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CLINICAL INVESTIGATION

Expression of clusterin in human renal diseases

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Expression of clusterin in human renal diseases. Clusterin, a glycoprotein with potent cohesive properties, is induced in a wide variety of acute and chronic experimental renal diseases. The purpose of this study was to examine clusterin expression in human renal diseases. Clusterin immunostaining was examined in nephrectomy specimens from patients with autosomal-dominant polycystic kidney disease (N = 5), autosomal-recessive polycystic kidney disease (N = 3), multilocular cyst of the kidney (N = 2), renal hypoplasia/dysplasia (N = 7), Wilms' tumor (nephroblastoma) (N = 6), renal cell carcinoma (N = 9), and acute and/or chronic renal transplant rejection (N = 15). No clusterin staining was detected in normal renal tissue distant from renal cell carcinomas. Increased expression of clusterin was found in epithelial cells lining cysts in all of the cystic disorders studied. Clusterin expression was found in some immature tubules in hypoplastic/dysplastic kidneys and in tubules of rejected renal allografts, but was not a prominent finding in renal neoplasms, although some renal cell carcinomas expressed clusterin in a focal manner. Common features of clusterin induction included exclusively epithelial production of clusterin in cysts, immature nephrons, and injured tubules, heterogeneity of clusterin expression, with only some tubules and/or cysts in a given area staining for clusterin, and uniform clusterin staining of epithelial cells in a given tubule or cyst in most cases. Based on its cohesive properties, we speculate clusterin functions to maintain cell-cell and cell-substratum interactions which become perturbed in the setting of renal injury and cystic diseases.

The injured cell mounts a number of unique responses directed at minimizing damage and initiating repair. The study of these injury responses has the potential to provide insight into mechanisms of injury and potential therapies to limit injury and accelerate repair.

Clusterin is a heterodimeric glycoprotein first isolated from ram rete testes fluid, and so named because of its ability to elicit clustering of Sertoli and red blood cells [1, 2]. Species and tissue homologues of clusterin have been isolated and/or cloned by a number of groups working in widely divergent areas resulting in a number of different names for clusterin including complement cytolysis inhibitor or CLI, sulfated glycoprotein-2 or SGP-2, testosterone repressed prostate message-2 or TRPM-2, dimeric acidic glycoprotein or DAG, SP-40,40, gp80, apolipoprotein J, NA1/NA2, and glycoprotein III [reviewed in 3–7]. In accordance with recent consensus regarding clusterin terminology, this protein will be referred to as clusterin in this manuscript [4]. The pathways leading to clusterin have included studies of Sertoli cell biology, fertility, apoptosis, complement regulation, renal tubular injury, lipid transport, Alzheimer's disease, and others.

Clusterin is induced during renal and other tissue injuries [3–7]. Despite its immediate and often prominent recruitment after injury, the role of clusterin remains elusive. We, and other investigators have demonstrated an increase in renal clusterin mRNA or protein in such experimental models of acute renal injury as ischemia/reperfusion, ureter obstruction, gentamicin nephrotoxicity, rhabdomyolysis and folic acid nephropathy [8–13]. Persistent increased expression of clusterin occurred in several chronic models of renal disease including renal ablation, tubulointerstitial disease induced by dietary deficiency of vitamin E and selenium, and in a mouse model of polycystic kidney disease [13–15]. Studies in human renal disease have demonstrated clusterin predominantly in glomerular immune deposits usually in association with other complement components [16, 17]. Tubular staining for clusterin has either not been a prominent feature of the diseases studied [16] or details regarding such tubular staining have not been provided [17]. Neither of these studies examined clusterin in cystic disorders.

Despite the marked tubular epithelial cell induction of clusterin in experimental models of renal injury, limited details regarding tubular expression of clusterin in human renal disease are available; therefore, we examined the expression of clusterin in acute and chronic renal transplant rejection, a common human example of tubular injury. Since the phenotype of the cells in human cystic diseases and renal cell cancers share certain characteristics with those of injured cells, including dedifferentiation and loss of cell-cell and cell-substratum interactions, we also studied the expression of clusterin in these disorders. Such cataloguing of clusterin was performed to clarify and extend our understanding of clusterin in human renal diseases, to evaluate the possible clinical relevance of clusterin, and to provide insight and allow speculation on the role of clusterin in kidney injury.

