Int. J. Exp. Path. (1998), **79**, 133–140

Expression of heat shock protein 47 is increased in remnant kidney and correlates with disease progression

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Received for publication 20 November 1997
Accepted for publication 5 March 1998

Summary. Glomerulosclerosis is characterized by accumulation of the mesangial extracellular matrix, including type I and IV collagen. The processing for the collagens in the glomeruli may play a critical role for development of glomerulosclerosis. We examined the expression of heat shock protein 47 (HSP47), a collagen-binding molecular chaperone in the progresive glomerulosclerosis model. Subtotally nephrectomized rats, unlike sham-operated rats, developed focal and segmental glomerulosclerosis. Immunological staining demonstrated an increased expression of HSP47 which paralleled the expression of type I and IV collagen in the glomeruli of the nephrectomized rats as the glomerulosclerosis developed. The mRNA levels encoding type I and type IV collagen and HSP47 were increased 3.4 fold, 3.6 fold and 2.8 fold, respectively, at week 7 after nephrectomy. By *in situ* hybridization, the expression of HSP47 mRNA was determined to be localized to the glomeruli with segmental sclerosis. These results suggest that HSP47 may play a central role in the process of extracellular matrix accumulation during the development of glomerulosclerosis.

Keywords: heat shock proteins, collagens, glomerulosclerosis

Most forms of progressive renal disease leading to end-stage kidney disease share a common pathological finding, glomerulosclerosis. Expansion of the tissue matrix and sclerotic lesions in the mesangium areas have been identified in glomerulosclerosis. These contain various extracellular matrix (ECM) components including type IV collagen, laminin, fibronectin, heparin sulphate proteoglycan, as well as interstitial collagens not normally present in glomerular ECM (Striker *et al.* 1984; Abrass *et al.* 1988; Doi *et al.* 1991). Overexpression of ECM components has been noted in both experimental and human glomerulosclerosis, but the precise mechanisms for the processing and secretion of ECM components under pathological conditions are not completely understood.

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The heat shock response is a highly conserved response of cells to a variety of stresses, which are induced under pathophysiological conditions such as embryonic development, cell differentiation, hormonal stimulation, microbial infection, ischemia, tissue trauma, toxin exposure, and oxidative stress. A 47 kD heat shock protein (HSP47) has been found to be a

collagen-binding glycoprotein, which is heat-inducible and sensitive to malignant transformation (Nagata *et al.* 1986; Nagata & Yamada 1986; Nakai *et al.* 1990). HSP47 is the heat-shock protein with the ability to bind to type I-V collagens and procollagens (Natsume *et al.* 1994). HSP47 is localized in the endoplasmic reticulum and transiently associates with procollagen. Thus it is involved in collagen processing and/or secretion under normal conditions. Under conditions of stress, HSP47 is part of the quality control system for procollagen, preventing the secretion of procollagen with abnormal conformation (Nakai *et al.* 1992). The synthesis of HSP47 has been correlated with collagen synthesis in several cell lines (Nagata & Yamada 1986; Nakai *et al.* 1990; Takechi *et al.* 1992; Kudo *et al.* 1994) and recently, a functional role for HSP47 during hepatic fibrosis in rats has been demonstrated (Masuda *et al.* 1994). In the hepatic model, the mRNA levels of genes encoding HSP47 and types I and III collagens are dramatically upregulated during the progression of fibrosis, which suggests the funtional involvement of HSP47 in collagen accumulation. However, it is not clear whether or not the processing and conformational changes of collagens play a critical role in glomerular damage. In this study, we report the augmented expression of HSP47 in parallel with the expression of type I and IV collagen and with the severity of sclerotic lesions in a progressive glomerulosclerotic model.

Methods

Disease model

Male Wistar rats weighing 180–250 g were used. Subtotal nephrectomy was performed by ligating two or three main branches of the renal artery in combination with a right nephrectomy (5/6 nephrectomy) (Chanutin & Ferris 1932). Sham operation was performed without destruction of renal tissue.

Renal histology and immunofluorescence staining

Kidneys were fixed in ethyl Carnoy's solution and were stained with haematoxylin and eosin (HE), periodic acid Schiff's reagent (PAS) and periodic silver methenamine. A semi-quantitative score was used to evaluate the degree of glomerular sclerosis, as described by Raiji *et al.* (1984).

Four mm thick cryostat sections of kidney were fixed in acetone. Tissue sections were incubated with rabbit anti-HSP47 antiserum (Takechi *et al.* 1994) (diluted 1:20), rabbit anti-rat type IV collagen antibody (HK681) (diluted 1:500) and guinea pig anti-rat type I collagen antibody (diluted 1:20) (Doi *et al.* 1991). They were then reacted with biotin conjugated secondary antibody against rabbit IgG (diluted 1:50, Tago Inc. Burlingame CA), or gunea pig IgG (diluted 1:200, Vector Laboratories, Inc. Burlingame CA). These sections were then incubated with FITC conjugated with streptoavidin (diluted 1:50, Zymed Laboratories, Inc. CA USA). Fluorescence intensity of the glomeruli was graded semiquantitatively as described by Floege *et al.* (1991).

