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Expression of decorin, biglycan, and collagen type I in human renal fibrosing disease

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Background. The extracellular matrix proteoglycans decorin and biglycan may have a pathogenic role in renal fibrosing disease via regulation of the activity of growth factors, such as transforming growth factor-β, and effects on collagen type I fibrillogenesis. The expression of decorin and biglycan in human glomerular diseases characterized by mesangial sclerosis is unknown.

Methods. Decorin, biglycan, and collagen type I were localized immunohistochemically in human renal biopsy cases of amyloidosis ($N = 18$), diabetic nephropathy ($N = 11$), fibrillary glomerulonephritis ($N = 5$), immunotactoid glomerulopathy ($N = 5$), light-chain deposition disease ($N = 4$), idiopathic mesangial sclerosis ($N = 4$), and nephrosclerosis ($N = 6$), and in morphologically normal tissues obtained from tumor nephrectomies ($N = 8$). Decorin and biglycan mRNA synthesis was evaluated by *in situ* hybridization.

Results. Decorin and biglycan protein were not identified in normal glomeruli. Decorin accumulated in amyloid deposits, but not in deposits of fibrillary glomerulonephritis or immunotactoid glomerulopathy. Biglycan weakly accumulated in amyloid deposits, and both decorin and biglycan weakly stained mesangial nodules in cases of morphologically advanced light-chain deposition disease and diabetic nephropathy. In all analyzed cases, irrespective of the underlying disease, decorin and biglycan accumulated in glomeruli in areas of fibrous organization of the urinary space and in areas of tubulointerstitial fibrosis. Biglycan, but not decorin, accumulated in the neointima of arteriosclerotic blood vessels. Decorin and biglycan mRNA synthesis was detected at sites of proteoglycan accumulation in glomeruli, interstitium, and neointima. Collagen type I colocalized with decorin and biglycan deposits.

Conclusions. Differences in extracellular matrix proteoglycan composition may be diagnostically useful in distinguishing morphologically similar diseases. Distinct patterns of proteoglycan expression may be related to modulation of specific growth factor activity in different glomerular diseases.

Key words: mesangial sclerosis, proteoglycans, amyloidosis, growth factor, extracellular matrix, glomerulosclerosis, arteriosclerosis, tubulointerstitial fibrosis.

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Extracellular matrix accumulation in mesangial areas may contribute to impaired glomerular function in diverse human renal diseases, including amyloidosis, diabetic nephropathy, light-chain deposition disease (LCDD), fibrillary glomerulonephritis, immunotactoid glomerulopathy, and idiopathic mesangial sclerosis. Proteoglycans are major components of the extracellular matrix that have diverse biologic functions, including binding and inactivation of growth factors, including basic fibroblast growth factor (FGF-2) and transforming growth factor-β (TGF-β), and regulation of collagen fibrillogenesis [1, 2]. Decorin and biglycan are members of the family of small, leucine-rich proteoglycans that may be particularly relevant to the pathogenesis of renal fibrosing injury [3]. *In vitro* studies have indicated that the synthesis of decorin and biglycan by fibroblasts, mesangial cells, and smooth muscle cells may be regulated by TGF-β and that decorin may bind and inactivate TGF-β [1, 2]. Further support for a role for decorin as a negative regulator of TGF-β activity comes from *in vivo* observations in the anti-Thy 1 rat model of mesangioproliferative glomerulonephritis, in which the administration of exogenous decorin [4], or decorin gene therapy [5], prevented the extracellular matrix accumulation that has been attributed to the action of TGF-β. Border and Noble have proposed that a relative "deficiency" of decorin may contribute to TGF-β–mediated renal injury [6]. The functions of biglycan are largely unknown. Besides possible roles in regulating TGF-β activity and collagen fibril formation, it may alter the bioavailability of decorin by competing for a shared re-uptake receptor [7]. Determining the expression of decorin and biglycan in human renal disease could provide insights into pathogenesis and could possibly lead to the design of novel therapeutic strategies.

In experimental renal injury, the expression of decorin and biglycan has been localized to glomerulosclerosis lesions and tubulointerstitial fibrosis [7–9]. Decorin, biglycan, and their shared endocytosis receptor have been detected in glomerulosclerosis lesions in diseased rat kid-

ney [7]. In human kidney, decorin has been immunolocalized to areas of interstitial fibrosis, but not in glomeruli [10–12]. Biglycan has been localized in glomeruli and collecting duct epithelial cells in developing human kidney [10]. However, the distribution of biglycan in adult human kidney has not previously been determined. A key role for decorin has been postulated in regulating collagen fibrillogenesis, as demonstrated by the finding of abnormal collagen fibrils in decorin-deficient mice [13]. Both decorin and biglycan interact with collagen type I *in vitro*, and codeposition of these molecules has been described in various models of fibrosing injury [14, 15].

In this study, we examined the expression of decorin, biglycan, and collagen type I in human renal tissues demonstrating a spectrum of glomerulopathies and tubulointerstitial fibrosis. Glomerular diseases characterized by accumulations of extracellular matrix (mesangial sclerosis) were selected to test the hypotheses (*a*) that decorin and biglycan are up-regulated in glomerular sclerosing injury, and (*b*) that differences in extracellular matrix proteoglycan composition can be used to distinguish morphologically similar diseases.

METHODS

Tissue selection and pathologic examination

Archived paraffin-embedded diagnostic renal biopsy tissues from cases of amyloidosis ($N = 18$), diabetic nephropathy ($N = 11$), fibrillary glomerulonephritis ($N = 5$), immunotactoid glomerulopathy ($N = 5$), LCDD ($N = 4$), nephrosclerosis ($N = 6$), and idiopathic mesangial sclerosis ($N = 4$) were selected for study. All cases were characterized by light microscopy, immunofluorescence microscopy, and electron microscopy, using standard techniques [16]. Clinical and pathologic data pertaining to the cases of immuntactoid glomerulopathy have been described in detail elsewhere [17]. Amyloid deposits were identified by green/red birefringent Congo Red staining under polarized light. Immunohistochemical staining of Congo Red material with a monoclonal antibody to amyloid A (AA) protein (Dako, Carpinteria, CA, USA) were positive in one biopsy. All other cases of amyloidosis were derived from κ or λ light chains (AL), as demonstrated by routine immunofluorescence microscopic studies. Idiopathic mesangial sclerosis was diagnosed by exclusion of the other causes of mesangial sclerosis listed previously in this article [18]. Macroscopically normal-appearing renal tissues were obtained from nephrectomies ($N = 8$) for localized renal carcinoma at sites away from the tumor. Renal biopsy tissues were fixed in 10% neutral-buffered formalin. Portions of nephrectomy tissues were fixed in both 10% neutral-buffered formalin and in methyl Carnoy's fixative (60% methanol, 30% chloroform, and 10% acetic acid).