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Table 1. Patient characteristics

Diagnosis	Age	Cr	Reason for nephrectomy	Clusrin staining
Cystic disease				
ADPKD	58	1.4	Previous transplant, pain	Cystic epithelium
ADPKD	53	9.9	EDRD, elective nephrectomy	Cystic epithelium
ADPKD	51	12.9	ESRD, hemorrhage	Cystic epithelium
ADPKD	4	12.0	ESRD, elective nephrectomy	Cystic epithelium
ADPKD	58	1.8	Pain, hypertension	Cystic epithelium
ARPKD	5	4.0	Elective, pre-transplant	Cystic epithelium
ARPKD	7 mo.	1.0	Elective, pre-transplant	Cystic epithelium
ARPKD	3 wk.	1.4	Elective, pre-transplant	Cystic epithelium
Multilocular cyst	68	7.9	Renal mass	Cystic and tubular epithelium
Multilocular cyst	57	1.2	Pain, hematuria, recurrent UTI	Cystic epithelium
Hypoplasia/dysplasia				
Renal dysplasia	2	0.7	Renal mass	Cystic and tubular epithelium
Renal dysplasia	13	4.8	Elective nephrectomy	Cystic epithelium
Renal dysplasia	1	4.0	ESRD, elective nephrectomy	Cystic epithelium
Renal dysplasia	1	1.2	Elective nephrectomy	Cystic epithelium
Renal dysplasia	17	3.4	Elective nephrectomy	Cystic and tubular epithelium
Renal dysplasia	1	4.4	ESRD, elective nephrectomy	Cystic and tubular epithelium
Renal dysplasia	1	5.9	Elective nephrectomy	Cystic and tubular epithelium
Renal tumors				
Wilms' tumor	5 mo.	0.4	Renal mass	Negative
Wilms' tumor	3	0.5	Renal mass	Negative
Wilms' tumor	2	0.5	Hematuria, renal mass	Negative
Wilms' tumor	4	0.5	Renal mass	Negative
Wilms' tumor-cystic	5 mo.	0.4	Renal mass	Cystic epithelium
Nephroblastoma	22 mo.	0.4	Renal mass	Negative
Renal cell-Grade III	55	1.6	Hematuria	Focal epithelial
Renal cell-Grade II	77	1.2	Renal mass	Focal epithelial
Renal cell-Grade II	73	1.2	Hematuria	Negative
Renal cell-Grade IV	66	1.3	Hematuria	Focal epithelial
Renal cell-Grade II	32	1.1	Renal mass	Focal epithelial
Renal cell-Grade IV	63	1.4	Hematuria	Negative
Renal cell-Grade II; cystic	79	1.0	Renal mass	Focal epithelial
Renal cell-Grade II	76	1.1	Renal mass	Focal epithelial
Renal cell-Grade III/IV	74	0.9	Hematuria	Negative
Transplant rejection				
Acute rejection	54	1.9	Ruptured kidney	Tubular epithelium
Acute rejection	33	2.5	Renal infarction	Tubular epithelium
Acute rejection	40	5.4	Intractable fever	Tubular epithelium
Acute rejection	69	5.0	Severe rejection, CMV	Tubular epithelium
Acute rejection	39	11.1	Severe rejection	Tubular epithelium
Chronic rejection	5	3.9	Severe rejection	Tubular epithelium
Chronic rejection	17	10.8	Rejection, lymphooele	Tubular epithelium
Chronic rejection	30	6.0	Elective, pretransplant	Tubular epithelium
Transplant glomerulopathy	46	1.5	Intractable hypertension	Tubular epithelium
Acute and chronic rejection	25	9.9	Hematuria, pain	Tubular epithelium
Acute and chronic rejection	42	8.0	Severe rejection	Tubular epithelium
Acute and chronic rejection	30	7.0	Severe rejection	Tubular epithelium
Acute and chronic rejection	45	11.3	Elective pretransplant	Tubular epithelium
Acute and chronic rejection	46	12.3	Severe rejection	Tubular epithelium
Acute and chronic rejection	25	1.5	Severe rejection	Tubular epithelium

