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An *in vitro* model of light chain deposition disease

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Nodular glomerulosclerosis results from increased deposition of extracellular matrix proteins and monotypic light chains. The inability of mesangial cells to degrade abnormal levels of tenascin-C—along with the increased expression of some growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β)—is crucial to the pathogenesis of light chain deposition disease (LCDD). In order to study the molecular processes contributing to LCDD, we grew mesangial cells in three-dimensional matrices and incubated the cells with free light chains purified from the urine of patients with biopsy-proven LCDD, immunoglobulin-associated amyloid deposits, or myeloma cast nephropathy. Light chains of the latter two cohorts served as controls. Mesangial cells incubated with light chains from patients with LCDD show a significant increase in tenascin-C expression, centrally located within newly formed nodules, along with increased expression of PDGF and TGF-βs, compared to mesangial cells incubated with control light chains. There was less extracellular MMP-7 even though its intracellular expression is markedly increased compared to the control. Addition of active MMP-7 degraded this excess tenascin-C *in vitro*, a process that could be prevented by an exogenous MMP inhibitor. Our *in vitro* model recapitulates *in vivo* findings in patients with LCDD, thus allowing definition of the sequential pathologic processes associated with glomerulopathic light chain interactions with mesangial cells.

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Light chain deposition disease (LCDD) is one manifestation of monoclonal immunoglobulin deposition in the kidney, first reported in 1973[1] and later confirmed by Randall *et al.*[2] The incidence of LCDD in patients with plasma cell dyscrasia is approximately 5%.[3–5] It is characterized by the presence of immunoglobulin components (light chains) deposits that are neither fibrillar nor Congo red positive. Ultrastructurally, the deposits are seen as punctate granular electron-dense material, which is typically present in the subendothelial glomerular areas and in the mesangium, and may also be present in other renal compartments.[3,6] Clinical manifestations of LCDD are varied and may include nephrotic syndrome (in less than 30% of patients), progressive renal insufficiency (in approximately 70% of patients), and hypertension (in more than 80% of the patients)[7] among others.

Nodular glomerulosclerosis, the classically described lesion in LCDD, is preceded by other morphological manifestations, including mesangial and membranoproliferative patterns.[8,9] The mesangial nodularity within the glomerulus results from the combined increased deposition of extracellular matrix (ECM) proteins mixed with monotypic light chain deposits, most commonly kappa (κ). Thickening of the glomerular peripheral capillary walls and tubular basement membranes results from the deposition of these monotypic light chains (LCs). Vascular deposits of monotypical LCs can also occur. The role of mesangial cells has been found to be critical in the pathogenesis of glomerulosclerosis.[3,10,11]

The interaction of mesangial cells with LCDD-LCs engages the autocrine activation of growth factors, such as platelet-derived growth factor-β (PDGF-β) and transforming growth factor-β (TGF-β).[10,11] These cells are stimulated initially by PDGF-β to proliferate, resulting in mesangioproliferative and membranoproliferative variants of LCDD. Increased matrix production and deposition of ECM proteins, such as type IV collagen, laminin, fibronectin, and, most importantly, tenascin-C,[10,11] under the influence of TGF-β in the later phases of the glomerulopathy, characterize LCDD (Figure 1). Apoptosis of mesangial cells accompanies glomerulosclerosis[12] and has been shown to be enhanced by glomerulopathic LCs.[13] Work performed on renal biopsies from patients with LCDD has supported a role for PDGF-β and TGF-β in the pathogenesis of this disorder,[14] although how these growth factors actually participate in the overall pathogenesis of this disease has remained unclear.

