Uromodulin Biology and Pathophysiology – An Update

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Abstract

Uromodulin (UMOD) is a glycoprotein expressed on the luminal surface of the apical membrane of renal tubular epithelial cells forming the thick ascending limb of Henle. Here, UMOD forms filamentous structures probably ensuring water impermeability and the countercurrent gradient. The multidomain structure, cellular topology of UMOD and clinical consequences associated with UMOD dysfunction, however, suggest that it may be involved in other biological processes such as receptor-mediated endocytosis, mechanosensation of urinary flow, Wnt-signaling, cell cycle regulation and planar cell polarity. A specific, but as yet unidentified, protease(s) releases UMOD into the urine, where it probably contributes to colloid osmotic pressure, retards passage of positively charged electrolytes, prevents urinary tract infection and modulates formation of supersaturated salts and their crystals. UMOD expression, biosynthesis and excretion are regulated in a complex manner, and dysregulation is found in a wide range of pathological conditions. It is strongly reduced or absent in cases with mutations in *UMOD*, *renin*, *HNF1B* and other genetic disorders causing autosomal dom-

inant hyperuricemic nephropathy. In contrast, elevated UMOD excretion may be associated with, and thus predictive of, chronic kidney disease. UMOD analysis is therefore of importance in all conditions with renal involvement and may be useful in the proper classification of renal diseases.

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Introduction

Uromodulin (UMOD) or Tamm-Horsfall protein (THP) is a protein selectively expressed by epithelial cells of the thick ascending limb of Henle's loop (TALH). Normally, the synthesized protein is cotranslationally translocated into the endoplasmic reticulum (ER), glycosylated, glypiated and secreted, and glycosylphosphatidylinositol (GPI) – anchored to the apical tubular cell membrane [1–3]. From this site the protein is released by a specific, but as yet unidentified, protease [4–6]. The released protein is excreted in the urine and represents the most abundant urinary protein in the healthy individual [7]. In the urine, the protein precipitates and is the main constituent of hyaline urinary casts [8–10].

UMOD expression and localization in kidney and its urinary excretion have been extensively studied in various pathophysiological states, and altogether more than 1,000 UMOD-related studies have been published. Unfortu-

nately, most of these studies used nonstandardized analytical methods for UMOD determination, resulting in a group of investigations which provide quite conflicting results and ambiguous conclusions. As a result, the exact biological roles of UMOD remain unclear and there has been a gradual decline of research interest in this protein.

The interest in UMOD biology and function was revived recently by the seminal work of Hart et al. [11] who identified causative *UMOD* mutations in a subset of families suffering from familial juvenile hyperuricemic nephropathy (FJHN) and medullary cystic kidney disease type 2 (MCKD2) [12]. Further stimuli have been undoubtedly provided by recently identified associations of common *UMOD* variants with chronic kidney disease, reduced estimated glomerular filtration rate and urinary UMOD excretion [13, 14], and by novel proteomic approaches allowing detailed fingerprinting of renal diseases [15–22].

The aim of this review is to provide an update on current knowledge (and guesses) of UMOD biology, function and pathophysiology, and to summarize the results of published studies reporting clinically relevant changes in UMOD expression and excretion.

Discovery of UMOD by Igor Tamm and Frank Lappin Horsfall

In 1950, Tamm and Horsfall [23] isolated a substance from human urine that acted as a potent in vitro inhibitor of virus-mediated hemagglutination. They determined its inhibitory and physicochemical properties and concluded that the substance was similar in structure to mucoproteins [24].

A number of chemical (elemental composition), biochemical (amino acid composition, carbohydrate content, supramolecular structure, molecular weight, pI and electrophoretic mobility), physicochemical (solubility, pH and thermal stability) and physical (viscosity, diffusion coefficient, ultraviolet and infrared absorption spectra, extinction coefficient, refractive index, and rheology) properties were determined in ongoing studies in the 1950s [25–30] and 1960s [31–40], and re-evaluated in the 1970s [41–45].

These studies showed that urinary THP has a molecular weight of 80–90 kDa, 20–30% of which is derived from high sialic acid content N-linked glycans. It contains a very high number of cysteine residues (48) that are completely engaged in disulfide bond formation. THP is the most abundant protein in normal urine, and is excreted

at the average rate of 50 mg/day. In low-salt solutions, it forms supramolecular filamentous aggregates of variable molecular weight ranging up to 7×10^6 kDa.

