared with 3-month-old group of the same genotype. †p < .05, compared with 12-month-old group of the same genotype. ‡p < .05, compared with wild type at the same age.
MMP-2, decreased activity of MMP-2, and increased activity of MMP-9 in transgenic mice indicated that TIMP-1 effects in vivo might be compensated for to a certain extent by the roles of gelatinases and other TIMPs.

In the present study, it was found that the protein expressions of collagen III and collagen IV in kidneys were increased in the two genotypes at the age of 24 months, which were much higher in the transgenic group than in the wild-type group. In the transgenic group of 24-month-old mice, the expression and activity of TIMP-1 were higher, whereas the activities of gelatinases were lower, than those of the corresponding wild-type mice, suggesting that TIMP-1 promotes age-related renal fibrosis by inhibiting gelatinases (6).

The number of macrophages infiltrated in kidneys at the age of 24 months increased as the expressions of ICAM-1 mRNA and protein were upregulated; these increases were much higher in the transgenic group than in the wild-type group, demonstrating that TIMP-1 might promote renal inflammation through increasing ICAM-1 expression in vivo. Fiore and colleagues (12) found that MMP-9 could degrade ICAM-1 on tumor cells. These results indicated that TIMP-1 may upregulate ICAM-1, at least partially, through inhibiting the degrading activity of MMP-9 in vivo. It is not clear yet whether TIMP-1 could directly increase the transcription of the ICAM-1 gene. Several groups have reported that TIMP-1 could translocate into nuclei of some cells, suggesting that TIMP-1 might act as a transcriptional factor to regulate some biological functions (34,35). However, it is not known whether there is a TIMP-1 binding site in the promoter of the ICAM-1 gene.

TGF-β1, a cytokine to promote ECM accumulation, played an important role in age-related renal fibrosis (5,36). It was found in our study that the expressions of TGF-β1 mRNA and protein were much more significant in the transgenic group than in the wild-type group at the age of 24 months, implying that upregulation of TIMP-1 could elevate the expression of TGF-β1, which might be due to the increase of ICAM-1. The fact that monocytes could synthesize TGF-β1 and stimulate renal mesangial cells and tubular epithelial cells to synthesize and secrete TGF-β1 (37–39), indicates that upregulation of ICAM-1 accelerates the expression of TGF-β1 by facilitating the adhesion and infiltration of monocytes and macrophages (40). Verrecchia and colleagues (41) and Hall and colleagues (42) reported that TGF-β1 could upregulate TIMP-1 through smad (an important family of transcription factors for TGF-β signal transduction) or the activator protein 1 (AP-1) signal transduction pathway. From the above results, we speculated that the age-related renal fibrosis promoted by TIMP-1 might be involved in such mechanisms, including inhibitions of MMP expression and activity, acceleration of inflammation, and upregulation of TGF-β1, all of which could influence each other, forming a vicious cycle.

**Summary**

Our results indicate that TIMP-1 could promote age-related renal fibrosis, which partly attributes to enhancing
inflammation through upregulation of ICAM-1. However, TIMP-1 also showed multiple other biological activities, such as regulating steroid hormone synthesis (43) and angiogenesis (44), altering cellular phenotype (46), and affecting apoptosis of many kinds of cells (45–47). The mechanisms of TIMP-1 promoting age-related renal fibrosis are worth exploring further.

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Address correspondence to Xiangmei Chen, MD, PhD, Department of Nephrology, Kidney Center and Key Lab of PLA, Chinese General Hospital of PLA, Fuxing Road 28, Beijing 100853, P.R. China. E-mail: xmchen@public.bta.net.cn

REFERENCES

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