Western immunobloting

Whole kidneys were removed from three nephrectomized rats and three sham-operated rats at week 7, and were homogenized in 100 mM Tris HCl pH 7.3, 4% SDS and 20 mM EDTA. Western immunoblotting was performed using polyclonal rabbit antibody against HSP47 followed by treatment with alkaline phosphatase conjugated goat antibody against rabbit IgG (Zymed, San Francisco, CA), and reactivity was visualized with NBT/X-phosphate (Boehringer Mannheim Biochemica, Mannheim, Germany).

Dot blot analysis of type I and IV collagen and HSP47 gene expression

Glomeruli were isolated by a serial sieving procedure. Total RNA was extracted from isolated glomeruli from five rats of each group at week 7 by acid guanidium thiocyanate phenol chloroform extraction (Chomczynski & Sacchi 1987). A series of three dilutions of each glomerular RNA sample (2.0, 0.67, 0.22 mg for HSP47 and type I and IV collagen, and 1.0, 0.33, 0.11 mg for 18S ribosomal RNA) were blotted onto nylon filters. The cDNA probes used for this analysis were as follows: (1) HSP47 cDNA. A 1.5 kb *EcoRI-HindIII* fragment of mouse HSP47 from plasmid pGEMSP47 (Takechi *et al.* 1992). (2) type I collagen cDNA (PGM101): 0.94 kb XhoI fragment of mouse type I collagen cDNA. (3) type IV collagen cDNA (p1234): 0.8 kb *EcoRI-HindIII* fragment of murine type IV collagen cDNA (Oberbäumer *et al.* 1985). (4) 18S ribosomal RNA. A pN29III (RN18S) was used to detect 18S ribosomal RNA (American Type Culture Collection, No63178, Maryland, USA) (Oberbäumer 1992). For quantification, densitometric analysis was performed using ImageMaster software (Pharmacia, Uppsala, Sweden).

In situ hybridization

The hybridization method employed was based on procedures described previously (Masuda *et al.* 1994), with

some modifications. Rat cryostat kidney sections (4 mm) were fixed in 4% paraformaldehyde. Sections were hybridized with dioxygenin-labelled RNA probes and visualized by Nucleic Acid Detection Kit (Boehringer). To generate probes for HSP47, the pGEM4Z plasmid containing a 1.5 kb mouse HSP47 cDNA (Takechi *et al.* 1992) was linearized with *Pst-I*, and labelled with digoxigenin-labelled UTP using the SP6/T7 transcription kit (Boehringer).

Statistical analysis

The data were expressed as the mean ± standard error, and were evaluated statistically by analysis of variance or nonparametric test (Mann–Whitney analysis).

Results

Progressive glomerulosclerosis and the accumulation of glomerular extracellular matrix

Light microscopic findings revealed focal and segmental glomerulonephritis, mesangial cell proliferation and glomerular hypertrophy at week 4. At week 7 the glomeruli were enlarged with proportional hypertrophy of the mesangium and dilatation of the capillaries. Most glomeruli showed global or segmental sclerosis with severe glomerular mesangium expansion, occlusion of the capillaries, or adhesion (Figure 1). Finally, at week 10, global sclerosis, complete adhesion to Bowman's capsule, interstitial fibrosis, tubular atrophy, and inflammatory cells were present.

Increased expression of type I and IV collagen, and HSP47 with glomerulosclerosis in the immunofluorescence study

To establish whether the expression of HSP47 could increase after 5/6 nephrectomy, Western blot analysis was performed on kidney tissue lysates. Samples were analysed by SDS-PAGE, and examined by immunoblot analysis using anti-HSP 47 antibody. As shown in Figure 2, a major band of 47 kD in molecular size was detected in 5/6 nephrectomized rat kidneys and in sham-operated rat kidneys at week 7, which is consistent with the specific size of HSP47. The intensity of the major band increased after 5/6 nephrectomy.