It is notable that these aggregates were directly visualized in several studies by electron microscopy [28, 31, 41, 46–48] and conditions and molecular mechanisms for their formation were established [46, 47, 49–52].

In the group of studies performed in parallel, THP was shown to be present in urine of other mammals [53–57].

In 1985, Muchmore and Decker [58] used lectin-based affinity chromatography to isolate a glycoprotein with in vitro immunosuppressive properties from the urine of pregnant women, and called the protein uromodulin. It underwent further independent characterizations [59–63] until Pennica et al. [64] proved, by complementary DNA (cDNA) analysis, that UMOD and THP are identical proteins. The term uromodulin (UMOD) will be used henceforth in this paper.

UMOD Gene

UMOD is located at cytogenetic band 16p12.3 according to major gene databases (Ensembl, UCSC, Entrez Gene, HGNC and GeneCards), except for the GeneAtlas database (<http://genatlas.medecine.univ-paris5.fr>), where it is located at 16p13.11. The physical position of *UMOD* is between 20,344,374–20,364,037 bp on chromosome 16 according to GRCh37/hg19 assembly. The gene is transcribed from the ‘plus’ strand (encoded on the ‘minus’ strand) into 2 major transcripts differing in their 5′ untranslated region, but encoding the identical protein (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>).

UMOD is transcribed exclusively in the kidney as shown by Northern blot analysis [64] and a number of tissue specific gene expression profiling studies (<http://biogps.gnf.org>). The transcription specificity of *UMOD* is determined by its promoter sequence. It was shown that 3, 3.9 and 1.5 kbp of mouse [65], bovine [66] and goat [67] genomic sequences, respectively, located upstream of the first *UMOD* exon lead to kidney-specific expression of reporter genes in transgenic animals. The same was shown for 5.6 kbp of human genomic sequence, consisting of 3.7 kbp promoter, exon 1, intron 1 and the untranslated part of exon 2 [68]. 5′ stepwise deletions of the bovine *UMOD* promoter revealed *cis* elements critical to transcription specificity are located within the first 600 bp adjacent to the transcription initiation site [66]. Zhu et al. [65] identified the first 589 bp of the *UMOD* promoter as being highly conserved in humans, rats, mice and cows.

Fig. 1. Schematic representation of uromodulin mRNA and protein. **A** Representation of *UMOD* mRNA with exons 1–11 in their actual proportions. **B** Scheme of *UMOD* precursor depicted as domains (vertical color boxes) on the protein backbone (horizontal thick black box) in their actual proportions. The domains' starts and ends are shown on their lower halves. The GPI attachment site is indicated by vertical magenta line at position 614. Mismatch mutations are indicated by vertical black lines in their actual

sites. Deletions in their actual ranges are indicated by horizontal black lines accompanied by letters: **a** (p.V93_G97del/insAASC), **b** (p.H177_R185del) and **c** (p.E188_L221del). **Y**= Potential N-glycosylation site; SP = signal peptide; EGF = epidermal growth factor-like; cbEGF = calcium-binding epidermal growth factor-like; D8C = 8-cysteine domain; ZP = zona pellucida. (Adapted and revised from M. Živnáč, PhD Thesis, 2010).

UMOD Protein

Primary and Secondary Structure

The *UMOD* precursor is composed of 640 amino acids, based on the cDNA sequence [64]. Motifs identified in the *UMOD* sequence (fig. 1) include signal sequence (residues 1–24), 1 epidermal growth factor-like and 2 calcium-binding epidermal growth factor-like domains (residues 31–64, 65–107 and 108–149), 1 zona pellucida domain (residues 334–585), 8 potential N-glycosylation sites and the GPI attachment site (residue 614). There are 48 cysteine residues involved in disulfide bond formation [42, 69] (http://smart.embl.de/smart/show_motifs.pl?ID=P07911). In the region between the 2nd calcium-binding epidermal growth factor domain and zona pellucida domain (residues 150–333), a 4th epidermal growth factor-like domain spanning residues 281–336 [70–72], or residues 297–335 [73], was proposed. A new domain, D8C (residues 199–287), common to a new family of proteins, including liver-specific zona pellucida domain-containing protein, glycoprotein 2, *UMOD* and several other uncharacterized proteins, was described by Yang et al. [74].