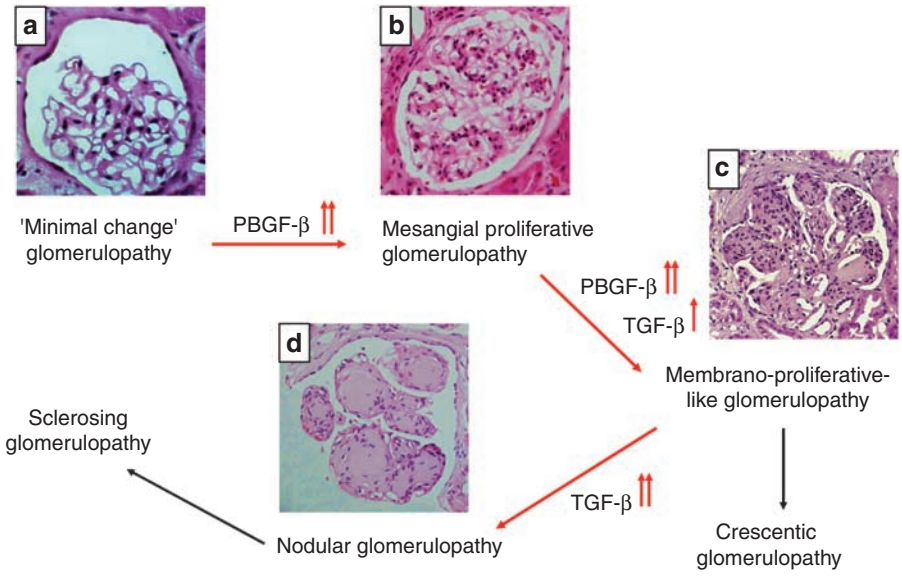

Figure 1 | Sequential glomerular changes associated with light chain deposition disease at different stages of disease process. Note the influences of PDGF-β and TGF-β at the different stages of the lesion. PDGF-β drives proliferation of the mesangial cells (**a,b,c**), whereas TGF-β is responsible for protein deposition and nodule formation (**c,d**).

The tenascins are a family of large oligomeric glycoproteins found in the ECM of vertebrate animals.[15] Tenascin is a cell adhesion molecule, which interacts with integrins, collagens, proteoglycans, and fibronectin.[16] They possess highly conserved sequences with the heptad repeats allowing them to assemble into timers or hexamers.[17] Tenascin-cytotactin was the first member of the family to be described[18] and exist in a hexabrachion[19–21] or octopus-like configuration. Splice variants of tenascin-C have been described,[22] with as many as 27 different mRNA variants identified in the developing mouse brain.[22] Subsequently, four other members were described, namely TN-R, TN-W, TN-X, and TN-Y.

Tenascin-C has been identified in a number of pathological conditions, including breast carcinomas,[23] keloids,[24] diabetic neuropathy,[25] pulmonary fibrosis,[26] and occupying the central areas of the mesangial nodules in LCDD and AL-amyloidosis (AL-Am) in the remaining mesangial matrix.[8,27] Its primary function has not been described; however, its presence in non-degradable fibrotic and sclerotic tissues suggests a fundamental role for this protein in progressive, difficult to reverse back to irreversible pathological processes.

Matrix metalloproteinase-7 (MMP-7), also known as matrilysin-1, is a hydrolytic protease belonging to the minimal domain group of human MMPs.[28] It is secreted as a pro-enzyme (proMMP-7) and is later activated by other MMPs or proteins[29–31] in a proteolytic cascade similar to the coagulation and thrombolytic systems in humans. Like other MMPs (Matrixins), it is a zinc-dependent endopeptidase involved in the regulation of ECM proteins.[32–38] It is made up of only three domains, namely a pre, pro, and catalytic zinc-containing domain. Although MMP-7 is capable of degrading and modulating several ECM proteins,[31,39,40] it has been shown to be the MMP most actively involved in the catabolism of tenascin.[41–43]

A previous paper addressed the overall role of MMPs in mesangial remodeling in AL-Am and LCDD.[38] *In situ* zymographic studies have demonstrated changes in MMPs and tissue inhibitor of metalloproteinases in the glomerular and interstitial renal compartment on tissue sections[44] that are consistent with fibrotic phenotype in LCDD. This paper expands on studies reported earlier and provides a unified and comprehensive understanding of the pathogenesis of LCDD.

This paper reports an *in vitro* model, which allowed the dissection of sequential pathologic processes that take place as glomerulopathic LCs interact with mesangial cells in LCDD.

Although *in vitro* events do not always accurately reflect *in vivo* events, this system has provided meaningful insights of clinical significance and has become an important research platform for understanding the pathogenesis of this disease.

RESULTS

Morphology of mature lesion in LCDD

Hematoxylin and eosin (H&E) staining of mature LCDD glomerular lesions showed characteristic mesangial nodular lesions and variable thickening of the peripheral capillary walls (Figure 2a). These nodules exhibited rather striking argyrophilia in most instances with Jones' silver methanamie stain (Figure 2b), central deposition of tenascin-C (Figure 2c), and peripheral deposition of collagen IV (Figure 2d). Ultrastructurally, punctate granular electron-dense material is present in the expanded mesangial areas (Figure 2e) corresponding to monoclonal light chain deposits.