Small proteoglycans of normal adult human kidney: Distinct expression patterns of decorin, biglycan, fibromodulin, and lumican

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Background. Among the members of the small leucine-rich proteoglycan family, decorin, biglycan, and fibromodulin have been proposed to be potent modulators of transforming growth factor-β (TGF-β) activity, thereby playing an important role in the pathogenesis of fibrotic kidney diseases. Furthermore, decorin expression influences the expression of p21$^{WAF1/CIP1}$, which has been related to kidney hypertrophy and hyperplasia. However, none of the members of this proteoglycan family have been investigated in normal adult human kidney cortex, thus making it impossible to correlate disease-mediated alterations of their expression with the normal situation in vivo.

Methods. The chondroitin/dermatan sulfate proteoglycans, decorin and biglycan, and the keratan sulfate proteoglycans, fibromodulin and lumican, were investigated in normal human adult renal cortex by immunohistochemistry on the light and electron microscopic level and by in situ hybridization. Northern blot and reverse transcription-polymerase chain reaction (RT-PCR) methods were used to get an estimate of their expression in isolated glomeruli. Decorin excretion with the urine was measured by Western blotting.

Results. Two bands of decorin and a single band of biglycan mRNA were identified in Northern blots of isolated glomeruli. Amplification by RT-PCR was required to detect the signals for fibromodulin and lumican. All four proteoglycans were preferentially expressed in the renal interstitium with accumulations around tubules. Weak expression was found in the mesangial matrix. Biglycan was expressed by glomerular endothelial cells and, together with fibromodulin, was synthesized and deposited in distal tubular cells and collecting ducts. Immunogold labeling indicated the presence of the proteoglycans in the glomerular basement membrane, which was interpreted as a result of glomerular filtration. Indirect evidence suggested tubular reuptake of decorin after glomerular filtration.

Conclusion. The data indicate that the different cells of the adult human kidney are characterized by a distinct expression pattern of the four small proteoglycans. It is suggested that these proteoglycans may have distinct pathophysiological roles depending upon whether they are expressed by mesangial cells, endothelial cells, epithelial cells, or cells of the tubulointerstitium.

Decorin, biglycan, fibromodulin, and lumican are members of a growing family of extracellular matrix molecules [reviewed in 1–3], which are characterized by core proteins with centrally located leucine-rich repeat motifs flanked by cysteine clusters. The family can be divided into three subfamilies that are characterized by the intron/exon organization and the arrangement of the cysteine clusters. Decorin and biglycan are the only members of the first subfamily, while fibromodulin and lumican belong to the second subfamily. Furthermore, decorin and biglycan both carry chondroitin/dermatan sulfate side chains, whereas keratan sulfate is the characteristic glycosaminoglycan moiety of the proteoglycan forms of fibromodulin and lumican. All four proteoglycans are able to interact with type I collagen, albeit biglycan does so with a lower affinity [4]. Fibromodulin and probably also lumican bind to a site that is different from the one used by decorin and biglycan [5, 6]. Targeted disruption of the genes for decorin, fibromodulin, and lumican all lead to abnormal collagen fibril morphology [7–9], indicating the need of an orchestrated action of several leucine-rich repeat proteins for fibril formation. Additionally, at least decorin and biglycan modulate cell adhesion to matrix glycoproteins like fibronectin and thrombospondin [10, 11].

It is becoming increasingly obvious that small proteoglycans are not only involved in matrix formation and cell adhesion, but also in the modulation of cytokine activity and in direct cell signaling. Decorin, biglycan, and fibromodulin have been shown to form complexes with transforming growth factor-β (TGF-β) [12], which

in certain models results in an inactivation of the cytokine [13]. In the model of anti-Thy-1–initiated glomerulonephritis, the dramatic up-regulation of TGF-β could be abrogated by administration of decorin [14] and by the induction of decorin by gene transfer [15]. Additional functions, which are most likely decorin-specific, concern growth suppression of tumor cells, which is explained by the interaction of the proteoglycan's core protein with the epidermal growth factor receptor [16] and the subsequent induction of p21[WAF1/CIP1] [17], a potent inhibitor of cyclin-dependent kinases. p21[WAF1/CIP1] has recently been proposed to be a regulator of the balance between hyperplasia and hypertrophy after renal ablation [18]. Finally, decorin expression by endothelial cells has been shown to occur during angiogenesis in vivo, and in vitro studies with endothelial cells demonstrated a correlation between decorin expression and the prevention of apoptosis [19].

In light of the great potential of small leucine-rich proteoglycans to be involved significantly in the pathogenesis of fibrotic and other kidney diseases, the scarcity of data on the expression of these macromolecules by the different cell types of human kidney is surprising. Bianco et al noted in fetal kidneys the expression of biglycan in endothelia and collecting tubules and the presence of decorin in the interstitium [20]. Additionally, decorin was localized in Bowman's capsule [21]. In an immunohistochemical study of several matrix proteins, including decorin, the latter was found to be the best predictor both of the severity of interstitial fibrosis and of renal failure [22]. A more recent study described the presence of biglycan in glomerular endothelial cells in both normal human kidney and in amyloidosis, and the almost complete absence of biglycan and decorin in the normal mesangial matrix. Decorin, too, was associated with amyloid fibrils [23]. In an attempt to study the role of small leucine-rich proteoglycans in the development of nephropathies in greater detail, we first investigated the expression of four prominent members of the family of these proteins in normal adult human kidney cortex. Unexpectedly, it had been found that biglycan and fibromodulin are synthesized by distal tubules and collecting ducts epithelia, whereas all four small proteoglycans were components of the tubulointerstitium and the mesangial matrix.

METHODS

Materials

Samples representing normal human kidneys were obtained from 11 patients [5 females and 6 males, median age 54.5 years (range of 38 to 62 years)] who were undergoing nephrectomy because of renal carcinoma. Only tumor-free samples were used for the present investigation. The samples were fixed immediately with 4% formaldehyde in 18 mmol/L sodium phosphate, pH 7.4, containing 0.15 mol/L NaCl [phosphate-buffered saline (PBS)] and embedded in paraffin under RNase-free conditions. Serial paraffin sections (2 to 6 μm) were stained with periodic acid-Schiff (PAS) reaction in order to exclude tumor infiltration. Paraffin sections of kidney biopsies with the histologic diagnosis of membranous nephropathy (three patients; 1 female and 2 males, mean age 56.3 ± 9.1 years) were kindly provided by the Department of Pathology for Immunohistochemical Studies (University of Muenster, Muenster, Germany). RNase-free material from human biopsies was not available.

Isolation of human glomeruli

Human glomeruli were isolated by differential sieving. Renal cortex homogenates were passed through stainless steel sieves of various pore sizes: 180, 250, and 150 μm (Linker, Kassel, Germany). Glomeruli collected on the 150 μm sieve were washed three times, examined by light microscopy (purity of isolates >95%), and processed further for RNA isolation.

