Expression of Megsin mRNA, a Novel Mesangium-Predominant Gene, in the Renal Tissues of Various Glomerular Diseases

DAISUKE SUZUKI,* TOSHIO MIYATA,* † MASAO NANGAKU, † HIDEO TAKANO,* NOBORU SAOTOME,* MASAO TOYODA,* YASUO MORI,* SHAO-YU ZHANG,* REIKO INAGI,† MASAYUKI ENDOH,* KIYOSHI KUROKAWA,* † and HIDEITO SAKAI*

*Division of Nephrology and Metabolism, and † Institute of Medical Sciences and Department of Internal Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan.

Abstract. Mesangial cells play an important role in maintaining a structure and function of the glomerulus and in the pathogenesis of glomerular diseases. Recently, we discovered a new mesangium-predominant gene termed “megsin.” Megsin is a novel protein that belongs to the serine protease inhibitor (serpin) superfamily. To elucidate the pathophysiologic role of megsin in the kidney, the expression and localization of megsin mRNA in renal tissues of patients with IgA nephropathy (IgA-N), diabetic nephropathy (DN), minimal change nephrotic syndrome (MCNS), membranous nephropathy (MN), and normal human kidney (NHK) was evaluated by in situ hybridization using digoxigenin-labeled oligonucleotide. Individual cells positive for megsin mRNA were observed only in glomeruli in all renal tissues. Their localization coincided with those of mesangial cells. The percentage of positive cells for megsin mRNA in total glomerular cells was significantly greater in IgA-N than in MCNS, MN, and NHK. It was also significantly greater in DN than in MCNS and NHK. In IgA-N, the percentage of megsin mRNA-positive cells was greater in tissues from those with mesangial cell proliferation and slightly mesangial matrix expansion (periodic acid-Schiff-positive area in the total glomerulus area, <30%; cell number in mesangial matrix area, >30; assessed in cross-sections through their vascular poles) than in tissues from those with severe mesangial matrix expansion (periodic acid-Schiff-positive area in total glomerulus area, >30%; cell number in mesangial matrix area, <30). In conclusion, megsin mRNA was predominantly expressed in glomerular mesangial cells in all renal tissues. The expression of megsin mRNA was upregulated in IgA-N and DN, both of which are diseases accompanied with mesangial cell proliferation and/or mesangial matrix expansion. These data suggest a link of megsin expression to the pathogenesis of IgA-N and DN, two major causes of end-stage renal failure.

Mesangial cells play a central role in maintaining a structure and function of the glomerulus. In the pathophysiology of glomerular diseases, the proliferation of mesangial cells and the accumulation of extracellular mesangial matrix are primary events leading to the progression to glomerulosclerosis in patients with a variety of glomerular disorders, such as chronic glomerulonephritis and diabetic nephropathy, two major causes of end-stage renal failure (1).

The determination and characterization of mesangium-specific genes is therefore essential to understand the pathogenesis of glomerular diseases. Recently, we reported the cloning and characterization of a new human mesangium-predominant gene, megsin, which was a new member of the serine protease inhibitor (serpin) superfamily (2,3). The amino acid sequences in the reactive loop site of megsin showed the characteristic feature of functional serpins. Northern blot and reverse-transcribed PCR analyses of various tissues and cells demonstrated that megsin was predominantly expressed in human mesangial cells. We also demonstrated that the expression of megsin mRNA was detected in mesangial cells from normal human kidney and IgA nephropathy (IgA-N) using in situ hybridization. However, the pathophysiologic role of megsin in normal and abnormal kidneys remains unknown.

In this study, to elucidate the role of megsin in the kidneys, the expression and localization of megsin mRNA in renal tissues of patients with IgA nephropathy (IgA-N), diabetic nephropathy (DN), minimal change nephrotic syndrome (MCNS), membranous nephropathy (MN), and normal human kidney (NHK) was evaluated by in situ hybridization using digoxigenin (DIG)-labeled oligonucleotide.