Antibodies

Polyclonal rabbit antisera LF-51, LF-30/136, and LF-67 (to human decorin, human biglycan, and human collagen type I, respectively) were kind gifts of Dr. Larry Fisher (National Institute of Dental Research, Bethesda, MD, USA). LF-30/136 was generated against a synthetic peptide corresponding to amino acids 5 to 17 of the core protein of human PGII/decorin, conjugated to keyhole limpet hemocyanin [19]. LF-51 was generated against a synthetic peptide corresponding to amino acids 11 to 25 of the core protein of the secreted form of human bone PGI/biglycan, conjugated to bovine serum albumin (BSA) [19]. LF-67 was generated against 26 amino acids of a synthetic C telopeptide antigen of the human collagen α I (I) chain [20]. The specificities of these antisera have been previously confirmed in Western blot and immunoprecipitation experiments [15, 19–21].

Immunohistochemistry

Sections were deparaffinized in xylene and rehydrated in a descending ethanol series. Endogenous peroxidase was quenched with 3% H_2O_2 for 10 minutes. For LF-30/136 and LF-51 staining, sections were digested with chondroitinase ABC lyase (ICN Biomedicals, Costa Mesa, CA, USA) at 250 mU/mL in 0.1 mol/L Tris, 0.01% BSA, for 60 minutes at 37°C, followed by blocking with normal goat serum for 10 minutes. For collagen type I staining, sections were digested in proteinase K (5 μg/mL at 37°C for 15 min; Sigma, St. Louis, MO, USA). Sections were incubated with primary antibody and diluted in 1% BSA/phosphate-buffered saline (PBS) for one hour at room temperature (LF-36/130, 1:250; LF-51 and LF-67, 1:500), followed by a biotinylated goat antirabbit antibody (Vector, Burlingame, CA, USA), ABC-Elite reagent (Vector). The reaction product was visualized with 3,3′ diaminobenzidine (Sigma) and nickel chloride enhancement. The slides were counterstained with methyl green. Negative controls for immunohistochemistry included substitution of the primary antibody with equal amounts of an irrelevant rabbit IgG (Dako).

Molecular probes

Two pBluescript SK plasmids containing either human bone decorin cDNA (plasmid P2) or human bone biglycan cDNA (plasmid P16) were generous gifts of Dr. Larry Fisher (National Institute of Dental Research). Plasmid P16 contains a 1658 bp insert with the complete protein encoding sequence of the human biglycan gene [21]. Plamid P2 contains a 1.6 kb insert with the protein encoding sequence of the human decorin gene [21]. The plasmids were linearized with Xba I and Kpn I (P16) or BamH I and Kpn I (P2) and transcribed into both antisense and sense (negative control) riboprobes in T3- or T7-primed reactions [21], using reagents from Pro-

Table 1. Localization of decorin, biglycan, and collagen type I in human glomerular disease

	Decorin core peptide	Decorin mRNA	Biglycan core peptide	Biglycan mRNA	Collagen type I
Normal	−	−	−	+ MC + PEC	−
Amyloidosis	+	+MC	+/−	+ MC + PEC	+
LCDD	+/−	−	+/−	+ MC + PEC	+/−
Fibrillary glomerulonephritis	−	−	−	+ MC + PEC	−
Immunotactoid glomerulopathy	−	−	−	+ MC + PEC	−
Idiopathic mesangial sclerosis	−	−	−	+ MC + PEC	−
Diabetic nephropathy	+/−	−	+/−	+ MC + PEC	+/−
Nephrosclerosis	+u	+MC	+u	+ MC + PEC	+u

Abbreviations are: MC, mesangial cells; PEC, parietal epithelial cells; LCDD, light-chain deposition disease; u, fibrous organization of urinary space; +, positive; −, negative; ±, weakly and/or focally positive

mega (Madison, WI, USA; except ^{35}S-UTP, obtained from New England Nuclear, Boston, MA, USA).

In situ hybridization

Formalin-fixed, paraffin-embedded tissues were deparaffinized following standard protocol. The sections were washed with 0.5 × standard sodium citrate (SSC; GIBCO, Grand Island, NY, USA) and digested with 5 μg/mL proteinase K (Sigma) in Tris buffer (500 mmol/L NaCl, 10 mmol/L Tris, pH 8.0) for 30 minutes at 37°C. Several 0.5 × SSC washes were followed by prehybridization for two hours in 50 μL of prehybridization buffer [50% formamide, 0.3 mol/L NaCl, 20 mmol/L Tris, pH 8.0, 5 mmol/L ethylenediaminetetraacetic acid (EDTA), 1 × Denhardt's solution, 10% dextran sulfate, 10 mmol/L dithiothreitol (DTT), 500 μg/mL yeast tRNA] at 50°C. The hybridization was started by adding 500,000 cpm of ^{35}S-labeled riboprobe in 50 μL of prehybridization buffer and was allowed to proceed overnight at 50°C. Sections were then washed with 0.5 × SSC, followed by RNase A (20 μg/mL, 30 min at room temperature), 2 × SSC washes (2 × 2 min), three high-stringency washes with 0.1 × SSC/0.1% Tween 20 (Sigma) at 50°C, and several 2 × SSC washes. The slides were dipped in NTB2 nuclear emulsion (Kodak, Rochester, NY, USA) and exposed in the dark at 4°C for one to four weeks. After developing, sections were counterstained with hematoxylin and eosin and were dehydrated and coverslipped. Positive cellular labeling was defined as five or more silver grains in a single cell. For negative controls, simultaneous hybridization with the sense riboprobe was performed on replicate tissue sections.