Northern blot analysis

Northern blot analysis was performed as described previously [24]. Briefly, RNA was extracted from glomeruli or whole renal cortex using TRI-zol (Life Technologies, Eggenstein, Germany), and 40 μg of total RNA were loaded on each lane of the gels. The cDNA probes for human decorin and biglycan were kindly provided by Dr. E. Schönherr (Muenster, Germany) and comprised a 533 bp fragment of human decorin (exons 2 to 3) 1028 to 259 bp (exon 7) cloned into the *Eco*RI/*Hin*d III site of pGEM 4Z (Promega, Madison, WI, USA) [25] and a 831 bp fragment of human biglycan 44 to 875 bp cloned into the *Hin*dIII/*Hin*cII site of pGEM 3Z (Promega) [26].

cDNA probes for human lumican and fibromodulin were obtained by reverse transcription-polymerase chain reaction (RT-PCR). RNA from human skin fibroblasts (1 μg) was reverse transcribed with Super Script II reverse transcriptase (Life Technologies) and random hexanucleotides for cDNA priming. Double-stranded cDNAs were generated by using primers that contained additionally appropriate sequences with restriction sites for *Eco*RI and *Not*I, respectively. For lumican, the forward primer was 5′-TGC TGG AAT TCT AAA CCA CAA CAA CCT GAC A-3′, and the reverse and complement primer was 5′-CTC GAG CGG CCG CAG AAA AAC ATA ACC ATA AAA-3′, thus yielding a human lumican cDNA encompassing bp 448 to 1138 [27]. For human fibromodulin, the respective primer pair was 5′-TGC TGG AAT TCC AAA CTT CCT CAC GGC CAT GT-3′ and 5′-CTC GAG CGG CCG CAA CTC ATT GAT CCT ATT GCC T-3′, which allowed the generation of the fibromodulin cDNA between bp 271 and 1003 [12]. After restriction endonuclease treatment, the PCR products were cloned into pGEM 11Z (Promega) and were verified by sequencing. GAPDH cDNA was obtained from ATCC (Rockville, MD, USA).

All probes were radiolabeled with [^{32}P]dCTP (Amersham-Buchler, Braunschweig, Germany) using the DECA prime DNA-labeling kit (Ambion, Austin, TX, USA). Hybridization was carried out overnight at 42°C. The filters were exposed to Kodak X-OMAT AR film at −80°C for one or more days or to a Storage Phosphor Screen (Molecular Dynamics, Uppsala, Sweden) for quantitation. After exposure to the Phosphor screen, the filters were hybridized with a cDNA probe for GAPDH. The Phosphor screens were analyzed by a STORM860 Phosphor Imager (Molecular Dynamics) using IQ Solutions Image Quant software (Molecular Dynamics). Each individual mRNA band was normalized for GAPDH to correct for the difference in RNA loading and/or transfer. Values are given as means ± SEM from three Northern blots.

Reverse transcription-polymerase chain reactions

Total RNA (1 μg) from isolated human glomeruli was reverse transcribed with Super Script II reverse transcriptase and oligo (dT)$_{12-18}$ primers (Life Technologies). cDNA was incubated with Taq PCR Master Mix (Qiagen, Hilden, Germany) containing Taq DNA polymerase and primers for the sequences of human lumican and fibromodulin (each at a final concentration of 0.2 μmol/L) as described previously in this article. The sample was kept for five minutes at 94°C before 35 cycles (denaturation at 94°C for 1 min; annealing at 60°C for 1 min; and elongation at 72°C for 1 min) and a final cycle with an elongation time of seven minutes were performed in a Stratagen Gradient Robo Cycler 40 (Stratagene, La Jolla, CA, USA).

In situ hybridization

In situ hybridization was performed as described previously [24]. The sense and antisense riboprobes were transcribed in vitro from the linearized plasmids containing human decorin, biglycan, lumican, and fibromodulin cDNAs.

Immunohistochemistry

The alkaline phosphatase anti-alkaline phosphatase (APAAP) technique was used for immunohistochemistry as described earlier [24]. In some cases, sections were pretreated with 0.004% pepsin (Sigma, Munich, Germany) in 0.1 mol/L HCl for 30 minutes at 37°C. The following primary antibodies were applied in this study, with their working dilution given in parentheses: rabbit α-PG-II (1:500), which was raised against core protein of human decorin [28] as well as rabbit α-human decorin (1:400), which was raised against native decorin purified from the supernatant of cultured human skin fibroblasts (kindly provided by Dr. H. Hausser, Muenster, Germany). Biglycan was immunostained using a rabbit antibody to human core protein of biglycan (1:200) [28]. A purified antibody raised in rabbits specifically recognizing the C-terminal region of human lumican (1:100) was kindly provided by Dr. P.J. Roughley (Shriners Hospital for Children, Montreal, Canada) [27]. A rabbit antiserum against fibromodulin from bovine cartilage (1:200) was a kind gift from Dr. A. Plaas (Shriners Hospital for Children, Tampa, FL, USA) [29]. All immunohistochemical stainings were carried out under identical conditions for each antibody. Fibromodulin was only detectable by a double APAAP sandwich technique. The specificity of immunolabeling was tested by omitting the primary antibody and by using nonimmune serum or "unspecific" IgG instead.

Postembedding immunogold electron microscopy

For postembedding immunocytochemistry, a small piece of kidney tissue was immersed in fixative consisting of 5% paraformaldehyde in PBS. Tissue blocks were processed for embedding in Lowicryl K4M (Chemische Werke Lowi, Waldkraiburg, Germany) as described previously [30]. Postembedding immunocytochemical labeling of sections of Lowicryl-embedded tissue was performed on floating grids, section side down, on 10 to 20 μL droplets placed on parafilm sheets. Pretreatment to block nonspecific binding sites and to quench aldehydes present on the section surface was carried out with 10% fetal calf serum (FCS) in PBS (pH 7.4) or with 1% bovine serum albumin (BSA) in PBS (pH 7.4) for 15 minutes.

The sections were incubated with decorin, biglycan, or lumican primary antibodies in 1% BSA/PBS for one hour, followed by four five-minute washes in PBS. Controls without the primary antibodies were processed in parallel. The specificity of immunolabeling for decorin was tested additionally using an antigen-preabsorbed (human decorin) antiserum. Incubation with gold-labeled secondary antibodies (12 nm colloidal gold, AffiniPure goat anti-rabbit IgG; Dianova, Hamburg, Germany) was then carried out for one hour, followed by four five-minute washes in PBS and five two-minute rinses in distilled water. Before examination, sections were stained with saturated aqueous uranyl acetate and lead citrate. The grids were examined with a Philips 410 electron microscope operated at 60 kV.