Materials and Methods

Patients

Open renal biopsy tissues were obtained from 17 patients with IgA-N, 10 type 2 diabetic patients with DN, five patients with MCNS, and five patients with MN. The presence of these diseases was

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Correspondence to Dr. Toshio Miyata, Molecular and Cellular Nephrology, Institute of Medical Sciences and Department of Internal Medicine, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa 259-1193, Japan. Phone: +81 463 93 1936; Fax: +81 463 93 1938; E-mail: t-miyata@is.icc.u-tokai.ac.jp

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confirmed by pathologic examination of renal biopsy specimens, such as light microscopy, electron microscopy, and immunofluorescence staining. No patients received steroids or immunosuppressive drugs before renal biopsy.

We also examined control samples obtained from five subjects using uninvolved portions of surgically removed kidneys affected with malignancies. After resecting, the samples were embedded in OCT compound (Tissue Tek, Miles, Elkhart, IN) and stored until use. These patients had no urinary abnormalities, and histopathologic examination of control tissues excluded any glomerular diseases.

To objectively quantify the mesangial matrix accumulation, we measured the total glomerulus area and the periodic acid-Schiff (PAS)-staining positive area in cross-sections of IgA-N through their vascular poles using an automatic image analyzer (4), and counted the cell number in mesangial matrix area. Accordingly, patients with IgA-N were divided into two subgroups: group I, those with mesangial cell proliferation and slightly mesangial matrix expansion (PAS-positive area in the total glomerulus area, >30%; cell number in mesangial matrix area, >30); cell number in mesangial matrix area, ≤30). In NHK, the PAS-positive area in total glomerulus area and the cell number in mesangial matrix area were 17.9 ± 1.6% (mean ± 1 SD) and 17.5 ± 1.7, respectively.

The study was approved by the Human Research Committee of Tokai University School of Medicine, and informed consent to open renal biopsy and in situ hybridization studies was obtained from each patient (5). The following clinical parameters were examined at the time of open renal biopsy: gender, age, serum creatinine, total protein, fasting plasma glucose, urinary protein, and 24-h creatinine clearance (Tables 1 and 2).

### In Situ Hybridization

Nucleotide sequences from 391 to 428 of human megsin cDNA (5’-ACGCTGGACTGAGGAGTGTGTCTTTCTACATATTAAT-3’) was used as a probe (3). One hundred picomoles of the oligonucleotide probe was labeled using a DIG oligonucleotide tailing kit according to the standard protocol (Boehringer 1417-231). Free DIG was removed by ethanol precipitation and dissolved in diethylpyrocarbonate-treated water.

**In situ** hybridization was performed according to the modified technique developed in our laboratory (6,7). Briefly, fresh kidney biopsy tissues were embedded in OCT compound and stored at −70°C until use. Specimens were cut to a thickness of 4 μm, and the sections were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) and were then deproteinized by HCl and digested with proteinase K (Sigma Chemical Co., St. Louis, MO). The specimens were prehybridized in a hybridization buffer, drained, and hybridized overnight with DIG-labeled oligonucleotide probe in the hybridization buffer. After hybridization, DIG-labeled probe was visualized by immunohistochemical staining using mouse monoclonal anti-DIG antibody (Boehringer Mannheim, Mannheim, Germany), horseradish