Fluorescence staining revealed a remarkable increase

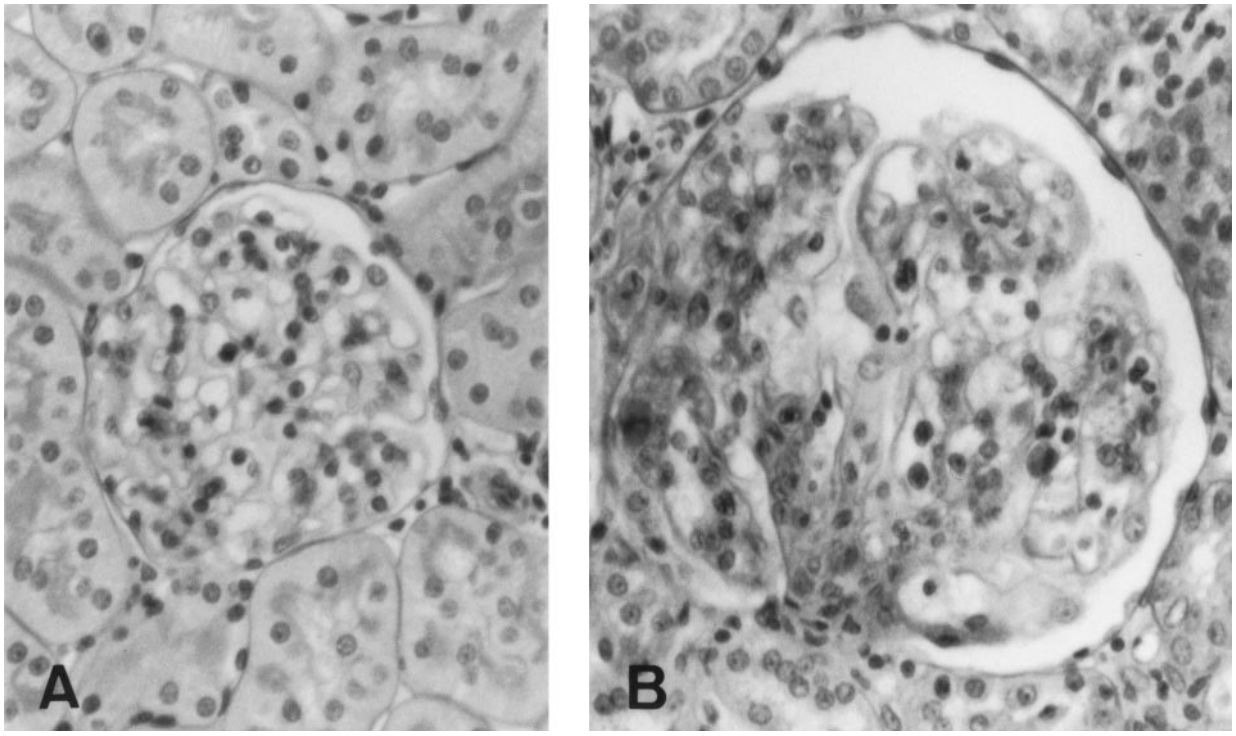

Figure 1. Glomerular periodic acid Schiff (PAS) staining obtained of rat kidney tissue (A) at week 7 after the sham-operation and (B) in rats with 5/6 nephrectomy at week 7 after the operation. Most glomeruli showed compensatory hypertrophy, diffuse mesangial proliferation and occlusion of the capillaries. The magnification of both photos are identical. (×590).

in the expression of HSP47 which paralleled the expression of type I and IV collagen in the nephrectomized rats, as glomerulosclerosis developed. At week 1, increased expression of type I and IV collagen and HSP47 was already detectable. At week 7, staining for type I collagen was observed in the mesangial area, Bowman's capsule, damaged tubular epithelial cells, and in the expanded mesagial area (Figure 3B). Deposits of type IV collagen increased in the glomerular mesangial area and in the expanded interstitial matrix (Figure 3D). However, no increased expression of these collagens was observed in sham-operated rats (Figure 3A,C). Staining for HSP47 was detected mainly in the tubular epithelial cells in the normal rats. Glomerular mesangial and epithelial cells were also stained, although expression was weak (Figure 3E). In the nephrectomized rats, staining of HSP47 was increased in the glomerular mesangial area and in the epithelial cells in the damaged tubules (Figure 3F). The expression of these proteins continued until week 10 (Figure 4).