Methods

Patients

Tissue for immunohistochemical studies was selected from nephrectomy specimens obtained at the University of Minnesota between 1985 and 1990. A total of 47 kidneys were studied encompassing a spectrum of renal disease with an emphasis on renal cystic disease, cancer of the kidney, and allograft rejection. The samples were classified according to disease based on clinical and pathologic criteria. Pathologic diagnoses were as follows (Table 1): autosomal-dominant polycystic kidney disease ($N = 5$), autosomal-recessive polycystic kidney disease ($N = 3$), multilocular cyst of the kidney (multilocular cystic nephroma) ($N = 2$), renal hypoplasia/dysplasia ($N = 7$), Wilms' tumor (nephroblastoma) ($N = 6$), renal cell carcinoma ($N = 9$), and acute and/or chronic renal transplant rejection ($N = 15$).

Immunohistochemistry

For detection of clusterin, a mouse anti-human monoclonal antibody (G7, a gift from Dr. Brendan Murphy, University of Melbourne, Melbourne, Australia) was used [18]. Specificity of the antibody was demonstrated by immunoprecipitation and

Western blot analysis using both human serum and seminal fluid [18, 19]. Sections from formaldehyde-fixed, paraffin embedded tissue were deparaffinized in Americlear (American Scientific Products, Minneapolis, Minnesota, USA). Endogenous peroxidase was blocked with 0.8% hydrogen peroxide in absolute methanol. The sections were stained with the avidin-biotin-peroxidase complex technique as previously described [20]. Briefly, after overnight incubation at 4°C with the primary antibody (mouse anti-human clusterin monoclonal antibody) the sections were incubated with biotinylated horse antiserum which recognizes mouse IgG, and subsequently, with avidin-biotin-peroxidase complex (Vector Laboratories, Inc., Burlingame, California, USA). The reaction was demonstrated with 3-3′ diaminobenzidine tetrahydrochloride (0.25 mg/ml) (Sigma, St. Louis, Missouri, USA) and 0.003% peroxide. The sections were counter stained with Harris' hematoxylin. Negative controls consisted of sections in which non-immune mouse ascites was substituted for primary antisera.

Results

Controls

Non-immune ascitic fluid, which was used as a negative control, did not result in any specific staining. No clusterin staining was seen in normal renal tissue distant from renal cell carcinomas (Fig. 1A).

Polycystic kidney disease

The kidneys of five patients with end-stage renal disease secondary to autosomal-dominant polycystic kidney disease were examined. In all five cases, clusterin staining was present in epithelial cells lining both large and small renal cysts. Intercyst heterogeneity was present with not all cysts in a given kidney staining for clusterin. There were no morphologic differences between cysts which stained for clusterin and those which did not. Often positive and negative cystic tubules were present in the same field (Fig. 1B and C). When one cell in a cyst expressed clusterin, all cells lining that cyst were immunoreactive. In addition, tubular casts and sloughed epithelial cells demonstrated clusterin staining (Fig. 1 C, D). In some sections (Fig. 1D) cells staining positive for clusterin were being sloughed into the tubular lumen.

An identical pattern of clusterin staining was seen in the kidneys from three patients with autosomal-recessive polycystic kidney disease, including the focal nature of clusterin staining with positive cysts interspersed with negative ones, the staining of all epithelial cells in a given positive cyst and the presence of clusterin in tubular casts. No glomerular, vascular, or interstitial staining was seen in any of the cases of polycystic kidney disease.

Multilocular cyst of the kidney

Similar to the findings in cases of polycystic kidney disease, clusterin staining was seen in the epithelial cells lining some of the cysts (Fig. 1E).

Renal dysplasia

The kidneys of seven patients with renal hypoplasia/dysplasia were examined. Many of the tubules from these kidneys exhibited cystic dilatation. The renal hypoplasia was characterized by lack of normal nephron differentiation, with loose aggregates of immature tubules surrounded by immature mesenchymal or fibromuscular collarettes. In some cases, focal aggregates of cartilage were identified. In many cases, superimposed changes of hydronephrosis and/or pyelonephritis were evident.

