Uromodulin and Chronic Kidney Disease

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Key Words

Uromodulin • Tamm-Horsfall protein • Chronic kidney disease • Glomerular filtration rate, reduced

Abstract

Uromodulin (Tamm-Horsfall protein) is produced in the kidney by cells of the thick ascending limb and distal tubule. Recent genetic studies suggest a role of uromodulin in chronic kidney disease. Mutations in the UMOD gene cause uromodulin storage disease. They code for amino acid substitutions that lead to misfolding of the molecule and its retention in the endoplasmic reticulum. Single nucleotide polymorphisms in the region of the UMOD gene have been shown to be associated with chronic kidney disease and reduced glomerular filtration rate. These polymorphisms affect uromodulin concentration in the urine, and lower genetically determined urinary uromodulin concentrations seem to protect against renal disease. Chronic kidney disease is associated with higher serum levels of uromodulin. From animal experiments and human studies it is hypothesized that uromodulin entering the renal interstitium either by basolateral secretion or urinary back-leakage in damaged tubuli interacts with and stimulates cells of the immune system and thereby causes inflammation and progression of chronic kidney disease.

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Introduction

Uromodulin or Tamm-Horsfall protein is a 95-kDa glycoprotein exclusively synthesized by the cells of the thick ascending limb (TAL) and early distal convoluted tubule in the kidney. It consists of 640 amino acids including 48 cysteine residues [1]. Seven of eight potential glycosylation sites are occupied by N-linked complex multiantennary glycans comprising one third of the molecular weight [2]. Uromodulin is produced in the endoplasmic reticulum (ER), shuttled to the apical cell membrane as a GPI-linked molecule and released into the urine by proteolytic cleavage. Healthy individuals excrete about 20–70 mg of uromodulin per day, making it the most abundant protein in the urine. Uromodulin forms a gel on the surface of the TAL cells, which is important for water impermeability. It protects against stone disease by preventing aggregation of calcium oxalate crystals. Uromodulin binds to type 1 fimbriae of *Escherichia coli* and thereby blocks colonization of urothelial cells [3]. Uromodulin knockout mice have an impaired capacity to concentrate urine and are prone to renal stone disease and urinary tract infections [4–6].

Recent genetic studies have renewed interest in the biology of uromodulin. Mutations in the UMOD gene on chromosome 16p12.3 cause the so-called uromodulin storage diseases familial juvenile hyperuricemic nephropathy, medullary cystic kidney disease type 2 and glomerulocystic kidney disease [7–10]. Second, two ge-

nome-wide association studies in the general population showed that several significant single nucleotide polymorphisms in the UMOD gene region were associated with chronic kidney disease (CKD) and estimated glomerular filtration rate [11, 12].

Uromodulin Storage Diseases

These autosomal dominant diseases clinically present with hyperuricemia and gout with a low renal fractional excretion of uric acid, and progressive renal failure leading to ESRD in adulthood [13]. Renal cysts are a frequent finding and renal histology shows tubular atrophy and interstitial fibrosis with mild inflammatory infiltration. Urinary uromodulin excretion decreases during childhood and reaches very low levels in adulthood [14, 15]. To date, more than 50 UMOD mutations have been identified. They are mainly localized in exons 3 and 4, and most of them are missense mutations or small inframe deletions. Many of them cause an amino acid change at cysteine sites. Cysteine residues form disulfide bonds and determine correct protein folding. Therefore, it is assumed that UMOD mutations causing uromodulin storage disease lead to defective protein folding. Misfolded immature uromodulin is retained in the ER and not expressed at or released by the apical cell membrane. In renal biopsies large, dense intracellular deposits of uromodulin in TAL cells can be visualized by immunostaining using antiuromodulin antibodies [9]. Electron microscopy of TAL cells shows bundles of hyperplastic ER containing moderately electron-dense storage material [16]. In vitro experiments of various renal epithelial cell lines transfected with mutant UMOD cDNAs indeed showed that mutated uromodulin is retained intracellularly [17–21]. Depending on the extent of maturation, Williams et al. [20] classified mutants into group A with reduced (50%) and group B with almost absent (25%) maturation. Group A mutants were also localized to the cell membrane, whereas group B mutants continued to be completely retained by the ER. There is, however, no correlation between the severity of the maturation defect and the clinical expression of the disease. Accumulation of misfolded proteins in the ER causes ER stress and the unfolded protein response with increased synthesis of chaperones and foldases and activation of ER-associated degradation in order to eliminate the misfolded proteins [22]. When the capacity of the cell to remove these molecules is working to full capacity, the unfolded protein response may trigger apoptosis and autophagy or alternatively lead