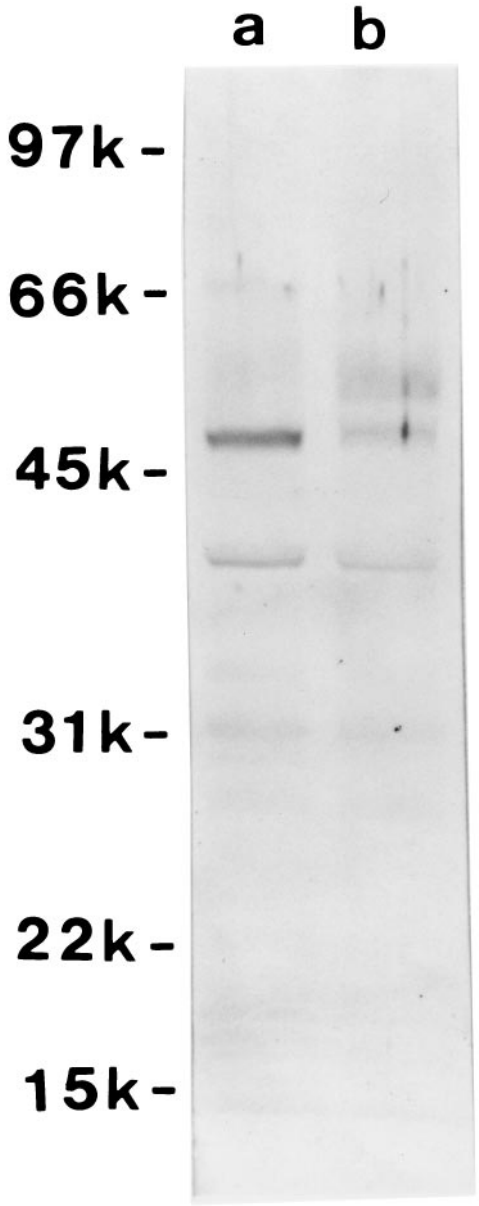

Figure 2. Immunoblot of HSP47 in rat kidneys. The lysate (50 μg) from total kidney tissue of (a) 5/6 nephrectomized rats and (b) sham-operated rats were applied on SDS-PAGE and were reacted with anti-HSP47 antibody.

We also calculated the intensity of immunofluorescence staining of glomeruli semiquantitatively and chronologically in the nephrectomized rats, as compared to the sham operated rats (Table 1). Immunostaining of type I and IV collagen and HSP47 increased chronologically in the nephrectomized rats compared to the sham-operated rats. Therefore, HSP47 expression was induced by 5/6 nephrectomy in parallel to the expression of type I and IV collagens.

Augmented expression of HSP47 mRNA correlated with the expression of type I and IV collagen mRNA during glomerulosclerosis as detected by dot blot analysis

We also investigated the transcripts for HSP47 and type I and IV collagen genes in rat glomeruli by RNA dot blot analysis. The augmented expression of HSP47 mRNA was observed at week 7 in the nephrectomized rats as compared to the sham operated rats. This increased expression paralleled the induced expression of type I and IV collagen mRNA as shown in Figure 5.

Densitometric analysis showed that HSP47 transcripts normalized for rRNA increased approximately 2.8 fold, and type I and IV collagen transcripts increased approximately 3.4 fold and 3.6 fold, respectively, at week 7 after 5/6 nephrectomy, when compared with levels in sham operated rats.

Increased expression of HSP47 mRNA during glomerulosclerosis as detected by in situ hybridization

In situ hybridization showed localization of HSP47 mRNA signals in glomeruli and tubules, that was more prominent in nephrectomized rats when compared to sham operated rats. At week 7, increased expression of HSP47 mRNA was observed in the nephrectomized rats, and the expression was mainly localized to the mesangial cells, epithelial cells, and Bowman's capsule of the glomeruli with segmental sclerosis, and to tubular epithelial cells exhibiting degenerative changes. In contrast, sparse signals were observed in the remaining