Biosynthesis, Trafficking and Cellular Localization

As *UMOD* is a GPI-anchored plasma membrane protein, its biosynthesis and intracellular trafficking must

proceed through the secretory pathway. During biosynthesis, the *UMOD* precursor is cotranslationally translocated into the ER. There the signal peptide is cleaved, and the protein is glycosylated on 7 of its 8 potential N-glycosylation sites [1, 75], disulfide bridges are formed and glypiation on its C-terminus (probably on S614) occurs. N-glycan moieties are further trimmed in the Golgi apparatus.

Both mature glycan moieties and GPI modification act as sorting signals [76, 77], routing the protein to the apical membrane of epithelial cells of the TALH, macula densa segment and distal convoluted tubule (DCT) as proven by immunohistochemistry [3, 70, 78–81], immunofluorescence [56, 82–87] and immunoelectron microscopy [78, 85, 88, 89] analyses.

Here the protein is finally GPI-anchored [1] and, facing the tubular lumen, it forms supramolecular structures [46], thereby ensuring some of its proposed physiological functions.

From the luminal side of the membrane, the protein is actively released by a specific, but currently unidentified, protease(s) [4, 5]. Proteolytic cleavage was originally proposed to occur after the residue F548 [5], but was later precisely assigned to the residue F587 [6]. This implies that urinary *UMOD* is composed of 563 amino acids and, depending on its glycosylation status, migrates as an 80–90 kDa band on SDS-PAGE.

In addition to this classical apical targeting, to a minor degree UMOD also sorts to the basolateral pole of tubular epithelial cells as shown by sensitive immunomethods [3, 78, 85, 90]. These observations are in line with the presence of UMOD in circulation [91] as proven by mass spectrometry analyses of the human plasma proteome (<http://www.peptideatlas.org>, <http://www.ebi.ac.uk/pride>).

Biochemical Analysis

Isolation of UMOD is usually performed by classical salt-out precipitation of urine [23], with several modifications in postprecipitation steps. The resulting preparation can be further purified by gel filtration [92]. As a quicker, as well as low-cost, alternative to precipitation, a method employing diatomaceous earth as a filter material [93, 94] was developed and evaluated for clinical purposes [95].

Qualitative and semiquantitative analysis of UMOD in complex samples such as urine, cultured cell lysates and tissue homogenates is usually performed by SDS-PAGE followed by detection with Coomassie brilliant blue staining, or by Western blotting and immunodetection [70, 80]. These methods are easy to set up, inexpensive, easily scalable and informative. UMOD antibodies are commercially available from several vendors; therefore, they can be routinely used to examine urinary UMOD excretion as a 1st step in the diagnostic process for UMOD-associated kidney diseases (UAKD; our experience). Qualitative and quantitative UMOD analyses by mass spectrometry offer promising but technically more demanding approaches [6, 80, 96].

Quantitative analysis of UMOD has been mostly performed by enzyme-linked immunosorbent assay (ELISA). Both indirect as well as direct ELISA setups have been reported for urinary [5, 97–101] and serum [91, 102, 103] measurements. In the 1990s, Syn^{elisa} THP immunoassay was available from Elias Medizintechnik GmbH (Freiburg, Germany; which later became Elias/Pharmacia & Upjohn), but its production was eventually discontinued. However, a new ELISA kit became available in 2009 from MD Biosciences GmbH (Zürich, Switzerland). Besides ELISA, radioimmunoassay [52, 92, 104–108], radial immunodiffusion [109, 110] and electroimmunoassay [111, 112] have also been used in the past. An elegant and highly quantitative immunomethod based on the Luminex platform has been described recently [14]. An analytical method based on high-performance liquid chromatography with native fluorescence detection [113] offers an alternative to immunoassay. Finally, SDS-PAGE and densitometry quantitation of bands after staining

has been used as a fully quantitative approach in several studies [114–116].

In situ detection of UMOD in kidney tissue specimens and cultured cells is performed using standard immunohistochemical, immunofluorescence and immunoelectron microscopy methods [70, 71, 91, 98, 117–120]. Quantitative in situ analysis of UMOD may be performed by immunogold-electron microscopy with particle counting [117]. Quantitative assessment of the UMOD display on the surface of cultured cells is performed mainly by fluorescence-activated cell sorter analysis [3, 70, 98, 117], but ELISA can be also used for this purpose [3].