Determination of urinary decorin excretion

Aliquots (25 mL) of 24-hour urine samples were tested for their conductivity and diluted with water, if necessary, to a conductivity of <15.5 mS/cm. They were then supplemented with stock solutions to give 10 mmol/L Tris/HCl, pH 7.4, 0.1% Triton X-100, and protease inhibitors [28]. After centrifugation, the samples and the appropriate standard solutions were loaded on 0.5 mL columns of DEAE Trisacryl (Serva, Heidelberg, Germany), prepared in Pasteur pipettes, and equilibrated with 20 mmol/L Tris/HCl, pH 7.4, containing 0.15 mol/L NaCl,
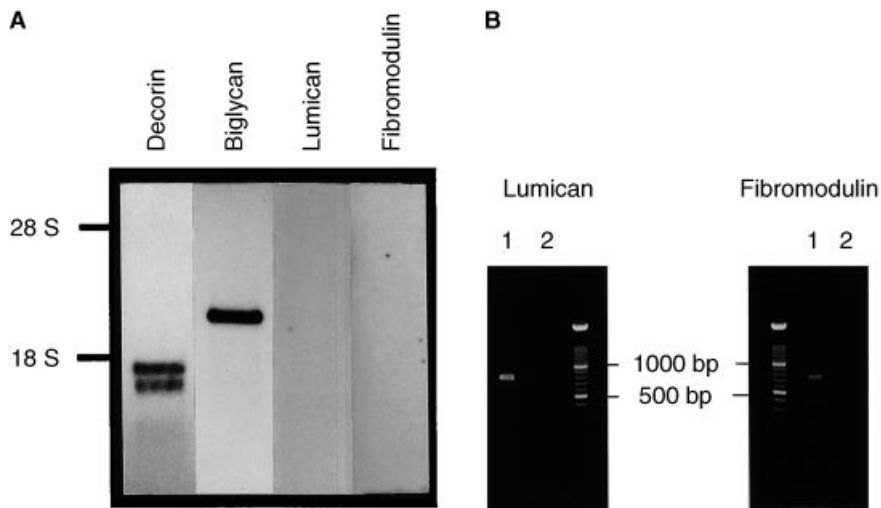
Fig. 1. Expression of small proteoglycans in isolated glomeruli of normal human kidney. (*A*) Northern blot analysis of decorin (1.8; 1.6 kb) and biglycan (2.8 kb) in isolated glomeruli from normal human kidneys. The glomerular expression of lumican and fibromodulin could not be detected by Northern blot analysis. 28 S and 18 S rRNA correspond to 4.7 and 1.9 kb, respectively. (*B*) Expression of lumican and fibromodulin mRNAs in isolated glomeruli from normal adult human kidneys as analyzed by RT-PCR (lane 1). Lane 2 represents negative controls (without RT product).

0.1% Triton X-100, and protease inhibitors (buffer 1). The columns were washed sequentially with 3 mL buffer 1 containing 7 mol/L urea, 3 mL urea-free buffer 1, and 3 mL buffer 1 containing 0.3 mol/L NaCl. Elution was achieved with 1.5 mL buffer 1 containing 1 mol/L NaCl. Upon adding 5 volume methanol and 1 volume chloroform, proteoglycans were collected at the interphase between chloroform and aqueous methanol and were washed with methanol. The samples were dissolved in 50 μL buffer, digested with chondroitin ABC lyase to remove the glycosaminoglycan chain, and subjected to polyacrylamide gel electrophoresis followed by Western blotting exactly as described [28]. The membrane was blocked with 10 mmol/L Tris/HCl, pH 7.4/0.15 mol/L NaCl (TBS) containing 3% casein, 1% goat serum, and 0.002% Tween 20. Incubation with the first antiserum (dilution 1:500 with TBS/1% BSA) was for 90 minutes at 37°C, whereas the second antibody, horseradish peroxidase-coupled goat anti-rabbit IgG (dilution 1:5000, EIA grade; Bio-Rad, Hercules, CA, USA) was applied for 90 minutes at ambient temperature. The probes were visualized by using the ECL Western blotting reagent kit according to the manufacturer's instructions (Amersham Pharmacia Biotech, Freiburg, Germany).

Using [35S]sulfate-labeled decorin from fibroblast secretions [10] as an internal standard indicated that the recovery after the ion exchange chromatography step on the DEAE Trisacryl matrix varied between 85 and 105%. The presence of 0.1% Triton X-100 was the critical component for achieving a good recovery. Additional control experiments, yielding the expected results, were performed by adding known quantities of decorin core protein to urine samples prior to polyacrylamide gel electrophoresis.

RESULTS

mRNA expression in isolated glomeruli and whole renal cortex from normal human kidneys

Northern blot analyses were performed using 40 μg of RNA extracted from isolated glomeruli. The signals expected for decorin mRNA (1.8 and 1.6 kb) and biglycan mRNA (2.8 kb) could easily be visualized (Fig. 1A). The use of probes for fibromodulin and lumican did not result in a detectable signal, although the probes were of a similar specific radioactivity as the ones for decorin and biglycan. Discrete expression of fibromodulin and lumican, however, could be verified by RT-PCR (Fig. 1B).

Using total RNA extracted from whole human renal cortex expression of all four small proteoglycans could be detected by Northern blot (data not shown). Quantitation of small proteoglycan mRNAs by a Phosphor Imager showed the following expression pattern in normal human renal cortex: biglycan (12.4 ± 1.4) > decorin (2.4 ± 0.3) > lumican (0.65 ± 0.12) > fibromodulin (0.39 ± 0.09). Data are expressed as mean (± SEM) ratios of small proteoglycan to GAPDH mRNAs, ×10 (N = 3).

Immunolocalization and in situ hybridization of small proteoglycans in glomeruli from normal human kidneys

From the overviews of the immunohistochemical stainings of decorin, biglycan, fibromodulin, and lumican in renal cortex as well as from the in situ hybridization results, it becomes evident that none of them are abundantly expressed in glomeruli (panels A and F of Figs. 2 through 5). Decorin could be detected in trace amounts in the mesangial matrix and in some podocytes. Glomerular endothelial cells were negative (Fig. 2B). In situ hybridization corroborated the weak expression by mes-
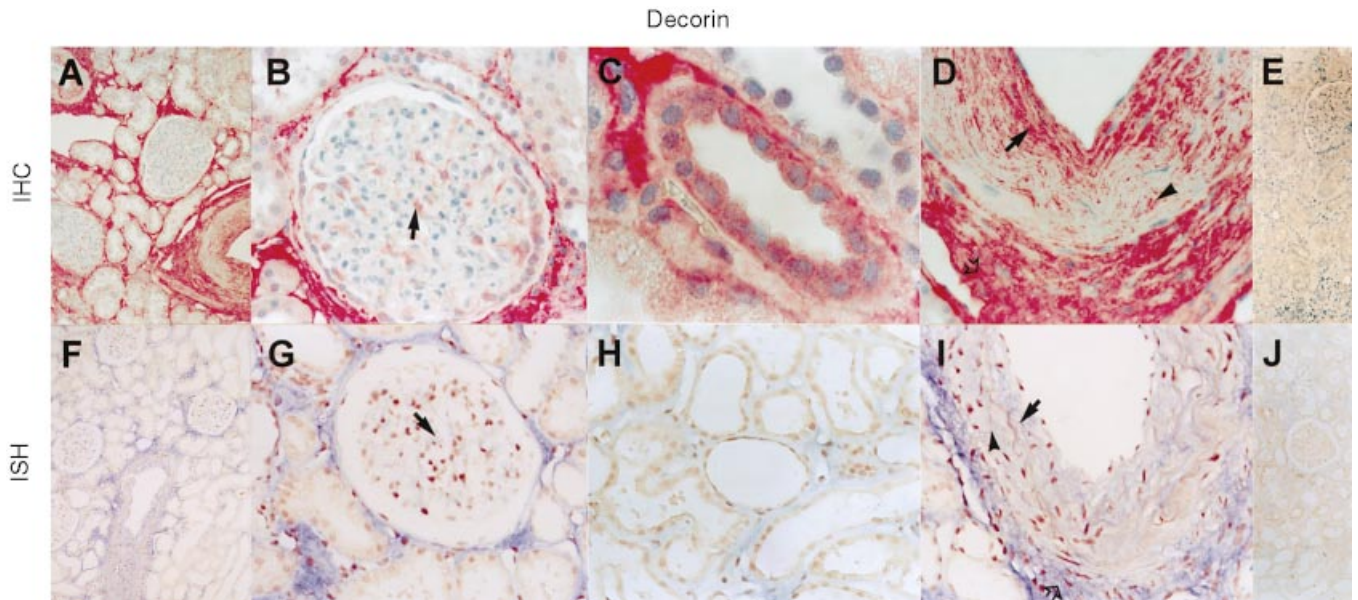
Decorin