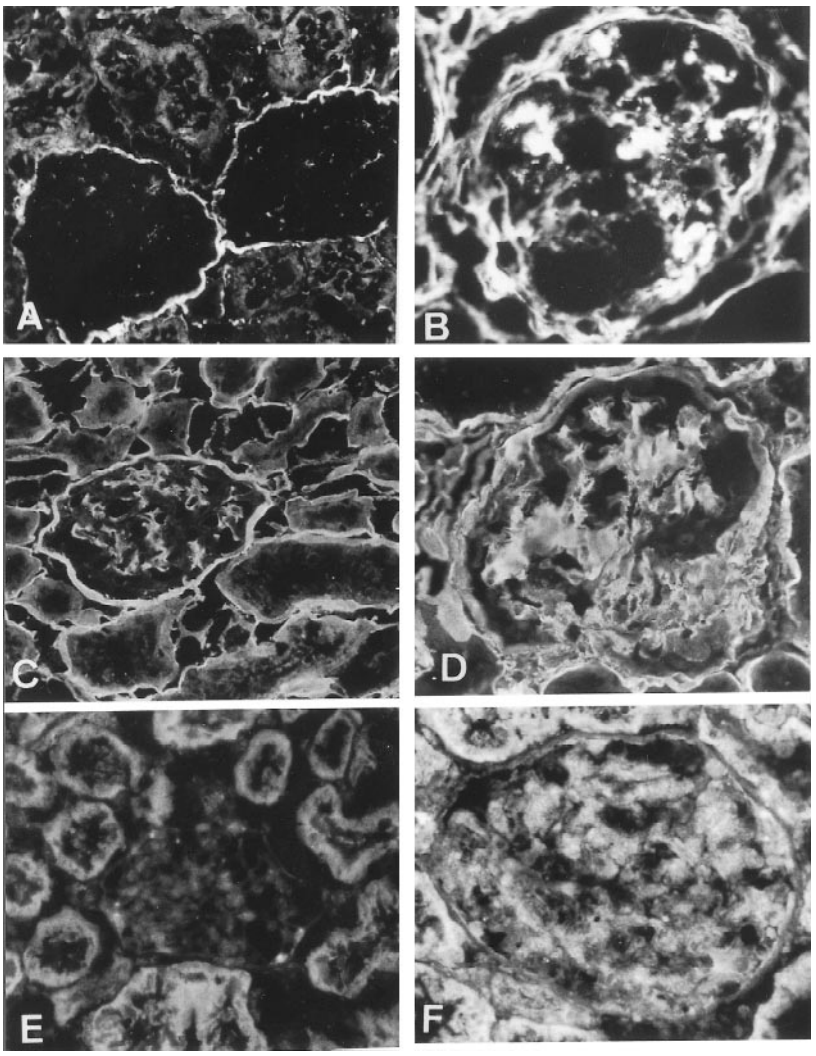

Figure 3. Immunohistochemical glomerular staining for type I collagen (A, B), type IV collagen (C, D), and HSP47 (E, F) in sham-operated rats (A, C, E) or in rats after 5/6 nephrectomy (B, D, F) at week 7. The expanded glomerular matrix stains positive for type I and IV collagen, and for HSP47. (×620).

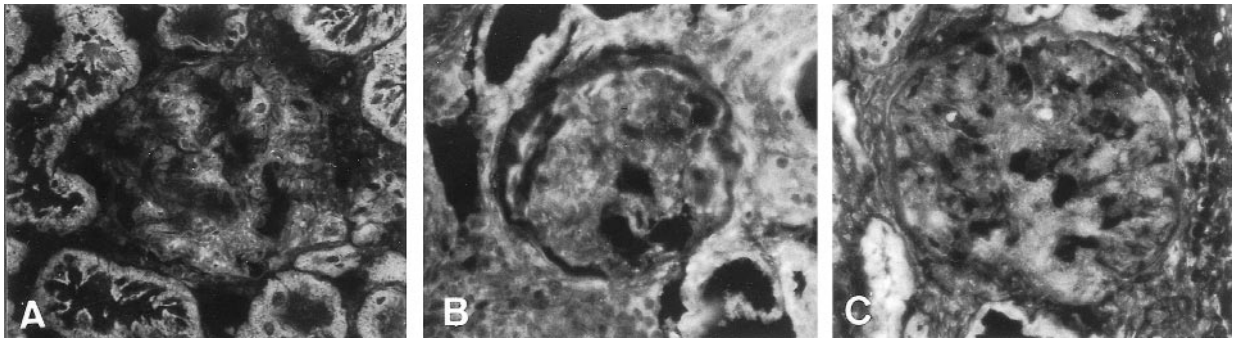

Figure 4. Immunohistochemical glomerular staining for HSP47 in rats at (A) week 1, (B) week 4 and (C) week 10 after 5/6 nephrectomy. At week 1, there are focal increases in mesangial glomerular staining for HSP47(A). Glomerular staining for HSP47 increased progressively in 5/6 nephrectomized animals to reach a maximum at week 10(C). (×620).

Table 1. Immunofluorescence scores for type I and IV collagen and for HSP47 in nephrectomized rats and sham-operated rats

	Sclerosis index		Type I collagen		Type IV collagen		HSP47	
Week	Sham	Ope	Sham	Ope	Sham	Ope	Sham	Ope
1			0.19 ± 0.06 (n=4)	0.67 ± 0.23 (n=6)	1.00 ± 0.07 (n=4)	1.72 ± 0.12** (n=6)	0.22 ± 0.07 (n=4)	0.67 ± 0.15 (n=6)
4	23.5 ± 3.9 (n=5)	196.9 ± 12.6** (n=5)	0.06 ± 0.05 (n=4)	0.90 ± 0.22 (n=5)	1.13 ± 0.11 (n=4)	1.93 ± 0.15* (n=5)	0.21 ± 0.11 (n=4)	0.73 ± 0.18 (n=5)
7	13.7 ± 5.1 (n=5)	246.5 ± 43.5** (n=4)	0.05 ± 0.05 (n=5)	1.42 ± 0.25** (n=6)	0.93 ± 0.06 (n=5)	2.92 ± 0.25** (n=6)	0.20 ± 0.11 (n=5)	1.31 ± 0.42** (n=6)
10	16.1 ± 0.9 (n=3)	306.6 ± 71.5 (n=3)	0.08 ± 0.08 (n=6)	1.08 ± 0.22** (n=6)	1.06 ± 0.05 (n=6)	2.83 ± 0.10** (n=6)	0.39 ± 0.18 (n=6)	1.25 ± 0.16* (n=6)

Values are mean values ± SE. * P<0.05 *vs.* control; ** P<0.01 *vs.* control

glomeruli, which showed minor changes in the nephrectomized rats, and in the glomeruli and the tubules of the sham operated rats. Sense probes showed only background signal levels. (Figure 6)

Discussion

The most extensively investigated model of progressive glomerulosclerosis is the 5/6 nephrectomy model in rats. In this model, progressive increases in ECM components has been noted in association with progressive glomerulosclerosis (Floege *et al.* 1992). This study demonstrated a pathological role for HSP47, a major collagen-binding protein, in the development of progressive glomerulosclerosis. We found that the activation of HSP47 correlated with the overexpression of type I and IV collagen and with the severity of glomerulosclerosis. These findings suggest that HSP47 plays a pivotal role for secretion, processing and conformational change of molecules for development of progressive glomerular damages.