Tubular epithelial clusterin was found in primitive tubules and epithelial cells lining cysts. As was seen in the other cystic disorders, clusterin was present in a focal manner with only some cysts demonstrating staining. In both cysts and tubules which stained for clusterin, all epithelial cells in a given section of a positive tubule or cyst stained for clusterin. No glomerular, vascular, interstitial, or stromal staining for clusterin was detected (Fig. 1 F, G, H).

Renal cancer

Clusterin staining was examined in the kidneys of six patients with Wilms' tumor (nephroblastoma), and in nine patients with renal cell carcinoma. Blastematous, epithelial and stromal cells in most cases of Wilms' tumor were negative for clusterin (Fig. 1I). The only positive staining was observed in one tumor in which the epithelial cells lining some neoplastic cysts were immunoreactive. Clusterin positivity was present in some normal appearing tubules entrapped by the tumor (Fig. 1J).

Focal epithelial cell staining for clusterin was present in some tumor cells in five patients with renal cell carcinoma (Fig. 1K). In addition, in one tumor with cystic changes, clusterin was seen in the epithelial cells lining the cysts.

Renal transplant rejection

Kidneys from 15 patients undergoing nephrectomy for renal transplant rejection were studied. Five patients had severe acute rejection, four patients had chronic rejection, and six patients had combinations of acute and chronic rejection. No qualitative differences were seen in clusterin staining in acute versus chronic rejection. Clusterin was present in both normal and injured tubular epithelial cells but not in glomeruli, vessels or interstitium. No relationship was present between histologic lesions of rejection and clusterin staining. As was seen in renal dysplasia and polycystic kidney disease, clusterin positivity occurred in a focal manner, with marked internephron heterogeneity, with some tubules staining positive whereas adjacent ones were negative (Fig. 1 L–P). In addition, intranephron heterogeneity of clusterin staining was present in some kidneys, with both positive and negative cells being present in a given tubule (Fig. 1O). Tubular casts, at times made up of sloughed tubular epithelial cells, also stained for clusterin (Fig. 1P). The cast material was usually present in tubules which stained for clusterin, although this was not always the case (Fig. 1L).

Discussion

Expression of clusterin was found in epithelial cells lining cysts in a variety of human renal diseases, including autosomal-dominant and autosomal-recessive polycystic kidney disease, multilocular cyst of the kidney, cystic renal dysplasia, and in neoplastic cysts found in some cases of Wilms' tumor and renal cell carcinoma. In addition, clusterin expression was seen in immature tubules in hypoplastic/dysplastic kidneys and in some tubules of rejected renal allografts, but was not a prominent finding in renal neoplasms, although some renal cell carcinomas expressed clusterin in a focal manner. Several common features

of clusterin induction were revealed by these studies and include the exclusively tubular epithelial production of clusterin in cysts, immature nephrons, and injured tubules. A heterogeneous pattern of clusterin staining was observed, with only some tubules and/or cysts in a given area staining for clusterin. In most cases, clusterin was present in all of the epithelial cells in a given tubule or cyst with the exception of some tubules in rejected kidneys where intratubular heterogeneity was seen. Many of these features of clusterin expression in human renal disease are similar to our observations in experimental renal diseases [12–14].