Fig. 2. Immunolocalization (IHC-APAAP, *A–E*) and in situ hybridization (ISH; *F–J*) of decorin in normal adult human kidney. Renal cortex (A and F, ×100); glomerulus (B and G, ×400, arrow indicates mesangium), tubulointerstitium (C, ×1000 and *H*, ×400) and renal blood vessels (D and I, ×400, full arrow, intima; arrowhead, media; and open arrow, adventitia). Negative control for IHC was performed using nonimmune rabbit IgG (E, ×100). As negative control for ISH, digoxygenin-labeled sense riboprobe for human decorin was used (J, ×100).
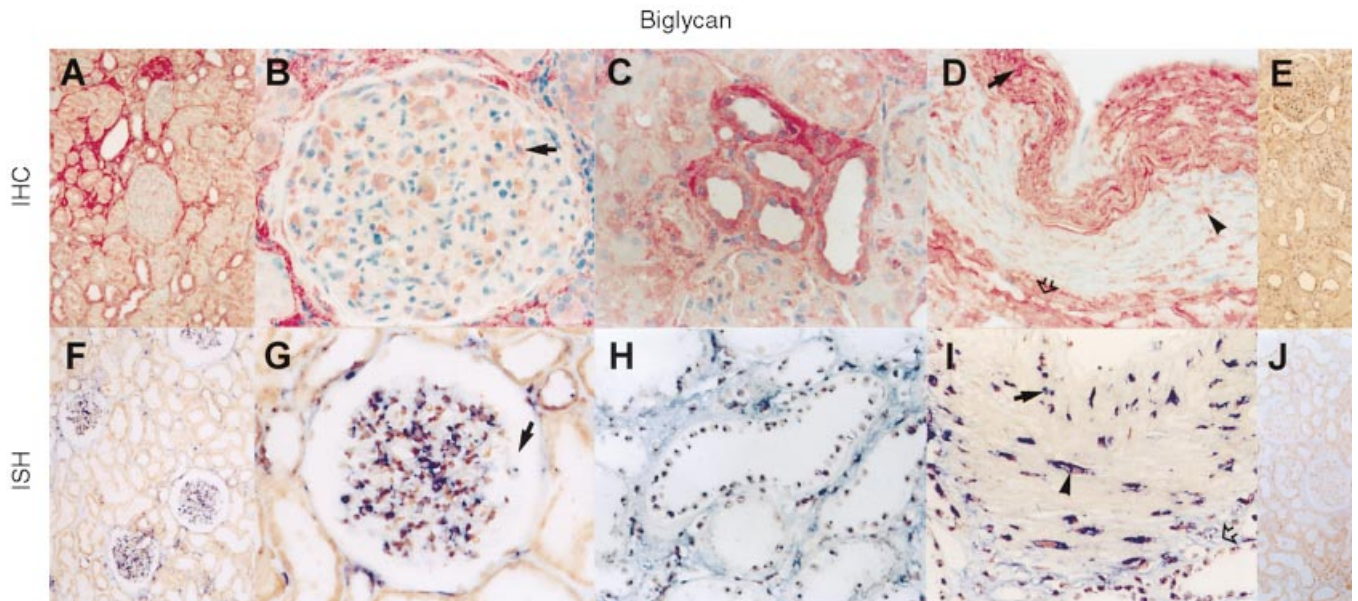
Biglycan

Fig. 3. Immunolocalization (IHC-APAAP; *A–E*) and in situ hybridization (ISH; *F–J*) of biglycan in normal adult human kidney: renal cortex (A and F, ×100); glomerulus (B and G, ×400, arrow indicates endothelial cells), tubulointerstitium (C and H, ×400) and renal blood vessels (D and I, ×400, full arrow, intima; arrowhead, media; and open arrow, adventitia). Negative control for IHC was performed using nonimmune rabbit IgG (E, ×100). As negative control for ISH digoxygenin-labeled sense riboprobe for human biglycan was used (J, ×100).

angial cells (Fig. 2G). In contrast, staining for biglycan was observed in endothelial cells, but also within the mesangial matrix and occasionally in epithelial cells (Fig. 3B). Correspondingly, mainly endothelial cells yielded a positive in situ hybridization signal (Fig. 3G). The expression

of fibromodulin was seen in the mesangium, but its presence in endothelial cells could not be shown (Fig. 4B, G). Lumican was confined to the mesangium (Fig. 5B, G). Specificity of immunostainings and in situ hybridizations were confirmed by negative controls (panels E and J of
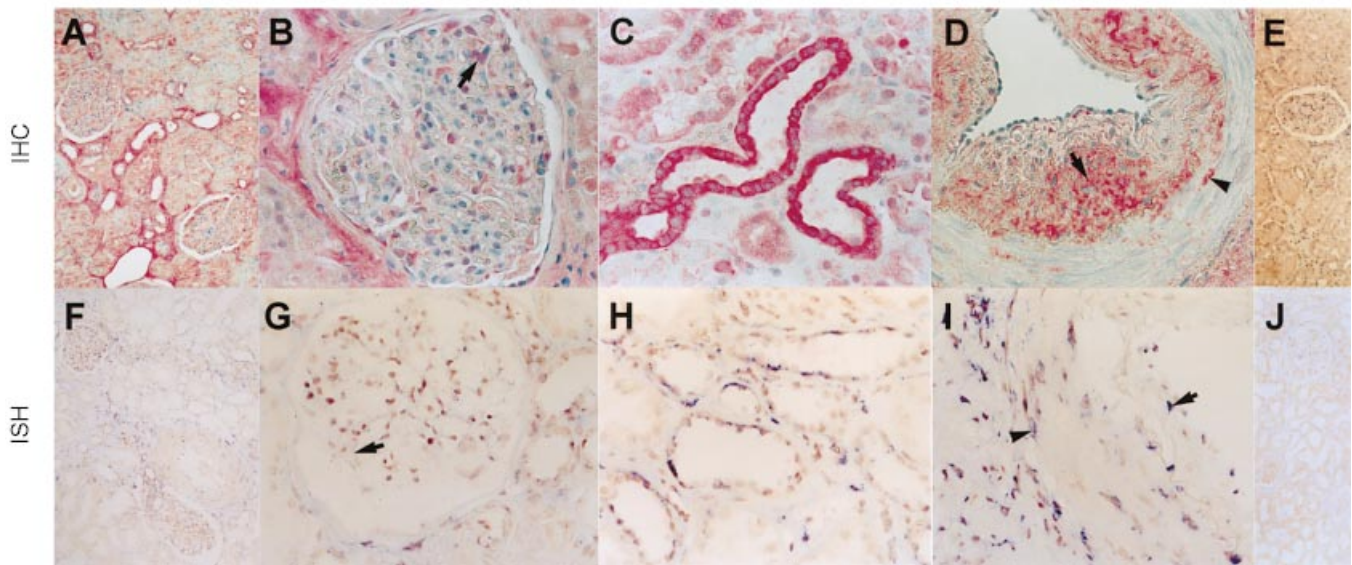
Fibromodulin