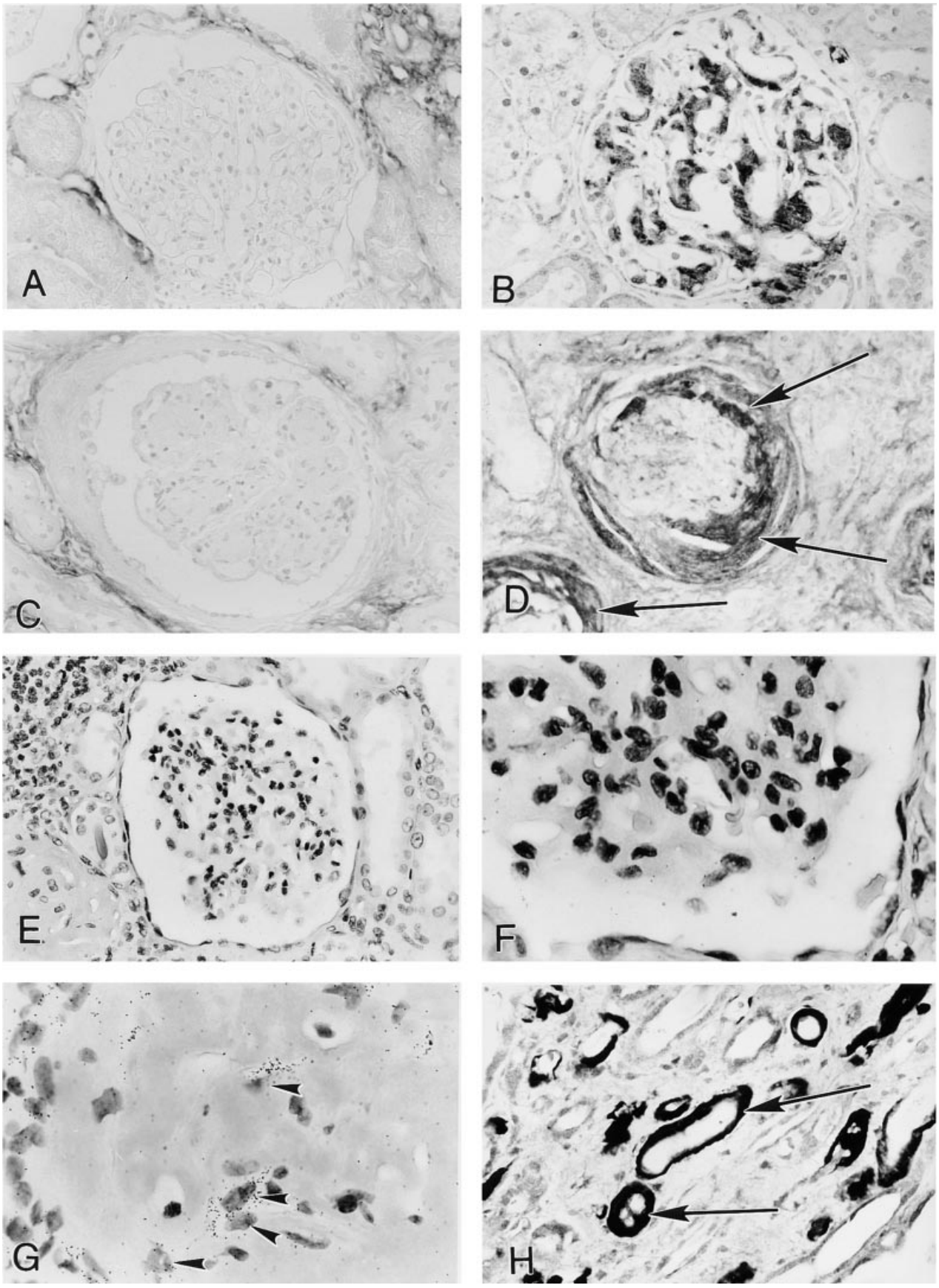
RESULTS

Histologic examination

Tissues demonstrated a spectrum of histologic patterns, including normal glomerular morphology (mostly in tumor nephrectomies), variably increased mesangial matrix and mesangial hypercellularity, capillary wall thickening, and nodular mesangial sclerosis. Many tissues also showed focal and segmental glomerulosclerosis and obsolescent (globally sclerotic) glomeruli. Some glomeruli showed ischemic changes consisting of wrinkling of glomerular basement membranes and fibrous thickening of Bowman's capsules. There was variable interstitial fibrosis, usually accompanied by a patchy, nonspecific mononuclear inflammatory cell infiltrate. Arterial vessels frequently showed arteriosclerosis. Arteriolar hyalinosis and arterial amyloid deposits were identified in individual cases. The severity of glomerulosclerosis, tubulointerstitial fibrosis, and arteriosclerosis was relatively consistent within individual cases.