### Table 1. Clinical parameters in IgA-N, DN, MCNS, MN, and NHK at the time of open renal biopsy

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gender (M/F)</th>
<th>Age (yr)</th>
<th>Serum Creatinine (mg/dl)</th>
<th>Total Protein (g/dl)</th>
<th>Fasting Plasma Glucose (mg/dl)</th>
<th>Urinary Protein (g/dl)</th>
<th>Creatinine Clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA-N (n = 17)</td>
<td>13/4</td>
<td>45.6 ± 5.6</td>
<td>0.96 ± 0.2</td>
<td>6.39 ± 0.29</td>
<td>83.0 ± 6.4</td>
<td>0.86 ± 0.67</td>
<td>82.2 ± 13.0</td>
</tr>
<tr>
<td>DN (n = 10)</td>
<td>7/3</td>
<td>44.6 ± 11.8</td>
<td>0.88 ± 0.22</td>
<td>6.22 ± 0.72</td>
<td>150.0 ± 40.8b</td>
<td>1.03 ± 1.13</td>
<td>96.0 ± 29.1</td>
</tr>
<tr>
<td>MCNS (n = 5)</td>
<td>4/1</td>
<td>23.8 ± 3.6c</td>
<td>0.90 ± 0.20</td>
<td>4.50 ± 0.45d,e</td>
<td>79.0 ± 10.4</td>
<td>5.45 ± 1.21c</td>
<td>87.0 ± 5.6</td>
</tr>
<tr>
<td>MN (n = 5)</td>
<td>4/1</td>
<td>53.0 ± 10.5</td>
<td>0.88 ± 0.05</td>
<td>5.30 ± 0.82f,g</td>
<td>83.0 ± 8.2</td>
<td>2.52 ± 1.65e,hi</td>
<td>92.8 ± 14.2</td>
</tr>
<tr>
<td>NHK (n = 5)</td>
<td>3/2</td>
<td>47.8 ± 3.0</td>
<td>0.84 ± 0.21</td>
<td>6.82 ± 0.58</td>
<td>91.0 ± 9.2</td>
<td>0.04 ± 0.02</td>
<td>95.0 ± 4.6</td>
</tr>
</tbody>
</table>

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### Table 2. Clinical parameters in IgA-N

<table>
<thead>
<tr>
<th>Group</th>
<th>Gender (M/F)</th>
<th>Age (yr)</th>
<th>Serum Creatinine (mg/dl)</th>
<th>Total Protein (g/dl)</th>
<th>Urinary Protein (g/dl)</th>
<th>Creatinine Clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n = 12)</td>
<td>9/3</td>
<td>45.2 ± 5.1</td>
<td>0.91 ± 0.21</td>
<td>6.45 ± 0.32</td>
<td>0.49 ± 0.27</td>
<td>88.2 ± 8.6b</td>
</tr>
<tr>
<td>II (n = 5)</td>
<td>4/1</td>
<td>46.6 ± 7.3</td>
<td>1.08 ± 0.15</td>
<td>6.26 ± 0.18</td>
<td>1.73 ± 0.45c</td>
<td>68.0 ± 10.7</td>
</tr>
</tbody>
</table>

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Note: *IgA-N, IgA nephropathy; DN, diabetic nephropathy; MCNS, minimal change nephrotic syndrome; MN, membranous nephropathy; NHK, normal human kidney.

*b P < 0.001 versus IgA-N, MCNS, MN, NHK.

*c P < 0.001 versus IgA-N, DN, MN, NHK.

*d P < 0.001 versus IgA-N, DN, NHK.

*e P < 0.05 versus MN.

*f P < 0.005 versus DN.

*g P < 0.001 versus NHK.

*h P < 0.005 versus IgA-N.

*i P < 0.05 versus DN.
peroxidase (HRP)-conjugated rabbit anti-mouse IgG antibody (Dako, Glostrup, Denmark), and HRP-conjugated swine anti-rabbit IgG antibody (Dako). Color was developed with diaminobenzidine tetrahydrochloride in 0.05 M Tris-HCl, pH 7.6, and 0.03% H2O2. Sections were briefly counterstained with hematoxylin, rinsed, dehydrated, cleared in xylene, and mounted.

To evaluate the specificity of the signals, two types of control experiments were carried out as described previously (6,7). First, a competitive study was performed by adding 100-fold excess amount of homologous or unrelated, unlabeled oligonucleotides to the hybridization buffer together with the antisense probe. Second, pretreatment with RNase was performed after proteinase K digestion, and thereafter sections were prehybridized and hybridized.