UMOD Physiology

GPI-anchoring, multidomain and supramolecular gel-like structures on the luminal side of the plasma membrane of epithelial cells, together with rich and highly variable post-translational protein modification and a massive presence of the polymerized protein in urine, suggest that UMOD may have multiple and site-specific physiologic functions. However, despite 60 years of UMOD research, these physiological functions still remain rather elusive, and they have been mostly predicted from the protein primary structure, localization and in vitro experiments. Below we recapitulate those proposed functions in the context of some of our purely hypothetical, mechanistic ideas.

UMOD Function in Biogenesis and Organization of the Apical Membrane of TALH Cells

GPI anchor, N-linked glycosylation and a tendency towards polymerization are well-established factors in the sorting of apical membrane proteins [121]. Regarding its massive production, it can be assumed that UMOD biosynthesis may play, either directly or indirectly, an important role in the formation of apical membrane-targeted cargo vesicles and vesicular trafficking. GPI-anchored proteins also associate with lipid raft domains that play a role in the organization and dynamics of the apical membrane and in signal transduction [122]. A highly ordered organization of apical membranes allows for close packing of GPI-anchored proteins on the cell membrane. In the case of UMOD, this probably promotes formation of complex filamentous gel-like structures, providing the water barrier on the luminal plasma membrane of TALH cells and serving as a physical barrier to water permeability. Such a barrier may play an important role in ion transport and maintenance of countercurrent gradient in the interstitium [78, 123].

UMOD-Mediated Endocytosis

Recent identification of GPI-enriched endocytic compartments [124, 125] have led to the hypothesis that UMOD may serve as a receptor for binding and endocytosis of yet to be defined ligands (cytokines, TNF, etc.). This may also play a role in modulation of cell surface events, receptor engagement and signal transduction along the corresponding nephron segment.

UMOD as a Component of Primary Cilia

Recently, it has also been shown that UMOD localizes to cilia [126], which suggests that it may contribute to mechanosensation of urinary flow, Wnt signaling, cell cycle regulation and planar cell polarity.

Urinary UMOD Functions

Following its protease-mediated shedding from the apical membrane of TALH cells, massive amounts of UMOD are released into the tubular fluid. One can imagine that the released protein maintains its gel-like and/or molecular sieve-like structures, interacting or associating with other UMOD molecules, which are still plasma membrane anchored. This structure may form a slowly moving polyanionic gel potentially serving several advantageous functions. It may contribute to the colloid osmotic pressure and retard passage of positively charged electrolytes (sodium and potassium) through the TALH and thus enhance their active transport and reabsorption in this segment.

Passing further along the distal tubule and collecting duct, this polyanionic phase-like structure may bind uropathogenic bacterial strains [127–129] and prevent their adherence to glycoproteins and glycolipids on luminal plasma membranes and, hence, urinary tract infections.

In this way, UMOD may also act as an inhibitor of urinary stone formation and adhesion [130–136], as well as a platform for the removal of proteins, cells and debris that are formed in the distal tubule.

Finally, not by direct binding as suggested [137], but through electrostatic (ionic) interaction, it may reduce uric acid transport in the distal tubule [138–140] and thus contribute to final tuning of uric acid excretion in kidney.

Lessons from Knockout Mouse Models

Some of the proposed physiological roles of UMOD were evaluated by the generation and examination of *Umod*^{-/-} mice models established by independent groups [141, 142]. In both cases, it was shown that knockout (KO) mice suffered significantly more frequently from urinary tract infections. Indeed, Raffi et al. [143, 144] showed that

UMOD could act as a general host defense factor against urinary tract infections.

Mo et al. [145] showed that a portion of KO mice spontaneously formed calcium oxalate stones primarily in the collecting duct of the deep medulla and papilla. The rate of stone formation and crystal abundance were substantially increased in KO mice on a special stone formation-inducing diet. Stone formation was never observed in wild-type littermates in either condition. Recently, it was also shown that these mice are more prone to ischemia-induced kidney injury than wild-type mice, and that the process was accompanied by altered tubular expression of Toll-like receptor 4 [146].

Although the examination of steady-state electrolyte handling showed no abnormalities in KO mice [147], creatinine clearance was significantly reduced. Impaired urine concentration ability was observed under water deprivation. Moreover, biochemical and histochemical quantitative approaches used to assess changes in the biosynthesis of proteins related to NaCl transport along the nephron revealed upregulation of major distal transporters and downregulation of juxtaglomerular apparatus components, consistent with the adaptation to altered NaCl reabsorption in the TALH.