Immunolocalization and mRNA expression of decorin

The results of immunohistochemical and *in situ* hybridization studies for decorin are summarized in Table 1 and Figure 1. Decorin protein and mRNA were not identified in morphologically normal glomeruli (Fig. 1 A, E, and F). A distinct pattern of decorin expression was evident in different glomerular diseases. Decorin localized to amyloid deposits, in glomeruli, tubulointerstitium, and arterial walls (Figs. 1 B, H, and J). The intensity and distribution of decorin immunostaining correlated with staining for Congo Red in individual cases. Deposits of both AA and AL amyloid showed a similar reaction. No significant immunostaining for decorin was identified in mesangial areas in most cases of diabetic nephropathy, LCDD, fibrillary glomerulonephritis, immunotactoid glomerulopathy, or idiopathic mesangial sclerosis (Fig. 1C). Occasional glomeruli with features of nodular mesangial sclerosis in some cases of LCDD or diabetic nephropathy demonstrated weak, focal staining for decorin (data not shown). Decorin pro-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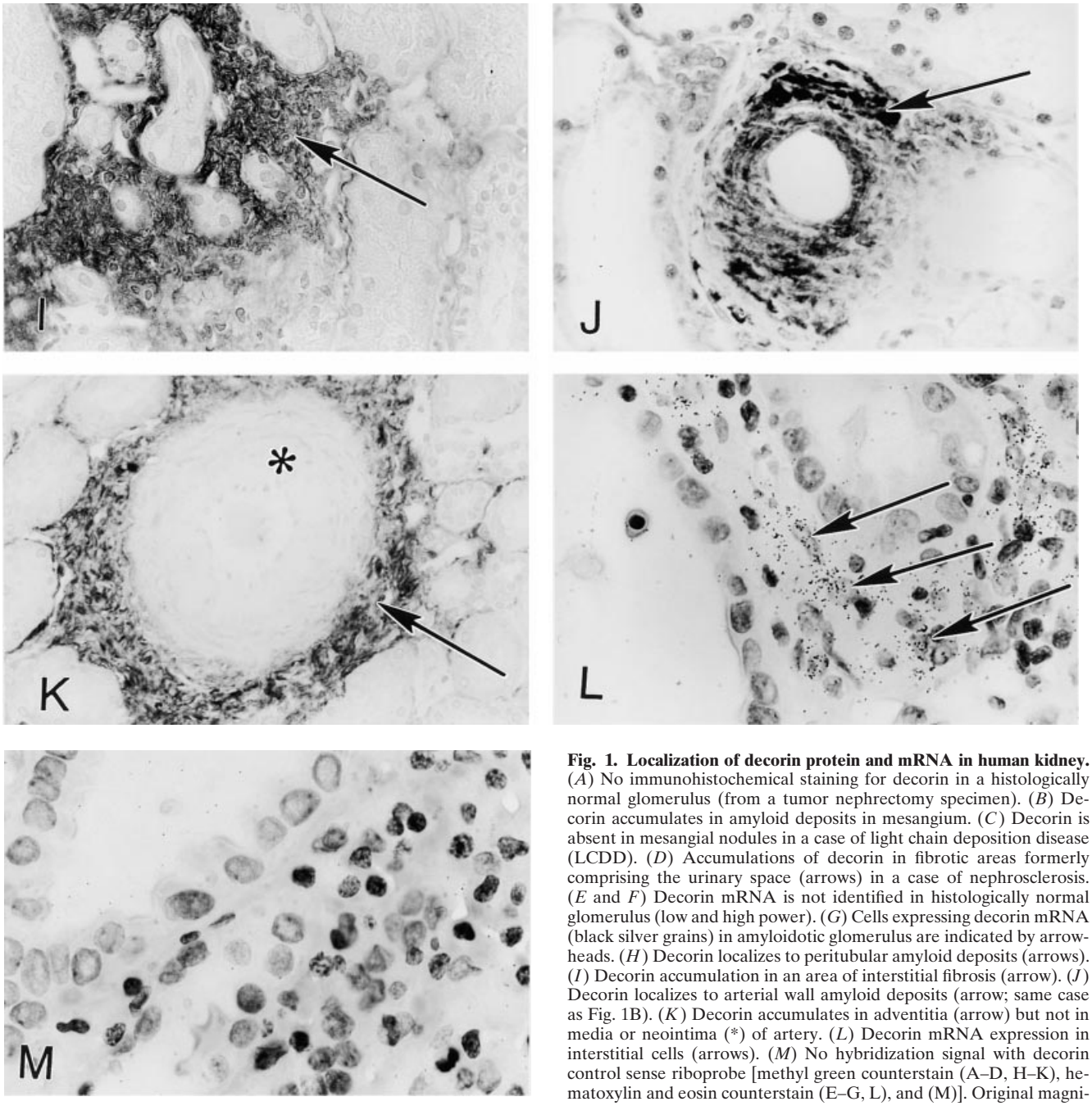
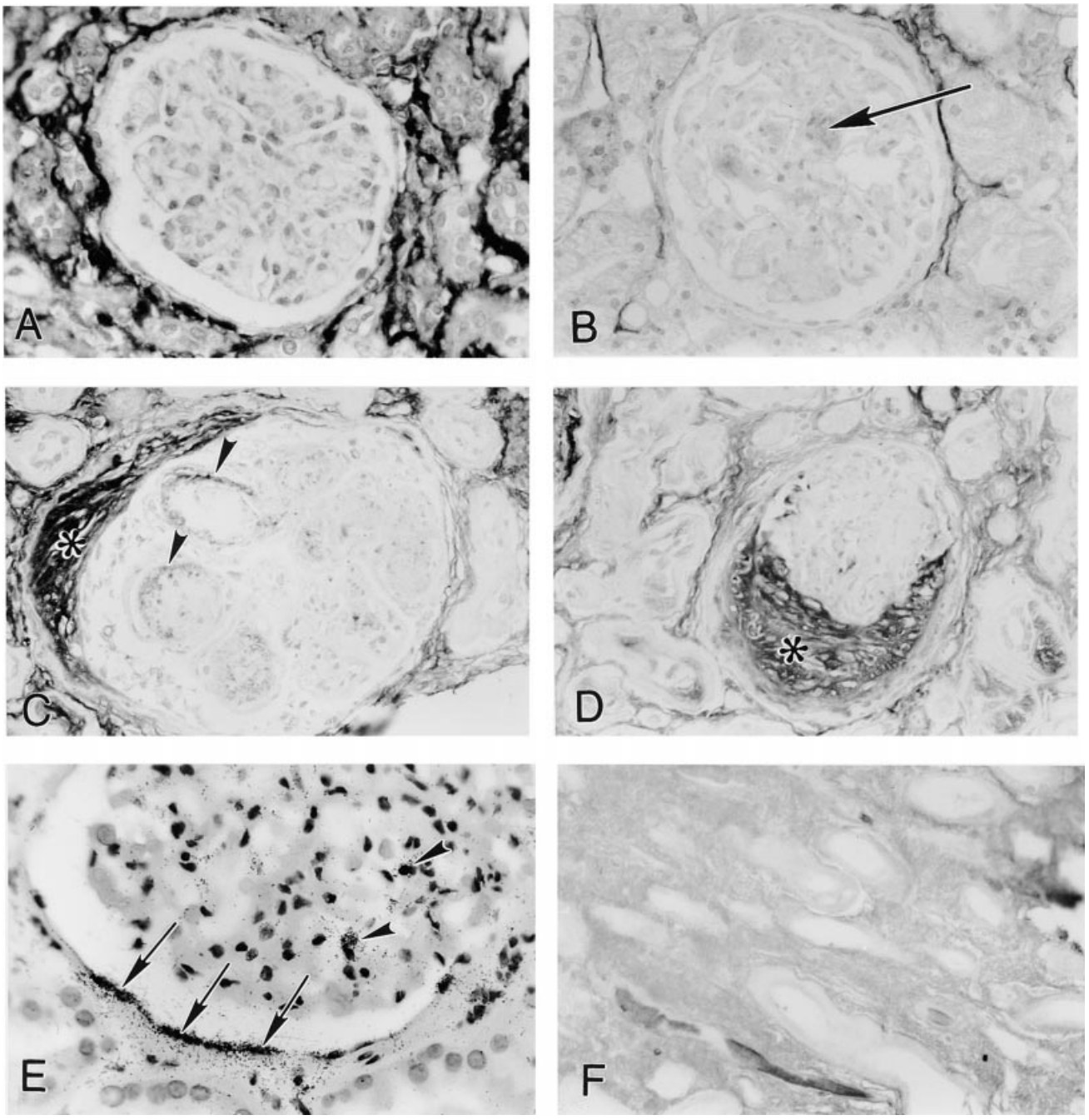