**Immunohistochemistry**

The above-mentioned frozen renal biopsy specimens from nine patients with IgA-N were sectioned at 4 μm and fixed with 4% paraformaldehyde in PBS on ice for 15 min. After washing with PBS, the sections were blocked with 4% skim milk for 60 min at room temperature and subsequently incubated with mouse monoclonal anti-α-smooth muscle actin antibody (Neo Markers, Lab Vision Corp., Fremont, CA) at 4°C overnight. After washing with Tris-saline buffer containing 100 mM NaCl and 150 mM Tris-HCl, pH 7.5, the sections were dehydrated through graded ethanol, incubated in methanol with 0.3% H2O2 at room temperature for 20 min to block endogenous peroxidase, and washed three times with Tris-saline buffer with 0.02% Tween 20. The sections were incubated with rabbit anti-mouse IgG conjugated with peroxidase (Dako). After washings with Tris-saline buffer containing Tween 20, they were developed by reactions with 3,3′-diaminobenzidine solution containing 0.03% H2O2, followed by the counterstaining with hematoxylin. Nonimmune mouse IgG was used as a negative control.

The formalin-fixed renal specimens embedded in paraffin were also stained with mouse monoclonal anti-proliferating cell nuclear antigen (PCNA) antibodies purchased from two companies (Dako and PharMingen, San Diego, CA). Briefly, sections were deparaffinized in xylene and irradiated using a microwave oven. They were preincubated with 10% fetal calf serum and 10% normal goat serum and incubated with mouse monoclonal anti-PCNA antibodies at 4°C overnight. After washing with PBS, sections were incubated with goat anti-mouse IgG conjugated with alkaline phosphatase (Dako) and reacted with alkaline phosphatase and mouse monoclonal anti-alkaline phosphatase (APAAP) (Dako) and then developed with the 4-nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate (Boehringer Mannheim).

**Statistical Analyses**

To quantify the expression of megsin mRNA, all nuclei as well as nuclei with surrounding megsin mRNA-positive cytoplasm in at least 10 randomly selected cross-sections of nonsclerotic glomeruli were

![Figure 1](https://example.com/figure1.png)
blindly enumerated. Results were expressed as the percentage of megsin mRNA-positive cells in total glomerular cells (8). Differences between groups were analyzed for statistical significance using ANOVA or Mann–Whitney U test. A P value, 0.05 denoted a statistically significant difference between groups.

**Results**

*In situ* hybridization identified cells positive for megsin mRNA in glomeruli of NHK (Figure 1A). In contrast, megsin mRNA was undetectable in the tubulointerstitium. *In situ* hybridization followed by PAS staining revealed that megsin mRNA localization coincided with those of mesangial cells in NHK (Figure 1B). The specificity of the megsin mRNA signal detected by *in situ* hybridization study was confirmed by two control studies. In a competitive experiment, the signals disappeared when a 100-fold excess amount of unlabeled homologous oligonucleotide was added to the standard hybridization mixture containing labeled probe (Figure 1C). Furthermore, the pretreatment of tissue with RNase before hybridization diminished the signals (Figure 1D).

Figure 2 shows the expression of megsin mRNA in IgA nephropathy (IgA-N) (A and B), diabetic nephropathy (C), membranous nephropathy (D), minimal change nephrotic syndrome (E), and normal human kidney (NHK) (F). Megsin mRNA were detected in glomeruli of all renal diseases, but were undetectable in the tubulointerstitium. Magnification: ×80.

![Figure 2. Detection of megsin mRNA in renal diseases. The expression of megsin mRNA in tissues from group I (A) and group II (B) IgA nephropathy (IgA-N), diabetic nephropathy (C), membranous nephropathy (D), minimal change nephrotic syndrome (E), and normal human kidney (NHK) (F). Megsin mRNA were detected in glomeruli of all renal diseases, but were undetectable in the tubulointerstitium. Magnification: ×80.](image-url)
megsin mRNA was greater in renal tissues from group I IgA-N than in MCNS, MN, and NHK. The number of positive cells for mesangial matrix accumulation and for PCNA was significantly greater in IgA-N than in MCNS, MN, and NHK. It was also significantly greater in DN than in MCNS and NHK. No significant differences in the percentage of megsin mRNA-positive cells was seen among MCNS, MN, and NHK.