Other heat shock proteins are also reported to change

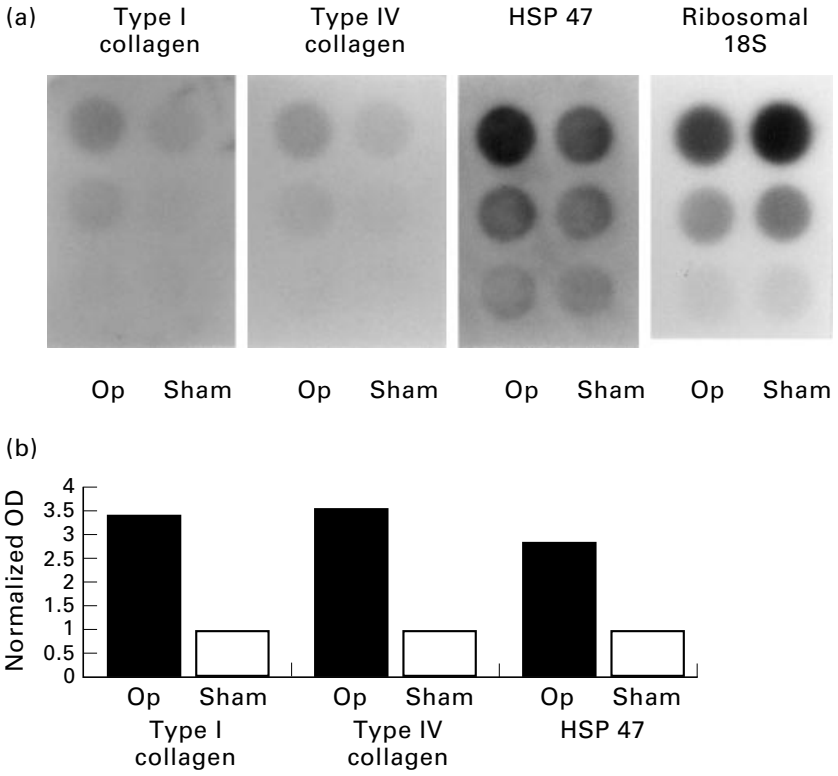

Figure 5. (a) Dot blot analysis of total glomerular RNA obtained in rats at week 7 after sham-operation or 5/6 nephrectomy, for the detection of type I and IV collagen mRNA, HSP47 mRNA and 18S RNA. (b) In comparison with the sham operated rats, type I and type IV collagen transcripts increased approximately 3.4 fold and 3.6 fold, and HSP47 transcripts increased approximately 2.8 fold respectively in 5/6 nephrectomized rats. Values are expressed as optical density units relative to specific mRNA levels observed in glomerular RNA from normal rats. Densitometry readings were normalized to equivalent amounts of 18S ribosomal RNA per dot to confirm equivalent loading of RNA.