Fig. 1. Localization of decorin protein and mRNA in human kidney. (*A*) No immunohistochemical staining for decorin in a histologically normal glomerulus (from a tumor nephrectomy specimen). (*B*) Decorin accumulates in amyloid deposits in mesangium. (*C*) Decorin is absent in mesangial nodules in a case of light chain deposition disease (LCDD). (*D*) Accumulations of decorin in fibrotic areas formerly comprising the urinary space (arrows) in a case of nephrosclerosis. (*E* and *F*) Decorin mRNA is not identified in histologically normal glomerulus (low and high power). (*G*) Cells expressing decorin mRNA (black silver grains) in amyloidotic glomerulus are indicated by arrowheads. (*H*) Decorin localizes to peritubular amyloid deposits (arrows). (*I*) Decorin accumulation in an area of interstitial fibrosis (arrow). (*J*) Decorin localizes to arterial wall amyloid deposits (arrow; same case as Fig. 1B). (*K*) Decorin accumulates in adventitia (arrow) but not in media or neointima (*) of artery. (*L*) Decorin mRNA expression in interstitial cells (arrows). (*M*) No hybridization signal with decorin control sense riboprobe [methyl green counterstain (A–D, H–K), hematoxylin and eosin counterstain (E–G, L), and (M)]. Original magnification A–D, H–K ×400; E–G, L, and M ×1000.

tein accumulated in scarred glomeruli in which the urinary space was obliterated, with fibrous adhesion of the glomerular tuft to Bowman's capsule (Fig. 1D). This finding was especially prominent in cases of nephrosclerosis, but was also present to a variable degree in many of the other diseased tissues examined. By *in situ* hybridization, decorin mRNA expression was detectable within some sclerosed glomeruli, including amyloidotic glomeruli (Fig. 1G).

In the tubulointerstitial compartment, decorin accumulated in expanded zones of interstitial fibrosis (Fig. 1I). The intensity of decorin immunostaining showed some variation between cases, but appeared to correlate with the degree of tubulointerstitial fibrosis, irrespective of disease category. Decorin mRNA expression was detectable in occasional peritubular interstitial cells resembling interstitial fibroblasts or myofibroblasts (Fig. 1L) [22]. Decorin mRNA-expressing cells appeared more



numerous in expanded areas of interstitial fibrosis that frequently showed mononuclear inflammatory cell aggregates, but the hybridization signal was not clearly identified in mononuclear inflammatory cells or in tubular epithelial cells.

In arterial vessels, decorin was localized predominantly in the adventitia (Fig. 1K), with only focal staining in the media, between smooth muscle cells, particularly in larger (arcuate-sized) arteries. Decorin was absent from the intima of morphologically normal arteries and was rarely identified in the neointima of arteriosclerotic vessels. Cells expressing decorin mRNA were identified in the adventitia but not in the media or intima of blood vessel walls (data not shown). Other than the association of decorin with amyloid deposits noted earlier in this article, no specific pattern of decorin immunostaining was identified in tubulointerstitium or arterial vessels in the other disease categories examined.