to cell activation via MAP kinases and NF- κ B. It is highly likely that these pathways eventually result in TAL cell damage and loss with progressive renal failure. We were able to show a significant number of apoptotic tubular epithelial cells in a biopsy of a patient with uromodulin storage disease [23]. In vitro, chaperones such as colchicine and sodium 4-phenylbutyrate increased uromodulin transport to the cell membrane and secretion in transfected cell lines and reduced apoptosis [17]. Therefore, such substances may be good candidates for the treatment of uromodulin storage disease. Jennings et al. [19] reported normal basolateral secretion of mutated uromodulin and increased serum levels in some patients. Higher basolateral secretion of uromodulin may cause an inflammatory response and tubulointerstitial damage (see below). Uromodulin is also located in primary cilia of TAL cells [24]. Studies in renal biopsies of patients and cell culture experiments in transfected cells showed decreased ciliary uromodulin expression in uromodulin storage disease [24]. As hereditary renal cystic diseases are caused by defects in ciliary proteins, this finding may explain the frequent presence of renal cysts in uromodulin storage diseases.

Why patients with uromodulin storage disease have a low fractional renal excretion of uric acid and consequent hyperuricemia remains a matter of debate. The common view is that hyperuricemia is a consequence of volume depletion. Scolari et al. [13] hypothesize that due to the lack of uromodulin on the luminal surface of the TAL, water reabsorption is increased. This would lead to a reduction of sodium and chloride reabsorption by the TAL, which is compensated by an increase in proximal tubular uptake, a process that is coupled to urate reabsorption. They also showed that a reduction in urine-concentrating capability was associated with higher uric acid serum levels in these patients. In contrast, Kotanko et al. [25] did not find any signs of volume depletion in their patients, who had normal renin and aldosterone levels. In addition, despite saline infusion they retained their low fractional uric acid excretion. Whatever the cause of hyperuricemia, treatment with uricosuric drugs such as benzbromarone can normalize renal uric acid excretion in these patients [23].

Uromodulin and CKD

As stated above, a genome-wide association study in 41,343 participants including 4,320 patients with CKD showed a significant correlation between SNPs in the

UMOD gene region and CKD and estimated glomerular filtration rate [11]. This association was confirmed in an even larger meta-analysis published recently [12]. In particular SNP rs12917707 located 3.6 kb upstream of the UMOD gene was associated with CKD. The minor T allele of that SNP conferred a 20% reduction in risk for incident CKD over almost 15 years of follow-up in the Atherosclerosis Risk in Communities (ARIC) Study. To follow this further, Köttgen et al. [26] found in a case-control study of 242 CKD patients that high urinary uromodulin excretion was associated with the development of CKD 10 years later. Cases had 50% higher uromodulin levels than did controls without CKD. In addition, uromodulin excretion was lower with each copy of the minor C allele of SNP rs4293393 in the UMOD gene region, which is associated with a lower risk of CKD. The frequency of the minor C allele is 0.18 and the mean urinary uromodulin concentrations are 5.5, 3.1 and 1.5 $\mu\text{g/ml}$ for 0, 1 or 2 copies of the C allele, respectively. This study suggests that high urinary uromodulin excretion, which is genetically determined, may be a risk factor for CKD.

The pathogenetic link between high uromodulin excretion and CKD is at present unknown. So far, low uromodulin levels have mostly been considered a consequence of TAL damage and correlate with reduced renal function in various nephropathies [27–30]. How could high uromodulin synthesis and excretion cause or accelerate CKD? There is evidence that the immune system may play a central role. Uromodulin reacts with various cells of the immune system, possibly by nonspecific binding of its carbohydrates with cell surface receptors of these cells. The binding of uromodulin to neutrophils induces synthesis of IL-8, provokes the respiratory burst and degranulation [31, 32] and stimulates chemotaxis and phagocytosis [33]. Monocytes are induced to secrete IL-1 β , IL-6 and TNF- α [34]. Uromodulin increases the expression of the IL-2 receptor and HLA class II molecules on lymphocytes and stimulates cell proliferation [34]. Immature dendritic cells are transferred to a mature phenotype via Toll-like receptor-4 signaling [35]. Incubation of venous blood with uromodulin causes a dose-dependent increase in synthesis and secretion of TNF- α , IL-1 β , IL-6 and IL-8 by leukocytes [36]. In animal models, repeated intravenous administration of uromodulin causes a specific immune response. Uromodulin autoantibodies are detected in these animals and tubulointerstitial nephritis affecting the distal nephron segments, where uromodulin is synthesized, develops [37–41]. This interstitial nephritis is mainly caused by a cellular im-