Previous studies examining expression of clusterin in human renal diseases have focused primarily on the relationship between clusterin and complement. Murphy et al examined clusterin staining in 90 renal biopsies of patients with a variety of renal diseases [16]. The major finding was co-localization of clusterin with complement components of the membrane attack complex (C6 and C9) and S-protein. This was particularly true in patients with immune complex glomerulonephritis, but was also found in glomeruli of patients with focal and segmental glomerulosclerosis, interstitial nephritis, and diabetic nephropathy. In contrast to our findings, vascular and tubular basement membrane clusterin staining was found in rejected allografts and in cases of interstitial nephritis, but tubular staining of clusterin was not a major feature of these disorders. French, Tschopp and Schifferli studied clusterin staining in 180 human renal biopsies and noted clusterin was usually found in association with components of the membrane attack complex, particularly when the membrane attack complex was associated with immunoglobulin [17]. Moderate to intense tubular and vascular staining for clusterin was noted in approximately 24% of biopsies but the diseases in which tubules stained for clusterin were not stated. The results of our study provide more details regarding the tubular expression of clusterin in human renal disease, and confirm experimental findings demonstrating predominant tubular induction of clusterin in a variety of injury states. Since clusterin can bind to the membrane attack complex [21], its localization in human renal diseases could be attributable to complement deposition, a possibility not tested for in our study. However, clusterin and complement can be dissociated in the kidney and other tissues [12, 22] and tubular epithelial cell deposition of complement is usually not a feature of many of the disorders studied [23].

Clusterin staining of the epithelial lining of renal cysts confirms the observations of Harding et al in the *cpk* mouse and extends these findings to human renal cystic diseases [15]. In the *cpk* mouse, progressive dilation of collecting duct cysts occurs leading to renal failure in most mice by three weeks of age. Clusterin mRNA was present in cystic collecting ducts and appeared to increase with progressive disease. Clusterin is developmentally regulated in the kidney, being detected in the ureteric bud, induced mesenchyme, S-shaped bodies, and collecting ducts of fetal and/or newborn mice [15, 24]. A decrease in clusterin expression occurs as the kidney matures, being detectable only in some distal tubular epithelial cells in the adult kidney. The re-expression of clusterin in the epithelial lining of collecting duct cysts of the *cpk* mice prompted Harding et al to suggest that clusterin is a marker of dedifferentiation of these epithelial cells [15].

The presence of clusterin in developing tubules, as well as in various cystic disorders, immature tubules in hypoplastic/dysplastic kidneys, injured tubules of rejected allografts, and in some renal cancer cells indicates some similarities between these diseased states and undifferentiated states. Common features defining these diseased states include enhanced proliferation, alterations in the cytoskeleton leading to loss of cell polarity, and abnormal cell-substratum and cell-cell interactions. For example, loss of polarity, best documented by redistribution of the sodium-potassium ATPase from the basolateral to apical cell membrane, is observed following renal tubular injury and in cystic epithelium [25, 26]. Abnormalities in cell-substratum and cell-cell interactions are found following renal tubular cell injury, and include loss of focal cell contacts, internalization of cell adhesion molecules and redistribution of integrins [27–29]. Perturbations in extracellular matrix components and decreased expression of cell adhesion molecules have been observed in renal cystic diseases and may play a role in the pathogenesis of the cysts [30–34]. The function of clusterin in renal disease remains undefined, but likely relates to one or more of these phenotypic alterations found in immature, cystic or injured tubules.

We speculate in the setting of renal disease/injury, clusterin acts as a cohesive factor to maintain proximity of cells (non-junctional cell contact) as well as cell adhesion to the substratum. Support for this hypothesis derives from the induction of clusterin in the wide array of renal tubular disorders presented and the potent ability of clusterin to aggregate cells. Clusterin induces aggregation of a variety of cells *in vitro* including erythrocytes, white blood cells, spermatozoa, mouse testes TM-4 cells, and primary cultures of rat Sertoli cells [1, 2, 35, 36]. Prolonged incubation of TM-4 cells with clusterin resulted in the formation of junctional contacts between cells in association with increased expression of the cell adhesion molecule N-CAM, suggesting clusterin may be involved with initial non-junctional cell aggregation which would then enable more stable cell-cell interactions to become established [36]. Clusterin is not a member of any known class of adhesion molecule (such as integrins or cadherins), and the biochemical basis by which it promotes cell interactions is poorly defined. Structural analysis does reveal several potential heparin binding sites which could facilitate interaction between clusterin and extracellular matrix [21, 37–39]. Furthermore, the state of cell-substratum interactions can influence clusterin-induced cell aggregation. Tung et al demonstrated that cell aggregation induced by clusterin was greatest when interactions between cells and substratum were inhibited, suggesting an anchorage related phenotypic alteration in cells which promotes interaction with clusterin [36].