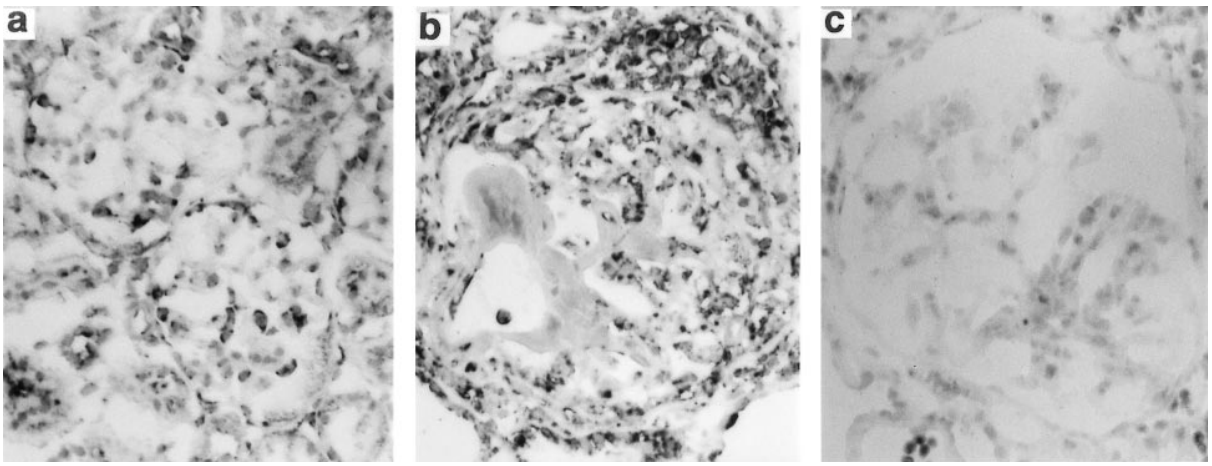

Figure 6. *In situ* hybridization for the detection of HSP47 mRNA in (a) the glomeruli of sham-operated and (b, c) 5/6 nephrectomized rats at week 7. In sham-operated rats, glomeruli and tubular epithelial cells showed weak expression of HSP47 (a). In sclerosed glomeruli, increased expression was obtained in the mesangial and epithelial cells of the glomeruli (b). Sense probe showed only background levels of signals (c). (×440).

their expression during glomerulonephritis. HSP73 is induced in cells in mesangial localization and cellular crescents in the glomeruli of puromycin aminonucleoside (PAN) nephrosis rats (Komatsuda *et al.*, 1993). Expression of HSP27 also increased in glomerular capillary loop in PAN-nephrosis rats (Smoyer *et al.*, 1996). Among various heat shock proteins, HSP47 is a unique stress protein with collagen-binding ability (Nakai *et al.*, 1989) and is synthesized only in collagen-binding cells (Takechi *et al.,* 1992)

HSP47 has been reported to be associated with the metabolism or processing of procollagen as a molecular chaperone specific to procollagen (Nagata *et al.* 1986). HSP47 is located in the endoplasmic reticulum (ER), and binds to types I-V collagens (Natsume *et al.* 1994) but does not bind to other extracellular matrix proteins such as fibronectin and laminin (Nakai *et al.* 1989). HSP47 transiently binds with and dissociates from procollagen before procollagen is secreted (Nakai *et al.* 1992). However, when the cells were heat shocked or treated with an iron-chelator, α,α'-dipyridyl, which inhibits the triple helix formation of procollagen, these conformationally abnormal procollagen species were shown to be bound with HSP47 and to be retained in the ER for much longer periods of time (Nakai *et al.* 1992). HSP47 is thus suggested to be involved in the processing or the transport of procollagen in the ER and to have molecular chaperone-like function under stress conditions.

The expression of HSP47 always correlates with that of collagens *in vitro*. The synthesis of HSP47 decreases after viral transformation and drastically increases during the differentiation of mouse F9 teratocarcinoma cells (Takechi *et al.* 1992). These changes in HSP47 are consistent with changes in the levels of collagen (Takechi *et al.* 1992).

Procollagen α1(I) chains have been associated with a number of proteins, including the glucose-regulated proteins GRP94 and BiP/GRP78, protein disulphide isomerase (PDI) (Nakai *et al.* 1992). These proteins appear to function in a successive series of reactions during procollagen processing, folding, and polymer assembly (Ferreira *et al.* 1994). Among these proteins, HSP47 is the only protein with the ability to bind specifically to collagens.

Recently, it has been showed that the mRNA of HSP47 was markedly induced in parallel with α1(I) and 1(III) collagen mRNAs during hepatic fibrosis in rats induced by the administration of CCl4 (Masuda *et al.* 1994). HSP47-positive cells were observed only along the collagen fibrils.

In this study, we have shown that HSP47 was markedly induced in parallel with type I and IV collagen in the glomeruli of the nephrectomized rats. It has been reported that antisense 'knock out' of HSP47 results in a diminished production of HSP47 and pro-α1(I) collagen in 3T6 cells (Sauk *et al.* 1994). These observations suggest that HSP47 may play an important role in the synthesis of collagens in the glomerulosclerosis model. We are now in the process of investigating inhibition of collagen expression by introduction of antisense HSP47 oligonucleotides in an anti Thy-1 glomerulonephritis model.

Acknowledgements

This work was supported in part by grants-in-aid from the Ministry of Education, Science and Culture, Japan (0655704, 05454341, 0504137), a grant for 'Progressive Renal Disease' from the Ministry of Health and Welfare research project for 'Specially selected diseases', Osaka Gas Group Welfare Foundation, Chiyoda Mutual Life Foundation and Ichiro Kanehara Foundation.

References

ABRASS C.K., PETERSON C.V. & RAUGI G.J. (1988) Phenotypic expression of collagen types in mesangial matrix of diabetic and nondiabetic rats. *Diabetes* **37,** 1695–1702.

CHANUTIN A. & FERRIS E.B. (1932) Experimental renal insufficiency produced by partial nephrectomy. I. control. diet. *Arch. Intern. Med.* **49,** 767.

CHOMCZYNSKI P. & SACCHI N. (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol.-chloroform extraction. *Anal. Biochem.* **162,** 156–159.

DOI T., STRIKER L.J. & KIMATA K. (1991) Glomerulosclerosis in mice transgenic for growth hormone. Increased mesangial extracellular matrix is correlated with kidney mRNA levels. *J. Exp. Med.* **173,** 1287–1290.