mune response and can be transferred with lymphocytes and spleen cells [41]. Whether a specific immune response to uromodulin plays a role in human disease is presently unknown. Anti-uromodulin antibodies are detected in acute pyelonephritis, reflux nephropathy and other nephropathies, but also in healthy individuals and patients with hepatitis [42–46]. These antibodies usually are of very low affinity [47]. Immunoglobulins also bind in an unspecific way to carbohydrates of uromodulin [48]. Therefore, it is likely that the described anti-uromodulin antibodies in humans are not specific and are most likely an artifact. Together, there is no clear evidence that a specific humoral or cellular immune response to uromodulin plays a role in human renal disease.

Recently Prajczek et al. [36] presented new data that may shed some light on the role of uromodulin in CKD. They measured uromodulin in serum and urine of 14 healthy individuals and 77 CKD patients. In serum, uromodulin levels lower by a factor of 1,000 as compared to urine were found in patients and controls (ng/ml range in serum vs. mg/l range in urine). In agreement with others, they found that the lower the GFR the lower the urinary uromodulin excretion. Low urinary uromodulin was also associated with tubular atrophy and interstitial infiltration as detected in renal biopsies. In serum, however, there was a trend towards higher uromodulin levels in individuals with low GFR. Furthermore, high serum uromodulin was associated with higher serum levels of the proinflammatory cytokines TNF- α , IL-1 β , IL-6, IL-8, and of vascular endothelial growth factor VEGF, but not hepatocyte growth factor HGF. The authors speculate that uromodulin entering the renal interstitium, either via basolateral secretion by TAL cells or via back-leakage of urine, may react with cells of the immune system and stimulate an inflammatory response, which then promotes further tubulointerstitial damage. Studying monolayers of uromodulin-transfected LLC-PK1 cells (porcine proximal tubular cells) Jennings et al. [19] found that around 10% of uromodulin is secreted basolaterally. Other studies that showed interstitial deposits of uromodulin in conditions such as reflux nephropathy, glomerulonephritis, interstitial nephritis and in renal allografts, often in the vicinity of disrupted tubules, support the presence of uromodulin back-leakage in damaged kidneys [42, 49–52]. The finding that these deposits are frequently surrounded by cellular infiltration also fits the concept.

Fig. 1. Uromodulin synthesized in the TAL is targeted primarily to the apical membrane of the TAL cells (1), where it forms a gel on the cell surface (2) and is eventually released into the tubular lumen (3). A small proportion of uromodulin may be secreted basolaterally (4). Uromodulin also enters the interstitium by tubular back-leakage (5), especially in damaged tubuli. Binding of uromodulin to bacteria or crystals in the urine is beneficial, whereas its interaction with cells of the immune system in the renal interstitium causes inflammation and further renal damage.

A Unifying Hypothesis

Figure 1 summarizes the different effects of uromodulin present in the urine or interstitium. Uromodulin is, by its carbohydrate structures, a very sticky multipurpose molecule. It binds and neutralizes all sorts of objects that might appear in urine such as crystals, bacteria, various proteins and exosomes [53]. Once uromodulin finds its way into the renal interstitium, either by cellular secre-

tion or urinary back-leak, this stickiness becomes dangerous. Uromodulin will bind to cells of the immune system such as neutrophils, monocytes, dendritic cells and lymphocytes and thereby stimulate in an unspecific way an already ongoing immune reaction that may lead to tubulointerstitial damage and progressive renal failure. The data of Prajczar et al. [36] and Köttgen et al. [26] suggest that high urine or serum levels of uromodulin are potentially dangerous. Therefore, downregulation of synthesis and secretion of uromodulin might be a therapeutic option for slowing CKD progression. To be able to achieve this, we have to understand the factors that, in addition to genetics, regulate uromodulin secretion. Data on this subject are scarce, but it appears that a high-salt or high-protein diet, loop diuretics and polyuria all may increase uromodulin secretion [54–57]. The recent data on uromodulin will certainly stimulate further research in this field and make it a prime target for further studies of the pathogenesis and progression of CKD.

Lastly, it should also be mentioned that uromodulin may have renoprotective properties. In the mouse, renal ischemia and reperfusion stimulate uromodulin expression. In this model, uromodulin knockout mice show more tubular necrosis and inflammation and a greater impairment in renal function than do normal mice [58]. Future research into the role of uromodulin in kidney physiology and disease certainly holds some surprises.

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