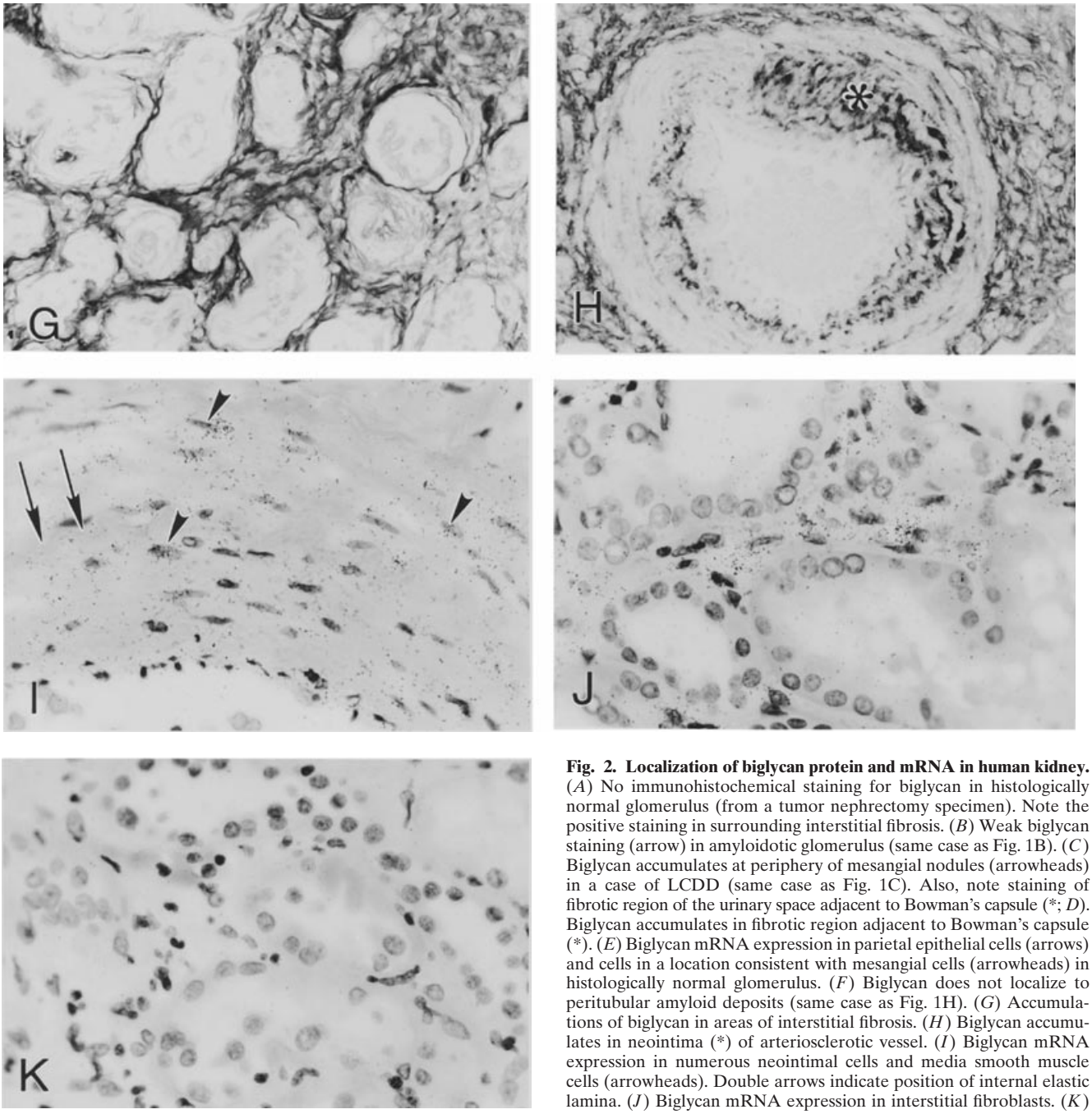
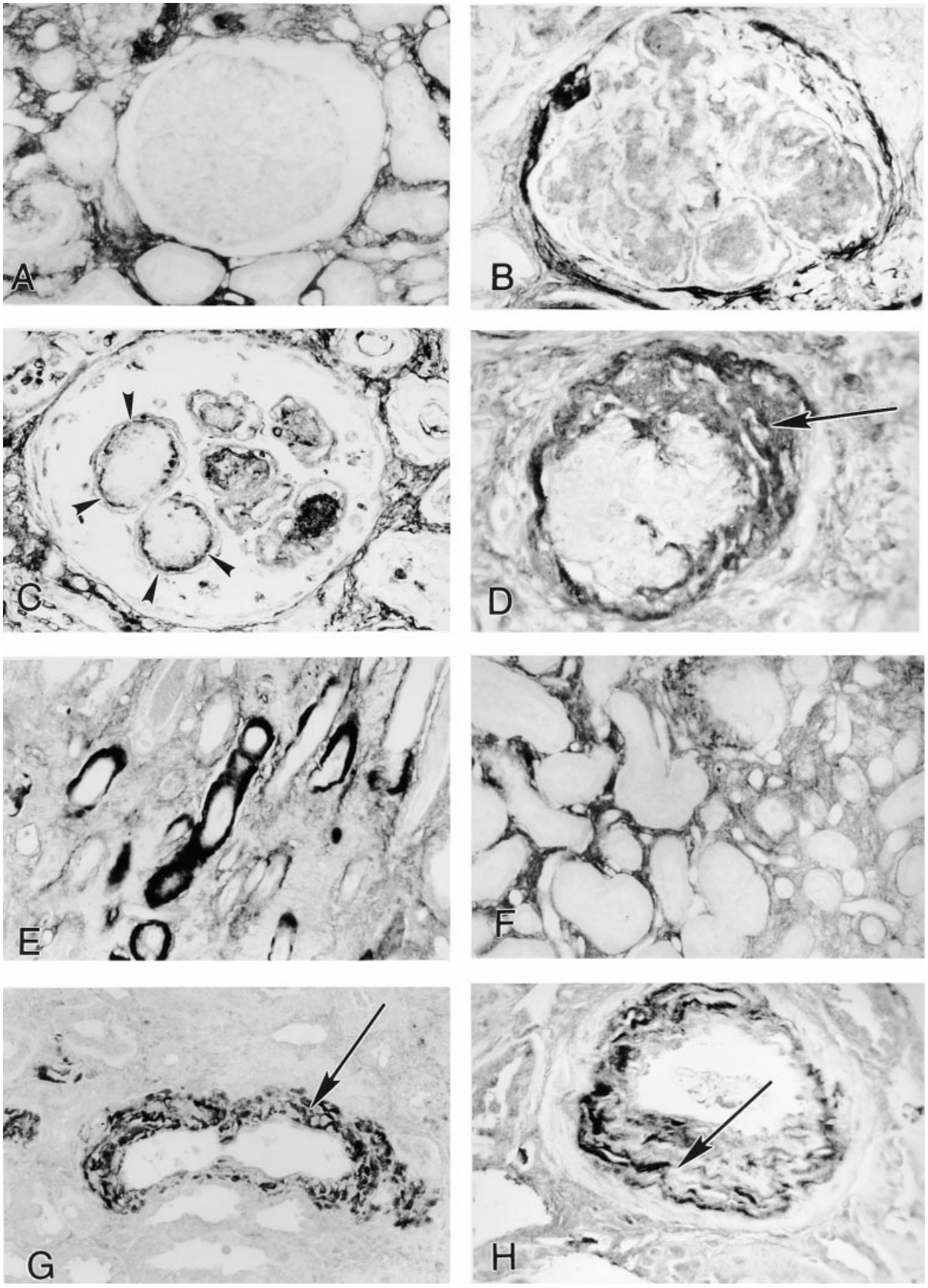