Fig. 4. Immunolocalization (IHC-APAAP; *A–E*) and in situ hybridization (ISH; *F–J*) of fibromodulin in normal adult human kidney: renal cortex (A and F, ×100); glomerulus (B and G, ×400, arrow indicates mesangium), tubulointerstitium (C and H, ×400) and renal blood vessels (D and I, ×400; full arrow, intima; and arrow head, media). Fibromodulin was detectable only with a double APAAP sandwich. Negative control for IHC was performed using nonimmune rabbit IgG (E, ×100). As a negative control for ISH, digoxygenin-labeled sense riboprobe for human fibromodulin was used (J, ×100).
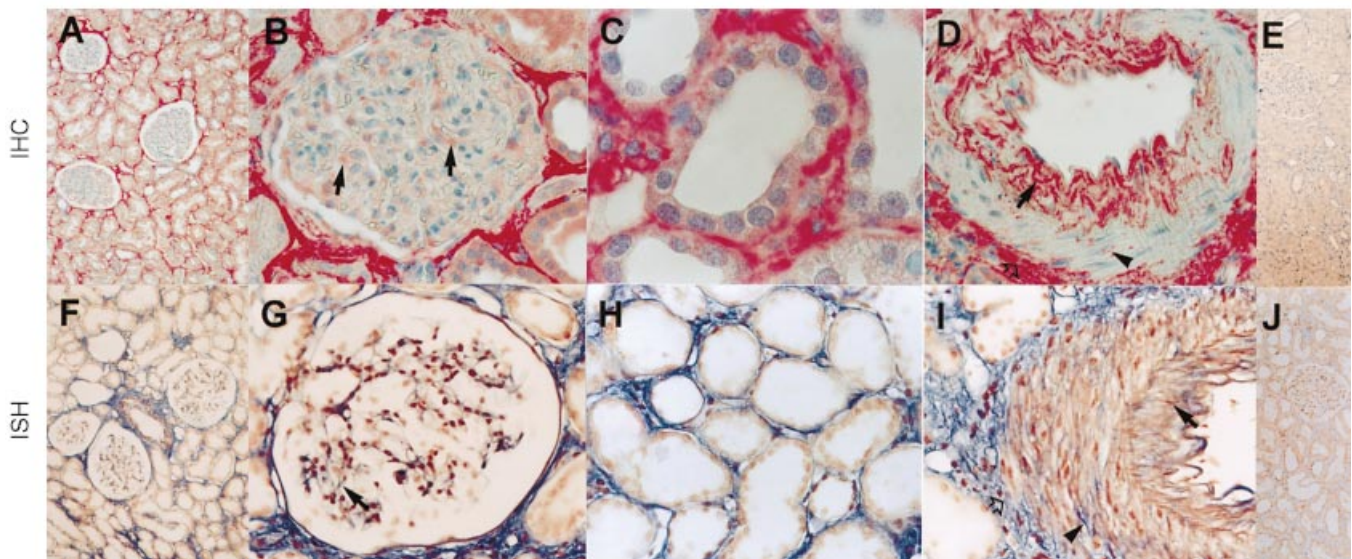
Lumican

Fig. 5. Immunolocalization (IHC-APAAP; *A–E*) and in situ hybridization (ISH; *F–J*) of lumican in normal adult human kidney: renal cortex (A and F, ×100); glomerulus (B and G, ×400, arrows indicate mesangium), tubulointerstitium (C, ×1000 and H, ×400) and renal blood vessels (D and I, ×400; full arrow, intima; arrowhead, media; and open arrow, adventitia). Negative control for IHC was performed using nonimmune rabbit IgG (E, ×100). As a negative control for ISH, digoxygenin-labeled sense riboprobe for human lumican was used (J, ×100).
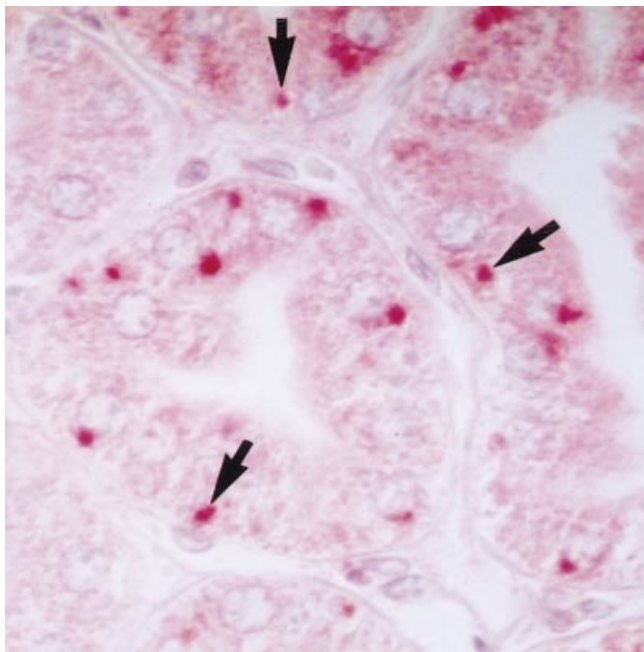
Fig. 6. Immunohistochemical detection of decorin in epithelial cells of proximal tubules from normal human kidney. Proximal tubules revealed punctate staining for decorin (indicated by arrows, APAAP, ×600). The sections were pretreated with pepsin.

Figs. 2 through 5). Results concerning glomerular expression of decorin, biglycan, fibromodulin, and lumican are summarized in Table 1.

In kidney sections from 11 different patients, reproducible glomerular stainings for the immunolocalization and expression of all four small proteoglycans were observed. The age difference between patients in the range of 38 to 62 years had no influence on the glomerular expression and immunolocalization of all four small proteoglycans.