Tables 3 and 4 show the results of megsin mRNA expression in various renal diseases, expressed as the percentage of positive cells for megsin mRNA in total glomerular cells. The percentage of megsin mRNA-positive cells was significantly greater in IgA-N than in MCNS, MN, and NHK. It was also significantly greater in DN than in MCNS and NHK. No significant differences in the percentage of megsin mRNA-positive cells was seen among MCNS, MN, and NHK.

We demonstrated previously that the megsin mRNA-positive cells in the proliferative stage of IgA-N was significantly greater than those in the sclerotic stage of IgA-N (3). Patients with IgA-N were divided into two subgroups according to the percentage of sclerotic glomeruli of total glomeruli (<30% versus >30%). In the present study, we objectively quantified the mesangial matrix accumulation and counted the cell number in mesangial matrix area. The number of positive cells for megsin mRNA was greater in renal tissues from group I IgA-N with mesangial cell proliferation and slightly mesangial matrix expansion (PAS-proliferation area in total glomerulus area, <30%; cell number in mesangial matrix area, >30%) than in those from group II IgA-N with severe mesangial matrix expansion (PAS-positive area in total glomerulus area, >30%; cell number in mesangial matrix area, <30) (Table 4).

To investigate whether megsin is expressed in “activated” mesangial cells, we examined in serial renal tissue sections from IgA-N the distributions of megsin mRNA and α-smooth muscle cell actin, a marker for activated mesangial cells (9–11). Figure 3, A and B, shows the representative pictures of megsin mRNA and α-smooth muscle cell actin staining. Both megsin mRNA and α-smooth muscle cell actin expression were upregulated in the expanded mesangial area, although their localizations were not completely identical. Furthermore, to know whether megsin expression is linked to the mesangial proliferation, we examined the distributions of megsin mRNA and PCNA, which is taken as a marker for cell proliferation (12–14). Anti-PCNA antibodies stained positive in some nuclei of NHK (Figure 3C) and IgA-N (Figure 3D) tissues fixed in formalin and embedded in paraffin. Like the megsin mRNA expression, the percentage of positive cells for PCNA was significantly greater in IgA-N than NHK.

Discussion

Our in situ hybridization studies of human kidneys localized megsin expression exclusively in glomeruli. Localization of megsin-producing cells was consistent with mesangial cells and did not differ among kidneys of healthy subjects and various renal disorders. These findings confirmed and extended our previous report of the predominant expression of megsin in mesangial cells (2,3).

Quantitative analysis of the in situ hybridization studies demonstrated that the expression of megsin was upregulated in IgA-N and DN. Furthermore, the expression of megsin mRNA was significantly upregulated in IgA-N of the proliferative stage compared with IgA-N of the late, sclerotic stage, showing different megsin expression dependent on disease stage. IgA-N, the most common primary glomerulonephritis, is characterized by mesangial cell proliferation and mesangial matrix expansion (15–17), while the critical lesion of DN that ultimately leads to renal failure is expansion of the mesangial matrix (18–20). In contrast, in both MN and MCNS glomerular epithelial cells are specifically injured. Our quantitative in situ hybridization studies clearly demonstrated that megsin was upregulated in diseases of mesangial injury. In agreement with this, rat megsin mRNA was upregulated in the glomeruli of rat anti-Thy1 nephritis model when mesangial cell proliferation and excessive production of mesangial matrix were at their peaks (at day 8) (M. Nangaku, T. Miyata, unpublished observation).