Fig. 2. Localization of biglycan protein and mRNA in human kidney. (*A*) No immunohistochemical staining for biglycan in histologically normal glomerulus (from a tumor nephrectomy specimen). Note the positive staining in surrounding interstitial fibrosis. (*B*) Weak biglycan staining (arrow) in amyloidotic glomerulus (same case as Fig. 1B). (*C*) Biglycan accumulates at periphery of mesangial nodules (arrowheads) in a case of LCDD (same case as Fig. 1C). Also, note staining of fibrotic region of the urinary space adjacent to Bowman's capsule (*; *D*). Biglycan accumulates in fibrotic region adjacent to Bowman's capsule (*). (*E*) Biglycan mRNA expression in parietal epithelial cells (arrows) and cells in a location consistent with mesangial cells (arrowheads) in histologically normal glomerulus. (*F*) Biglycan does not localize to peritubular amyloid deposits (same case as Fig. 1H). (*G*) Accumulations of biglycan in areas of interstitial fibrosis. (*H*) Biglycan accumulates in neointima (*) of arteriosclerotic vessel. (*I*) Biglycan mRNA expression in numerous neointimal cells and media smooth muscle cells (arrowheads). Double arrows indicate position of internal elastic lamina. (*J*) Biglycan mRNA expression in interstitial fibroblasts. (*K*) No hybridization signal with sense riboprobe. Methyl green counterstain (A–D, F–H), hematoxylin and eosin counterstain (E, I–K). Original magnification A–D, F–H, ×400; E, I–K, ×1000.

Immunolocalization and mRNA expression of biglycan

Biglycan peptide was not detected in morphologically normal glomeruli (Table 1 and Fig. 2A). No distinct pattern of biglycan expression was identified in the different glomerular diseases examined. Mesangial areas focally showed weak staining for biglycan in some cases of amyloidosis (Fig. 2B), diabetic nephropathy, and LCDD. In some of these glomeruli, biglycan staining was localized to the periphery of mesangial nodules (Fig. 2C). Biglycan did not accumulate in mesangial areas in cases of fibrillary glomerulonephritis, immunotactoid glomerulopathy, or idiopathic mesangial sclerosis. Accumulations of biglycan were identified in scarred glomer-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uli in which the urinary space was obliterated, with fibrous adhesion of the glomerular tuft to Bowman's capsule, in a similar distribution to that observed for decorin (compare Fig. 1D and Fig. 2D). By *in situ* hybridization, biglycan mRNA localized to parietal epithelial cells and, focally, to intraglomerular cells (Fig. 2E) in both morphologically normal and sclerosed glomeruli.

Biglycan did not localize to peritubular or interstitial amyloid deposits (Fig. 2F). The distribution of biglycan in the tubulointerstitium overlapped with that of decorin (compare Fig. 1H and Fig. 2G), with accentuation in vascular adventitia and diffuse staining in expanded areas of interstitial fibrosis. Biglycan mRNA expression was seen in peritubular interstitial fibroblasts or myofibroblast-like cells, which were more numerous in areas of interstitial fibrosis (Fig. 2J). No hybridization signal for biglycan was identified in tubular epithelial cells or in infiltrating mononuclear inflammatory cells.

Biglycan peptide localized to the adventitia and, focally, to the media of morphologically normal arteries, but was not detected in normal intima (data not shown). Accumulations of biglycan were diffusely present in the neointima of arteriosclerotic vessels (Fig. 2H). Biglycan mRNA was identified in occasional smooth muscle cells in the media and in neointimal cells (Fig. 2I), but not in endothelial cells.

Immunolocalization of collagen type I

Collagen type I protein was not identified in morphologically normal glomeruli (Table 1 and Fig. 3A). In glomerular disease, collagen type I colocalized with decorin and biglycan. Intense immunostaining for collagen type I was observed in amyloid deposits, in glomeruli, tubular basement membranes, and artery walls, in a pattern identical to that of decorin (Fig. 3 B, E, and G). Collagen type I expression was similar in cases of AA and AL. Weak immunostaining for collagen type I was identified in mesangial nodules in some cases of LCDD and diabetic nephropathy that also showed staining for decorin and/or biglycan (Fig. 3C). No immunostaining for collagen type I was identified in mesangial areas in cases of fibrillary glomerulonephritis, immunotactoid glomerulopathy, or idiopathic mesangial sclerosis. Collagen type I also colocalized with decorin and biglycan in glomeruli with features of fibrous organization of Bowman's space (compare Fig. 1D, Fig. 2D, and Fig. 3D).

In morphologically normal kidneys, collagen type I localized to the interstitium, particularly in the medulla (data not shown). Collagen type I accumulated in expanded zones of interstitial fibrosis (Fig. 3F). Collagen type I localized to the adventitia and, focally, to the media of morphologically normal arteries, but was not detected in normal intima. In the neointima of arteriosclerotic vessels, diffuse accumulations of collagen type I were observed in a parallel distribution to that observed for biglycan (compare Fig. 2H and Fig. 3H).

DISCUSSION

The present study is the first to our knowledge to describe the synthesis and deposition of the proteoglycans decorin and biglycan in human glomerular diseases characterized by accumulations of extracellular matrix. The demonstration of decorin and biglycan expression at sites of glomerular fibrosing injury and tubulointerstitial fibrosis supports a pathogenic role for these proteoglycans in progressive human renal disease. Differences in proteoglycan composition may be diagnostically useful in distinguishing morphologically similar glomerular diseases. The finding that decorin colocalizes with amyloid deposits, but not in diabetic nephropathy, LCDD, fibrillary glomerulonephritis, and immunotactoid glomerulopathy, supports the existence of different pathways of mesangial sclerosis in these diseases, which may be related to the modulation of specific growth factors by proteoglycans.