Immunolocalization and in situ hybridization of small proteoglycans in tubuli, in the peritubular matrix, and in arterial vessel walls from normal human kidneys

Decorin predominated by immunohistochemistry and in situ hybridization in the renal interstitium with accumulations around tubules and in all layers of arterial blood vessels (Fig. 2A, C, D, F, H, and I). Occasionally, a punctate staining pattern was observed in the epithelia of distal tubules (Fig. 2C). Single cells of collecting ducts were also strongly positive (data not shown). Decorin mRNA was not detectable in tubular epithelial cells by in situ hybridization (Fig. 2H).

When tissue sections were subjected to a stronger proteolytic pretreatment by using pepsin instead of the protease from *Streptomyces griseus*, some proximal tubules contained punctate immuno-positive inclusions, although

decorin expression by in situ hybridization was never found in tubular epithelium (Fig. 6).

Biglycan was immunolocalized in the tubulointerstitium. There was strong positivity peritubularly as well as in association with epithelial cells of distal tubules and collecting ducts (Fig. 3C). In preglomerular arterioles, biglycan staining was observed in endothelial and smooth muscle cells and in the adventitia (Fig. 3D). In situ hybridization revealed biglycan mRNA in peritubular fibroblasts, distal tubules, and collecting ducts as well as in endothelial cells, smooth muscle cells, and the adventitia of blood vessels (Fig. 3H, I).

Fibromodulin was only weakly stained (detectable only with the double APAAP sandwich) and expressed in the normal human kidney. The strongest staining was found in the tubulointerstitium: peritubular cells and in cells of cortical collecting ducts and medullar distal tubules (Fig. 4C). Renal blood vessels stained for fibromodulin in the intima and media (Fig. 4D). In situ hybridization for fibromodulin gave signals in peritubular fibroblasts and weak expression in some epithelial cells of distal tubules and collecting ducts (Fig. 4H), as well as in the intima and media of blood vessels (Fig. 4I).

Staining for lumican in the normal adult human kidney was present mainly within the tubulointerstitium with an accumulation around the tubules (Fig. 5A, C, H). As in case of decorin, occasionally a punctate staining pattern for lumican was present in the epithelial cells of distal tubules (Fig. 5C). In renal blood vessels, lumican was found in smooth muscle cells of the media in the intima and adventitia (Fig. 5D). In situ hybridization studies confirmed the immunostaining in terms of interstitial cells and blood vessels (Fig. 5F, I). Tubular epithelial cells were negative for the expression of lumican (Fig. 5H). Results concerning tubulointerstitial expression of decorin, biglycan, fibromodulin, and lumican are summarized in Table 1.

These patterns of expression and immunolocalization of small proteoglycans in the tubulointerstitium and in vessel walls were reproducible in all 11 cases examined with some rather quantitative than qualitative differences, which appeared to be age-dependent. In some kidneys (particularly in those from patients older than 50 years), there was an increase of peritubular matrix with the accumulation of all four small proteoglycans in fibrotic areas. Age-related changes in vessel walls such as thickening of the neointima and enlargement of the peri-adventitia were associated with enhanced expressions of decorin, biglycan, fibromodulin, and lumican (in situ hybridization). In parallel, immunohistochemistry showed increased accumulation of all four proteoglycans in the peri-adventitia, while only biglycan, fibromodulin, lumican, and, to a much lesser degree, decorin were detected in the neointima of blood vessels.

Table 1. Expression of decorin, biglycan, fibromodulin, and lumican in the normal human kidney

	Glomerulus			Tubulointerstitium		
	Mesangial cells	Endothelial cells	Epithelial cells	Peritubular mesenchymal cells	Proximal tubule	Distal tubule
Decorin	±	−	−	+	−	−
Biglycan	±	+	±	+	−	+
Fibromodulin	±	−	−	+	−	+
Lumican	±	−	−	+	−	−

Abbreviations are: (+) = positive; (−) = negative; (±) = weakly or occasionally positive.
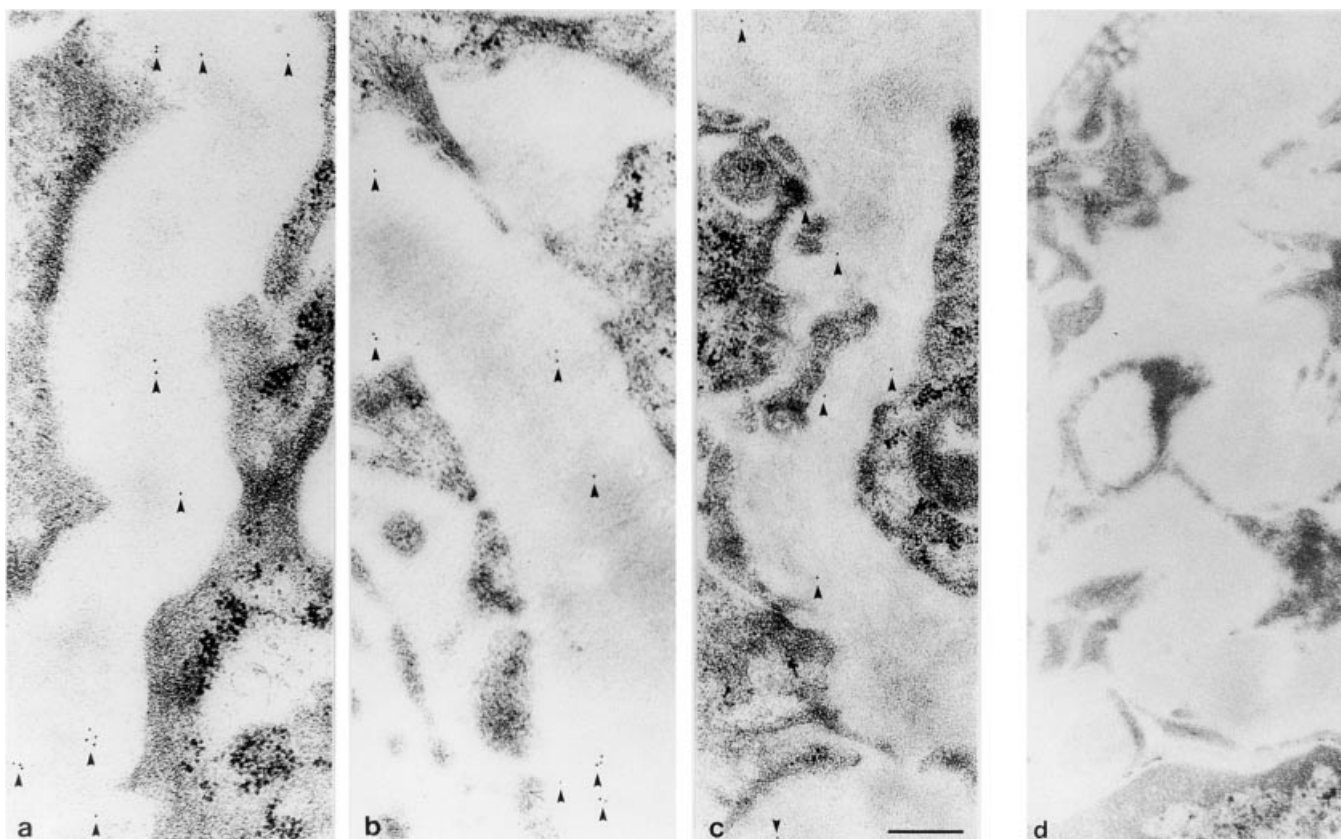

Fig. 7. Immunogold localization of biglycan (*a*), decorin (*b*), and lumican (*c*) in normal adult human kidney. The gold label is distributed uniformly over the basement membranes of glomeruli (as indicated by arrows). Bar, 0.25 μm. (*d*) A negative control for decorin where an antiserum preabsorbed to human decorin was used.