Maintenance of cell-cell and cell-substratum contact has obvious advantages to the kidney, as it would prevent loss of viable cells into the tubular lumen which would not only disrupt the epithelial barrier leading to backleak of glomerular filtrate, but the detached cells could also cause tubular obstruction [29, 40, 41]. In addition, maintenance of cell-cell contact would allow the normal junctional contacts to become re-established once the injury subsides.

Other proposed roles for clusterin in the setting of tissue injury include complement defense, apoptosis and membrane protection. Clusterin can inhibit complement mediated cytolysis by binding to nascent C5b-7 complex preventing insertion of

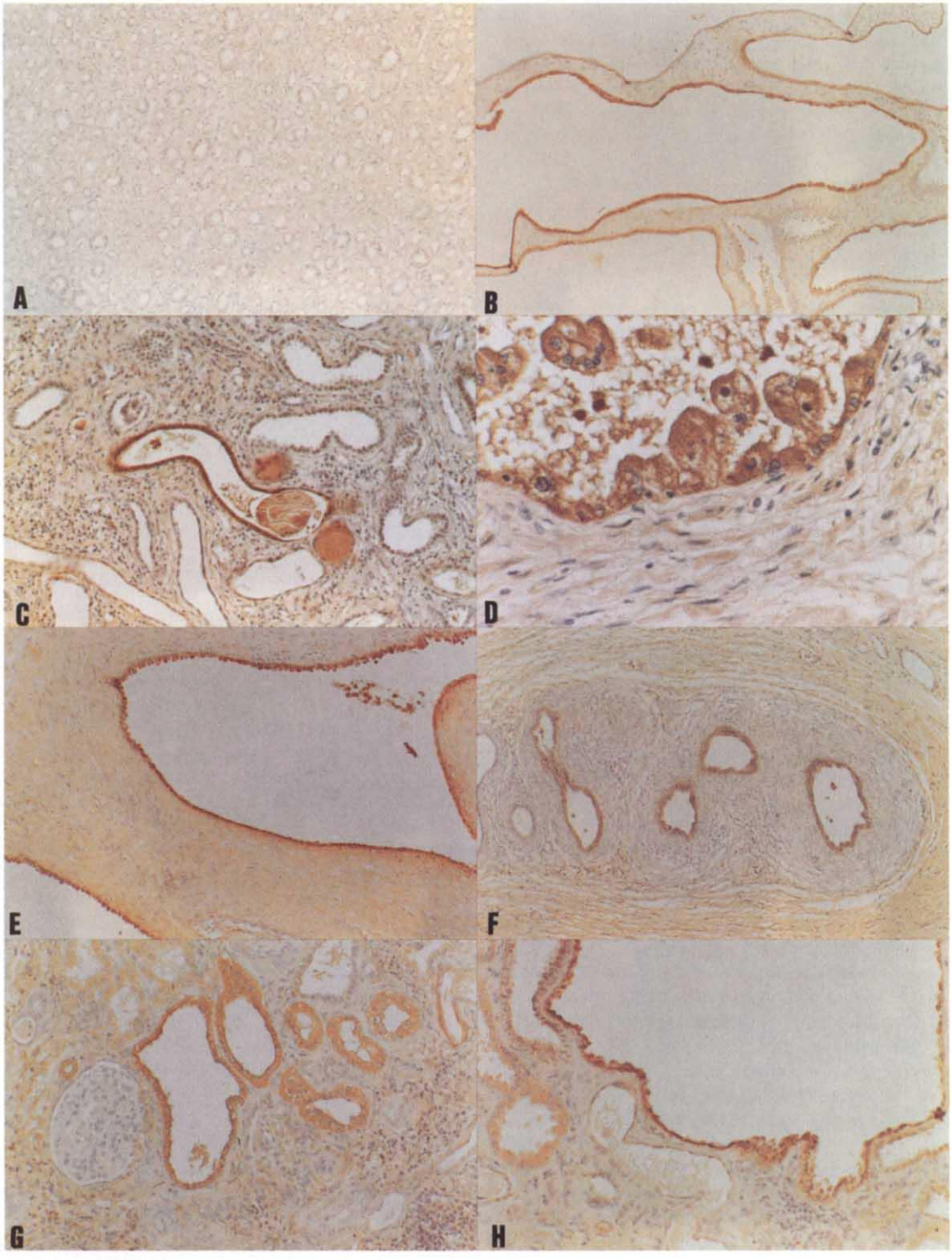
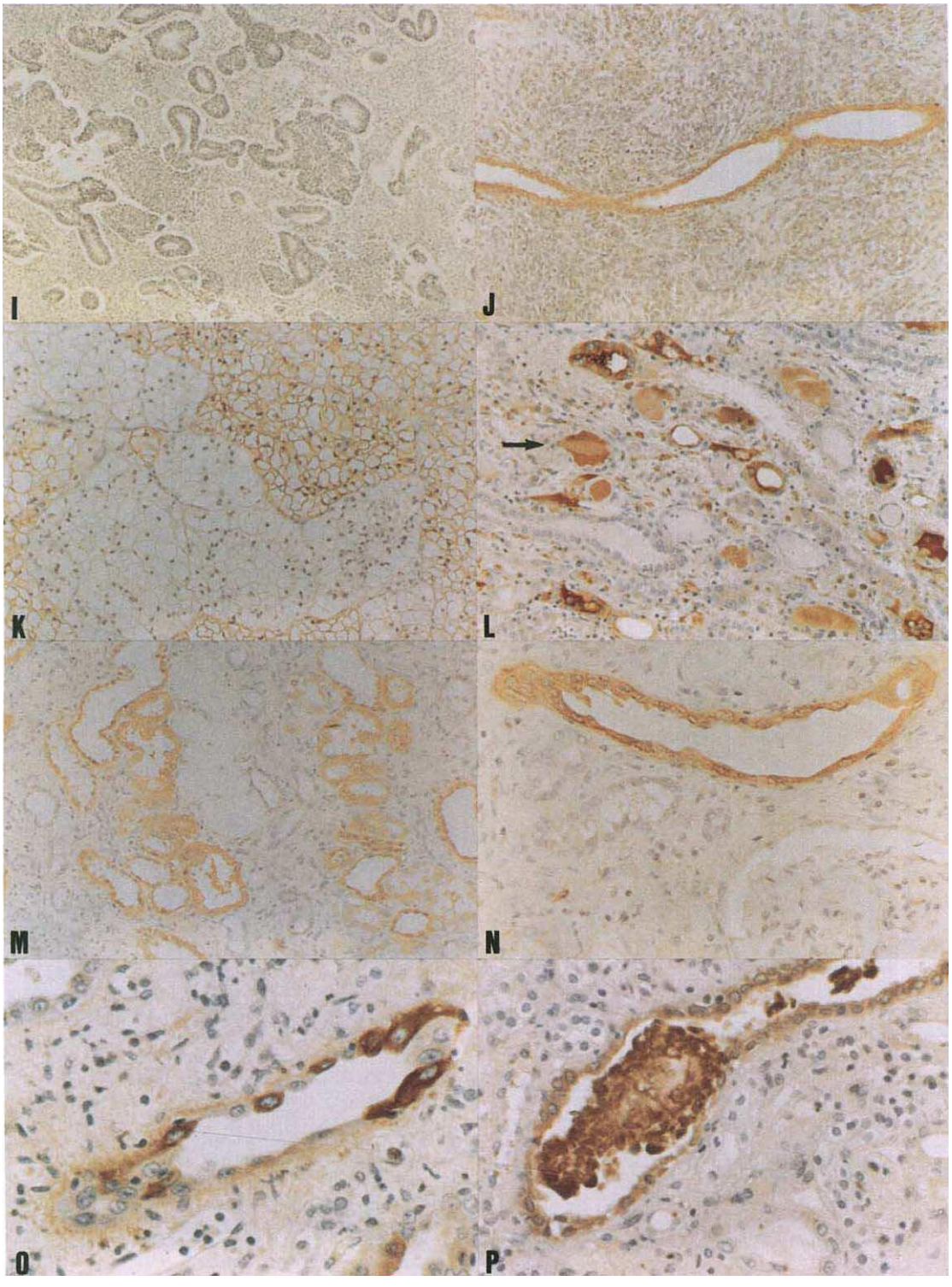