For the precise localization of megsin protein expression in glomeruli, immunohistochemical studies using anti-human megsin antibody are required. We raised anti-human megsin antibody by immunizing rabbits with human megsin fusion protein with maltose-binding protein expressed in Escherichia coli.
coli (3). This obtained anti-human megsin antibody reacted with expressed human megsin protein. However, megsin is highly homologous to other members of the serpin: Amino acid homology search by the FASTA program throughout the Swissprot database revealed that megsin has 27.2% identity with human plasminogen activator inhibitor-1 (PAI-1) and 35.2% identity with human PAI-2 (3). The polyclonal rabbit anti-human megsin antibody also reacted with human PAI-2 protein expressed in Escherichia coli and purified human PAI-1 (our unpublished observation). Therefore, the preparation of polyclonal antibodies specific for human megsin has been hampered by its high homology to other serpins. For the immunohistochemical studies for megsin, the specific monoclonal mouse anti-human megsin antibody will be necessary.

Whether megsin is preferentially expressed in “activated” mesangial cells is of particular interest. The present immunohistochemical studies using the antibody to α-smooth muscle cell actin, a marker for activated mesangial cells, revealed the upregulation of both megsin mRNA and α-smooth muscle cell actin staining in the expanded mesangial area of IgA-N. However, their localizations were not completely identical. This may be partially accounted for by the discrepancy of the methodology between megsin mRNA detection and α-smooth muscle cell actin staining. Additional immunohistochemical studies for megsin protein expression and α-smooth muscle cell actin localization will be required.

Another interesting issue is the link of megsin expression to the mesangial cell proliferation. The present study could not...

Figure 3. Detection of megsin mRNA, α-smooth muscle cell actin, and proliferating cell nuclear antigen (PCNA) in NHK and IgA-N. Both megsin mRNA (A) and α-smooth muscle cell actin (B) expression were detected in the expanded mesangial area of IgA-N, although their localizations were not completely identical. Anti-PCNA antibodies stained positive in some nuclei of NHK (C) and of IgA-N (D) tissues fixed in formalin and embedded in paraffin. Magnification: ×80.
reveal the double staining studies in the same frozen section for megsin mRNA detection by in situ hybridization and for markers of mesangial cell proliferation by immunohistochemistry. Therefore, we did not examine whether megsin mRNA completely colocalizes with the markers of mesangial cell proliferation. Nevertheless, the present results demonstrate that like the megsin mRNA expression, the percentage of positive cells for a marker for mesangial cell proliferation, such as PCNA, was significantly greater in IgA-N than NHK, suggesting a link of megsin expression to the mesangial proliferation.

Megasin is a new member of the serpin superfamily. Serpins play a central role in the regulation of a wide variety of physiologic and pathologic processes, including coagulation, fibrinolysis, matrix metabolism, development, BP, malignancy, and inflammation (21,22). The enzymatic activity of a protease is regulated by a balance among proteinases and their inhibitors. Studies using transgenic mice that either lack or overexpress PAI-1 revealed a correlation between the level of PAI-1 and the extent of lung fibrosis after injury (23). In kidney diseases, inhibition of proteases led to accumulation of extracellular matrix as a consequence of decreased degradation, eventually leading to glomerulosclerosis. Mesangial cells from transgenic mice for growth hormone have no metalloproteinase 9 production, and these mice develop progressive glomerulosclerosis (24). In DN, increased megsin expression may participate in accumulation of mesangial matrix. In contrast, some serpins, including PAI-2, maspin, and early population doubling level cDNA-1 (EPC-1), are involved in processes of cell proliferation and survival. HeLa cells that overexpress PAI-2 were protected from apoptosis (25,26). Maspin has been shown to have tumor suppressor activity (27,28). EPC-1, also called pigmented epithelial differentiation factor, was induced upon G0 growth arrest in human fibroblast-like cells (29).

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In conclusion, megsin mRNA was expressed in glomerular mesangial cells in both healthy and diseased kidneys. The expression of megsin mRNA was upregulated in IgA-N and DN. These data suggest a link of megsin to the mesangial proliferation and/or mesangial matrix expansion in glomerular diseases accompanied by mesangial injury.

Acknowledgments
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References
21. Potempa J, Kozrus E, Travis J: The serpin superfamily of pro-

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