Localization of decorin, biglycan, and fibromodulin in the glomerular basement membrane from normal human kidney by immunogold electron microscopy

Small proteoglycans do not belong to the growing number of established basement membrane components. The glomerular basement membrane, however, showed a weak immunogold labeling for biglycan, decorin, and lumican core proteins (Fig. 7A–C). In controls without primary antibodies (data not shown) or by using an antiserum that was antigen-preabsorbed to human decorin (Fig. 7D), immunogold labeling was not detected. Because the antibody against fibromodulin was not suited

for use in Lowicryl-embedded tissue, fibromodulin could not positively be identified.

Urinary excretion of decorin

The detection of decorin, biglycan, and lumican in the basement membrane could simply reflect their filtration through the basement membrane. We therefore attempted to detect decorin, which can easily be purified by anion exchange chromatography, in the urine itself. However, very sensitive assay methods provided evidence that the urine of healthy subjects contained <1 μg/L of decorin core protein, which was below the
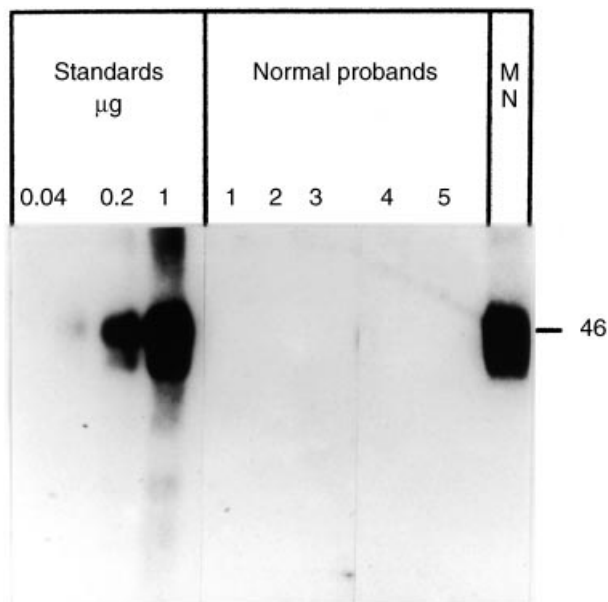
Fig. 8. Urinary decorin excretion detected by Western blot analysis. Urinary concentrates from 25 mL of urine were applied per lane. Lanes 1 through 5 represent urine from normal subjects. MN denotes urine from a patient with membranous nephropathy. Purified decorin core protein (0.04 to 1 μg) was used as a standard.

limit of detection. Urine from a patient with membranous nephropathy was included for control purposes. This urine did contain >40 μg core protein/L (Fig. 8). Biglycan was not detected in the urine either from the healthy controls nor from the patient with membranous nephropathy.

Immunolocalization of decorin, biglycan, fibromodulin, and lumican in renal biopsies showing membranous nephropathy

All four small proteoglycans were immunolocalized in renal biopsies from three patients suffering from membranous nephropathy (mean serum creatinine, 178.5 ± 38.3 μmol/L) in order to give an example of the localization of decorin, biglycan, fibromodulin, and lumican in renal pathology. Figure 9 represents the renal biopsy specimen from one of the three patients with the most advanced stage of disease (serum creatinine, 255 μmol/L) showing diffuse glomerulosclerosis and tubulointerstitial fibrosis. As can be seen, there was a remarkable increase in antigen positivity for all four small proteoglycans in the tubulointerstitium that was especially pronounced in areas of fibrosis (Fig. 9, left panels). Comparable changes were also observed in the two other cases, albeit to a lesser degree (data not shown). Despite of the changes in glomerular morphology, there was only a moderate accumulation of decorin, biglycan, fibromodulin, and lumican in areas of glomerular fibrosis, even when different techniques of antigen demasking (proteolytic digestion or

microwave treatment) were used (Fig. 9, "glomerular" panels). Only in scarred glomeruli did all four proteoglycans strongly accumulate in Bowman's capsule and the periglomerular region as well as in areas of fibrous organization of the urinary space (the "fibromodulin" panel in Fig. 9).

DISCUSSION

To our knowledge this is the first report showing that in addition to decorin and biglycan, further members of the small proteoglycan family, that is, fibromodulin and lumican, are expressed in the adult human kidney. In isolated glomeruli, mRNA of the latter two proteoglycans could be detected only after RT-PCR. However, the half-lives of these mRNA species are not known, and the immunohistochemistry and in situ hybridization data demonstrated the presence of all four proteoglycans in the mesangial matrix of glomeruli, although the signal intensity was low. Biglycan was the only small proteoglycan that could be shown to be expressed in glomerular endothelial cells. As was to be expected for extracellular matrix proteins, decorin, biglycan, fibromodulin, and lumican were all present in the renal interstitium. Biglycan and fibromodulin had in common that they were synthesized and deposited in the epithelia of distal tubules and collecting ducts. Surprisingly, decorin and lumican occasionally showed a punctate staining pattern in tubular epithelia, and some cells of the cortical collecting ducts were also positive for decorin. It could not be determined whether these cells represent one or more of the different subtypes of intercalated cells or individual principal cells. However, in no case did epithelial cells give a positive in situ hybridization signal for decorin or lumican. It is therefore likely that either these two proteoglycans were synthesized by nearby interstitial cells or that they were endocytosed from the lumen of the tubuli. The latter possibility is in accordance with (1) the presence of small proteoglycans in the glomerular basement membrane, (2) the presence of the decorin/biglycan endocytosis receptor in the distal tubuli of rat kidney [24], and (3) the absence of decorin core protein in the urine. The immunohistochemical appearance of decorin in proximal tubules is compatible with its presence in large secondary lysosomes, but further ultrastructural studies are required to prove this hypothesis.