Fig. 1. *Clusterin expression in human renal disease.* **A.** Normal kidney. No clusterin staining is seen in normal tubular epithelial cells in renal tissue distant from a renal cell carcinoma. **B.** Autosomal-dominant polycystic kidney disease. Clusterin positivity in some cysts highlights the focal nature of its distribution. In a positive cyst, all epithelial cells stain for clusterin. No clusterin staining is present in the interstitium. **C.** Autosomal-dominant polycystic kidney disease. Some cysts and tubular casts stain for clusterin. **D.** Autosomal-dominant polycystic kidney disease. A high power view of a cyst demonstrating positive staining for clusterin in lining cells and in detached epithelial cells; the latter contribute to the casts seen in cysts and dilated tubules. **E.** Multilocular cyst. Clusterin staining of cyst epithelium and detached epithelial cells. **F.** Renal dysplasia. A collarette of negatively staining mesenchymal cells encases immature tubules variably staining for clusterin. Adjacent tubules are non reactive. **G.** Renal dysplasia. Dilated and normal appearing tubules stain for clusterin. No glomerular staining is seen. **H.** Renal dysplasia. Clusterin



staining in the epithelial lining of a cyst in an area of cystic dysplasia. **I.** Wilms' tumor. No clusterin staining is seen in tumor cells. **J.** Wilms' tumor. Positive staining for clusterin is observed in a non-neoplastic tubule entrapped by non-reactive blastematous tumor cells. **K.** Renal cell carcinoma. Focal, membrane-based staining for clusterin is observed in some cells. **L.** Acute renal transplant rejection. Clusterin staining is observed in some tubules and tubular casts. Tubules containing positively staining casts do not necessarily show immunoreactive lining epithelium (arrow). **M.** Chronic renal transplant rejection. Positive clusterin staining is noted in some tubules. No interstitial staining is observed. **N.** Chronic renal transplant rejection. A positively staining tubule is seen next to negatively staining tubules and glomerulus. **O.** Acute and chronic renal transplant rejection. Intratubular variation in clusterin staining is observed, with positive and negative epithelial cells present in this tubule. **P.** Acute and chronic renal transplant rejection. An epithelial cell cast positive for clusterin is present in the lumen of this tubule; the lining epithelium is moderately positive. Publication in color was made possible by educational grants from Sandoz Pharmaceuticals, Ortho Biotech, Pfizer Laboratories, and Amgen, Inc.

the membrane attack complex into cell membranes [21, 42]. The pathophysiologic relevance of this property of clusterin has not been determined. The role of clusterin in apoptosis is controversial. Evidence for an association between clusterin and apoptosis has been provided from studies demonstrating expression of clusterin at times of apoptosis such as during prostate atrophy, limb bud regression or treatment of thymocytes with dexamethasone [43–45]. However, many examples exist where clusterin has been dissociated from apoptosis [4, 7, 46–48]. Clusterin is probably not necessary in initiating apoptosis, but may appear as a consequence of apoptosis perhaps to maintain cell interactions during the apoptotic process. A role for clusterin as a membrane "policeman" has been proposed by Jordan-Starck et al based on its ability to interact with hydrophobic molecules, its association with membranes, and its location at interfaces between epithelia and "harsh" environments [6].

In conclusion, clusterin was expressed in renal tubular epithelium in a number of human renal diseases including autosomal-dominant and -recessive polycystic kidney disease, multilocular cysts, renal hypoplasia/dysplasia, and acute and chronic renal transplant rejection. No clusterin was detected in Wilms' tumors, except in one case associated with cystic changes. In renal cell carcinoma, focal staining for clusterin was present in some tumors. Based on its potent cohesive properties, we speculate clusterin functions to maintain cell-cell and/or cell-substratum contacts which in the setting of renal cystic disease, tubular injury or immaturity would not only preserve the integrity of the tubular epithelium but also prevent loss of potentially viable cells into the tubular lumen.

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