FERREIRA L.R., NORRIS K. & SMITH T. (1994) Association of HSP47, Grp78, and Grp94 with procollagen supports the successive or coupled acton of molecular chaperones. *J. Cell. Biochem.* **56,** 518–526.

FLOEGE J., ALPERS C.E. & BURNS M.W. (1992) Glomerular cells, extracellular matrix accumulation, and the development of glomerulosclerosis in the remnant kidney model. *Lab. Invest.* **66,** 485–497.

FLOEGE J., JOHNSON R.J. & GORDON K. (1991) Increased synthesis of extracellular matrix in mesangial proliferative nephritis. *Kidney Int.* **40,** 477–488.

KOMATSUDA A., WAKUI H. & SATOH K. (1993) Altered localization of 73-kilodalton heat-shock protein in rat kidneys with gentamicin-induced acute tubular injury. *Lab. Invest.* **68,** 687–695.

KUDO H., HIRAYOSHI K. & KITAGAWA Y. (1994) Two collagen-binding proteins, osteonectin and HSP47, are coordinately induced in transformed keratinocytes by heat and other stresses. *Exp. Cell. Res.* **212,** 219–224.

LAEMMLI U.K. (1970) Cleavage of structural proteins during the assembly of the band of bacteriophage T4. *Nature* **227,** 680–685.

MASUDA H., FUKUMOTO M. & HIRAYOSHI K. (1994) Coexpression of the collagen-binding stress protein HSP47 gene and the a1 (I) and a1 (III) collagen genes in carbon tetrachloride-induced rat liver fibrosis. *J. Clin. Invest.* **94,** 2481–2488.

NAGATA K., SAGA S. & YAMADA K.M. (1986) A major collagen-binding protein of chick embryo fibroblasts is a novel heat shock protein. *J. Cell. Biol.* **103,** 223–229.

NAGATA K. & YAMADA K.M. (1986) Phosphorylaton and transformation sensitivity of a major collagen-binding protein of fibroblasts. *J. Biol. Chem.* **261,** 7531–7536.

NAKAI A., HIRAYOSHI K. & NAGATA K. (1990) Transformation of BALB/3T3 cells by simian virus 40 causes a decreased synthesis of a collagen-binding heat-shock protein (hsp47). *J. Biol. Chem.* **265,** 992–999.

NAKAI K., HIRAYOSHI H. & SAGA S. (1989) The transformation-sensitive heat shock protein (HSP47) binds specifically to feutin. *Biochem.. Biophys. Res. Commun.* **164,** 259–264.

NAKAI A., SATOH M. & HIRAYOSHI K. (1992) Involvement of the stress protein HSP47 in procollagen processing in the endoplasmic reticulum. *J. Cell. Biol.* **117,** 903–914.

NATSUME T., KOIDE T. & YOKOTA S. (1994) Interactions between collagen-binding stress protein HSP47 and collagen. *J. Biol. Chem.* **269,** 31224–31228.

OBERBÄUMER I. (1992) Retroposons do jump: B2 element recently integrated in as 18S rDNA gene. *Nucl Acids Res.* **20,** 671–677.

OBERBÄUMER I., LAURENT M. & SCHWARZ U. (1985) Amino acid sequence of the non-collagenous globular domain (NC1) of the a1 (IV) chain of basement membrane collagen as derived from complementary DNA. *Eur. J. Biochem.* **147,** 217–224.

RAIJ L., AZAR S. & KEANE W. (1984) Mesangial immune injury, hypertension and progressive glomerular damage in Dahl rats. *Kidney Int.* **26,** 137–143.

SAUK J.J., SMITH T. & NORRIS K. (1994) HSP47 and the translation-translocaton machinery cooperate in the production of a1 (I) chains of type I procollagen. *J. Biol. Chem.* **269,** 3941–3946.

SMOYER W.E., GUPTA A. & MUNDEL P. (1996) Altered expression of glomerular heat-shock protein 27 in experimental nephrotic syndrome. *J. Clin. Invest.* **97,** 2697–2704.

STRIKER L.M.-M., KILLEN P.D. & CHI E. (1984) The composition of glomerulosclerosis. I.Studies in focal sclerosis, crescentic glomerulonephritis, and membranoproliferative glomerulonephritis. *Lab. Invest.* **51,** 181–192.

TAKECHI H., HIRAYOSHI K. & NAKAI A. (1992) Molecular cloning of a mouse 47-kDa heat-shock protein (HSP47), a collagen-binding stress protein, and its expression during the differentiation of F9 teratocarcinoma cells. *Eur. J. Biochem.* **206,** 323–329.

TAKECHI H., HOSOKAWA N. & HIRAYOSHI K. (1994) Alternative 5′ splice site selection induced by heat shock. *Mol. Cell. Biol.* **14,** 567–575.