The colocalization of decorin with amyloid deposits in glomeruli, tubulointerstitium, and arterial blood vessel walls is striking and strongly suggests a pathogenic role for this proteoglycan in the formation of amyloid deposits. Although there is considerable experimental evidence to support a role in amyloidogenesis for basement membrane-derived extracellular matrix components, including heparan sulfate proteoglycan, laminin, and collagen type IV [23–25], few studies have examined the distribution of nonbasement membrane proteoglycans in amyloidotic tissues. Decorin has previously been localized to the periphery of Aβ amyloid deposits in the brain [26], and both decorin and biglycan have been localized by immunoelectron microscopy in glomerular deposits of AA [27]. The identification of decorin mRNA synthesis in amyloidotic glomeruli, but not in morphologically

Fig. 3. Localization of collagen type I at sites of fibrotic injury. (*A*) There is no immunohistochemical staining for collagen type I in a histologically normal glomerulus. (*B*) Collagen type I accumulates in amyloid deposits in mesangium (same case as Fig. 1B). (*C*) Collagen type I localizes to the periphery of mesangial nodules (arrowheads) in a case of LCDD (compare with Fig. 2C). (*D*) Accumulations of collagen type I in fibrotic glomerulus (arrows; compare with Fig. 1B and Fig. 2B). (*E*) Collagen type I localizes to peritubular amyloid deposits (compare with Fig. 1H). (*F*) Collagen type I staining in areas of interstitial fibrosis. (*G*) Collagen type I localizes to amyloid deposits (arrows) in arterial wall (compare with Fig. 1J). (*H*) Collagen type I accumulates in neointima (arrow; compare with Fig. 2H). Methyl green counterstain (A–H). Original magnification A–H ×400.

normal glomeruli, suggests that local up-regulation of decorin synthesis contributes to the accumulation of proteoglycan in these cases. The colocalization of collagen type I with amyloid has not been reported in the literature. Our study raises the possibility that codeposition of decorin and collagen type I may have a role in stabilization and persistence of amyloid deposits.

The finding of weak staining for decorin and biglycan in some cases of advanced LCDD and diabetic nephropathy was also related to the focal finding of collagen type I deposits in these tissues. Although there is experimental evidence that mesangial cell synthesis of decorin is up-regulated in hyperglycemic cell culture media [28] and in rodent models of diabetic glomerular disease [29], the results of our study do not support a role for up-regulated synthesis and deposition of decorin and biglycan in the pathogenesis of human diabetic nephropathy, and in most clinically important human glomerular diseases characterized by mesangial sclerosis. An important caveat is that the tissues examined in this study were obtained from individuals with symptomatic renal disease, and these investigations do not address the possibility that decorin and biglycan may have functions in earlier stages of glomerular injury. Further studies to examine the expression of these extracellular matrix molecules in more acute forms of glomerular injury, characterized by cellular proliferation and mesangiolysis, will help to define better the roles of decorin and biglycan in human glomerular disease.

The codistribution of decorin and biglycan with collagen type I in glomerulosclerosis and interstitial fibrosis suggests that synthesis and/or deposition of these extracellular matrix moieties may be pathogenically related. Decorin has a key role in regulating collagen fibril assembly and growth, as demonstrated by the finding of abnormal collagen fibrils in decorin-deficient mice [13], and interactions between biglycan and collagen type I have also been described *in vitro* [14]. The codeposition of decorin and biglycan with collagen type I has been described in other instances of fibrosing injury, including arteriosclerotic blood vessels [15, 30, 31], myocardial infarction [32], and keloids [33]. Proteoglycan-collagen type I interactions may contribute to stabilization of the remodeled extracellular matrix at sites of fibrosing injury. Decorin and biglycan bound to collagen fibrils may be sequestered from the normal pathway of reuptake and receptor-mediated endocytosis [2], thus contributing to the formation of immunohistochemically detectable deposits at sites of collagen type I deposition. This interpretation is consistent with *in vitro* observations of delayed turnover of proteoglycans synthesized by fibroblasts grown on a collagenous matrix [34]. It is interesting to speculate that binding of proteoglycans to collagen may lead to reduced availability of these molecules for interactions with growth factors, such as TGF-β, leading to unregulated TGF-β activity at these sites. Alternatively, binding of growth factors to extracellular matrix proteoglycans may serve a reservoir function, increasing the availability of growth factors at sites of fibrosing injury.

Peritubular interstitial fibroblasts expressing decorin mRNA and biglycan mRNA were more numerous in areas of interstitial fibrosis compared with morphologically normal tubulointerstitium, suggesting that proteoglycan synthesis may be up-regulated in areas of interstitial fibrosis. Biglycan mRNA, as distinct from biglycan peptide expression, was identified in parietal epithelial cells and in occasional intraglomerular cells in both morphologically normal and sclerotic glomeruli. The role of increased biglycan synthesis in the formation of biglycan accumulations in glomeruli remains undetermined. Of note, other investigators have described a somewhat different distribution of biglycan in the kidney. In rat kidney, biglycan mRNA and core protein have been localized to glomerular capillaries, mesangial cells, and podocytes and in collecting ducts and distal tubules [7, 35]. In human fetal kidney, Bianco et al have localized biglycan protein on the surface of endothelial cells and collecting tubules and "very occasionally" in parietal epithelial cells, and they have also localized mRNA to these cells [10]. The results of this study, in which biglycan mRNA synthesis was not identified in endothelium or collecting ducts, suggest that biglycan expression by endothelial cells and tubular epithelial cells may be developmentally restricted, perhaps reflecting different biologic functions in developing and mature human kidney.

Biglycan, but not decorin, was identified in the neointima of arteriosclerotic blood vessels. Distinct patterns of localization of decorin and biglycan have also been described in human coronary atherosclerotic plaques [15, 36], in restenosis lesions following coronary angioplasty [15], and in coronary transplant arteriopathy [31]. Based on the demonstration of specific interactions between biglycan and apolipoprotein E *in vitro*, and the immunohistochemical colocalization of biglycan with apolipoprotein E in human coronary atherosclerotic plaques, it has been proposed that interactions between proteoglycans and lipoproteins may contribute to the pathogenesis of atherosclerosis by trapping lipoproteins in the artery wall [36]. The finding of distinct and overlapping patterns of decorin and biglycan accumulation in the kidney suggests specific, albeit as yet undetermined pathogenic roles in human renal fibrosis that may be related to different biologic properties of these molecules. However, the pathogenesis of proteoglycan accumulation in fibrotic disease is complex and multifactorial, and other factors not examined in this study, such as local differences in activity of profibrogenic cytokines [37] and matrix degrading enzymes, are likely to be important in

determining the topographic distribution of proteoglycans in human renal fibrosing injury.

In summary, decorin and biglycan contribute to the pathologic accumulations of extracellular matrix that characterize fibrosing human renal disease, possibly reflecting a pathogenic role related to modulation of TGF-β activity. The codeposition of other matrix components, such as collagen type I, may have a significant effect on localization and biologic activity of these molecules in human renal disease.

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