The colocalization of all four proteoglycans in the mesangium and in the tubulointerstitium as well as the distinct presence of selected family members in glomerular endothelium and in tubules clearly raises the question of the conditions where either all or only a particular small proteoglycan play a distinct pathophysiological role. While this question has not been addressed in the present article, the data provide a basis for interpreting pathological situations. It had been shown, for example,
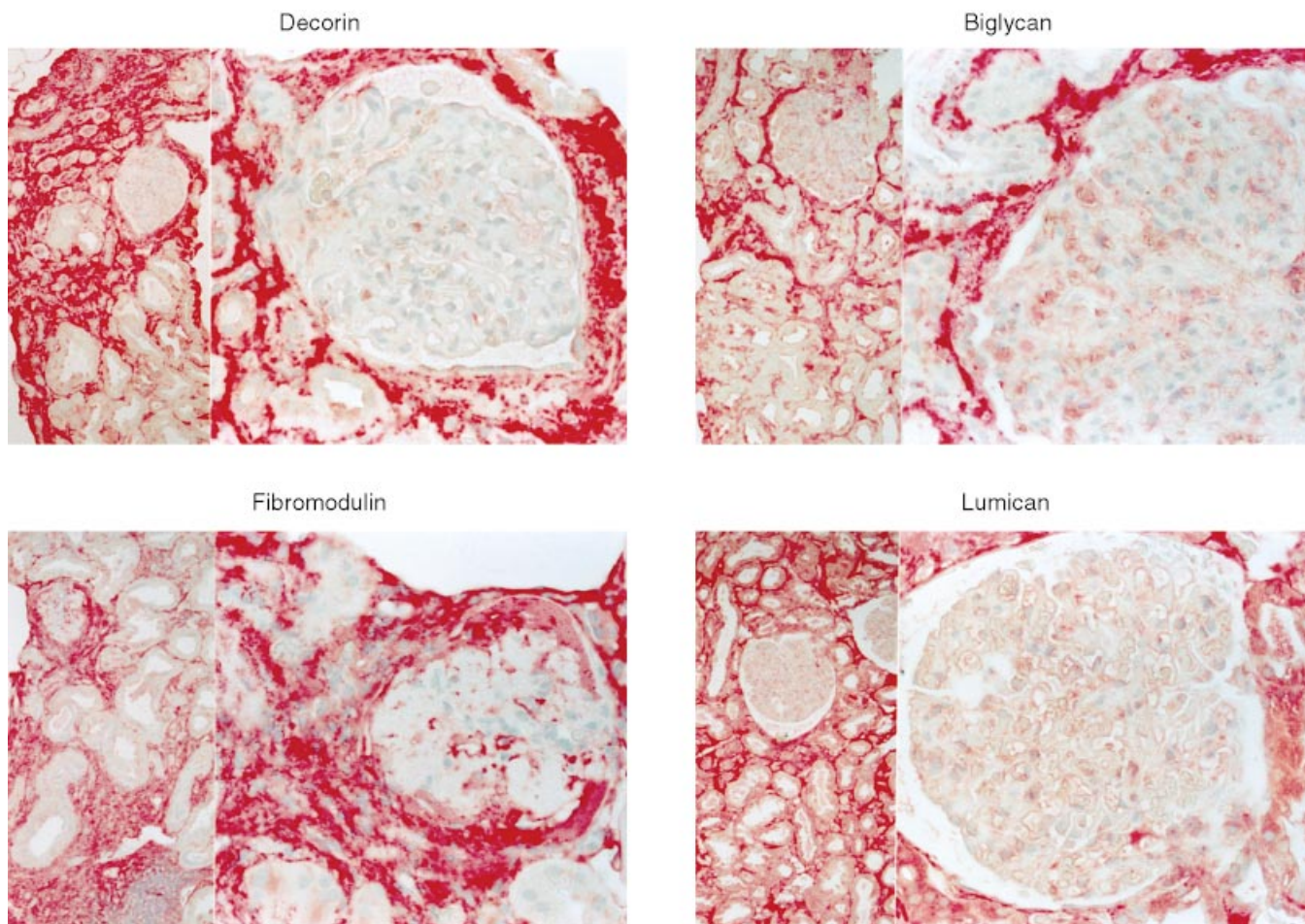
Fig. 9. Immunolocalization of decorin, biglycan, fibromodulin, and lumican in a renal biopsy showing membranous nephropathy. Left panels represent immunostaining for all four small proteoglycans in cortical sections and show expanded areas of fibrosis in the tubulointerstitium (APAAP, ×100). Right panels represent glomerular immunolocalization of small proteoglycans (APAAP, ×400). Fibromodulin was detected using a double APAAP sandwich technique. All sections were pretreated with 0.05% protease type XIV from *Streptomyces griseus* (EC 3.4.24.31) for eight minutes at 37°C followed by 7.5 mU/mL of chondroitin ABC lyase (EC 4.2.2.4) for one hour at 37°C.

that glomerular endothelial cells participate in the repair of glomerulonephritis [31]. Biglycan, which is constitutively expressed by endothelial cells, plays a role during endothelial cell migration [32], whereas the induction of decorin in endothelial cells is an angiogenesis-related and apoptosis-preventing event [19]. In light of the conflicting data as to whether complex formation of TGF-β with decorin leads to an inactivation [13], an activation [33], or to a sequestration without an activity change of the cytokine [34], one could also hypothesize that the beneficial effects of decorin in the anti–Thy-1 model of acute mesangioproliferative glomerulonephritis could be due to improved endothelial regeneration. This could be considered as an analogous situation to the VEGF$_{165}$-mediated repair seen in the same model of glomerulonephritis [35].

In renal biopsies from patients suffering from membranous nephropathy, a remarkable increase in antigen posi-

tivity of all four small proteoglycans was observed in the tubulointerstitium, and was especially pronounced in areas of fibrosis, while in the glomerulus, there was only very moderate accumulation of decorin, biglycan, fibromodulin, and lumican. In end-stage glomerulosclerosis, all four proteoglycans strongly accumulated in Bowman's capsule and in areas of fibrous organization of the urinary space. These findings provide good evidence of the importance of all four small proteoglycans in renal disease, since even fibromodulin, which is normally present in the kidney only in very limited amounts, strongly accumulated in areas of fibrosis.

Biglycan and decorin received specific attention in studies of fibrotic kidney diseases because of the proposed regulatory loop between TGF-β elevation, increased matrix production, and complex formation between small proteoglycans and the cytokine. The presence of fibromodulin and lumican in the mesangial matrix and in the

tubulointerstitium suggests that this loop is even more complex. On the one side, fibromodulin is known to interact with TGF-β, too [12], whereas at present, there are no reports on the respective property of lumican. The structural similarities among the small proteoglycans, however, suggest that lumican also binds TGF-β. On the other hand, TGF-β affects the expression of small proteoglycans differently. In all models studied so far, the cytokine led to a marked up-regulation of biglycan. Minor effects were seen with decorin expression, and fibromodulin expression was not influenced in any of the cells investigated [36]. Chronic exposure to circulating TGF-β, however, also caused an up-regulation of decorin in mouse kidney [37].

The functional importance of the expression of biglycan and fibromodulin by the epithelial cells of distal tubules and collecting ducts remains unknown at present. While these cells most likely express a unique extracellular matrix [38], the architecture of this matrix and the binding partners of the two small proteoglycans remain to be determined. Candidate molecules are type V and type VI collagens, among others [39].

In conclusion, the present study provides evidence for a distinct expression pattern of four members of the small leucine-rich proteoglycan family in adult human kidney cortex, thereby providing a basis for elucidating the role of the individual family members in the pathogenesis of various kidney diseases.

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