The same authors further observed a significant difference in the ratio of urinary sodium concentrations and urinary urate concentrations between KO and wild-type mice [148] – KO mice excreted less uric acid for a given amount of sodium, a result consistent with previously observed dysregulation of NaCl transport-related proteins. Interestingly, mRNA levels of PT urate transporters were found to be normal, suggesting that the upregulation of their expression is not responsible for increased urate reabsorption. It is important, however, to remember that the transporters for urate are very different between mice and humans, and that mice normally produce and reabsorb only small amounts of urate [149].

UMOD in Inflammation and Immunity

A number of studies have suggested that UMOD plays a role in inflammation processes and modulation of immune system responses [3, 58–63, 150–159]. This notion, however, comes mostly from in vitro studies or in vivo studies performed under artificial conditions, and their relevance for physiology and particularly for pathophysiology is debatable. For this reason, this topic is not further discussed in this review.

UMOD Pathophysiology

UMOD function(s), especially in humans, can be deduced to some extent from clinical and biochemical coincidences and/or consequences associated with changes in UMOD primary structure, transcription, expression, post-translational modification, localization and urinary excretion that have been identified and studied in a number of pathological states.

UMOD Mutations

UMOD mutations have been identified in patients suffering from FJHN (OMIM 162000), MCKD2 (OMIM 603860) or glomerulocystic kidney disease (GCKD) (OMIM 609886) [11, 12, 70]. At this time, 58 different mutations in the *UMOD* gene have been reported in the literature [11, 12, 70, 71, 73, 96–98, 117, 119, 126, 160–168]. Most of the mutations occur in exons 3 and 4. Fifty-five are missense mutations and 3 are in-frame deletions leading to the loss of 5–33 amino acids (fig. 1). Interestingly, mutations reducing either *UMOD* transcript amount or encoding for completely truncated protein have not yet been identified as a cause of UAKD.

The observed mutational spectrum implies a dominant-negative effect of the mutations. Indeed, identified mutations in *UMOD* affect biosynthesis of the protein, which leads to its aberrant intracellular trafficking, ER storage, altered formation of supramolecular domains on the apical plasma membrane of TALH cells, abnormal UMOD expression in the kidney and decreased urinary UMOD excretion [70, 91, 96, 98, 117, 120, 160]. These events may initiate various physiologic reactions, such as ER stress and tubulointerstitial injury, and lead to progressive renal damage eventually requiring dialysis and renal transplantation. The hyperuricemia and reduced urinary concentration, biochemical hallmarks of FJHN/MCKD, are likely related [72]. As described previously, UMOD is likely important in keeping the TALH watertight. Loss of the integrity of the TALH would decrease the concentrating ability of the loop of Henle and result in decreased urinary concentration. The decreased ability to reabsorb sodium in the thick ascending limb would be balanced by an increase in PT reabsorption of sodium and secondarily of uric acid, resulting in hyperuricemia.

Uromodulin-Associated Kidney Diseases

Initial linkage mapping studies have shown that FJHN/MCKD is genetically heterogeneous [163, 169–172]. Following the discovery of *UMOD* mutations, subsequent sequencing studies revealed that *UMOD* muta-

tions are present in at most 30% of families presenting with symptoms fulfilling diagnostic criteria of FJHN/MCKD, i.e. autosomal dominant inheritance of hyperuricemia resulting from decreased fractional excretion of urate, impaired urine concentration ability, attacks of gouty arthritis and progressive renal insufficiency associated with early onset, nonspecific tubulointerstitial lesions, hypertension and inconstantly with renal corticomedullary cysts.

While the term ‘uromodulin-associated kidney disease’ was first proposed by Hart et al. in 2002 [11] to describe families with mutations in the *UMOD* gene, we believe that UMOD abnormalities are an important component of the pathophysiology in other individuals with the clinical syndrome of autosomal dominant hyperuricemia and kidney disease.

In our research [98, 173, 174], we have shown that altered UMOD biology may account for this clinical syndrome in many individuals, despite different underlying genetic defects. We performed qualitative and quantitative analysis of urinary UMOD excretion in 12 and 15 families, respectively. Both Western blot and ELISA revealed significantly decreased urinary UMOD excretion in all but one of the families examined. In addition, we performed immunohistochemical analysis of UMOD and epithelial membrane antigen mucin (MUC1), the protein transported through the same secretory pathway as UMOD, in kidney sections representing putatively 4 different genetic defects leading to UAKD.

In individuals with *UMOD* mutations, urinary UMOD is usually normal during childhood (despite hyperuricemia being present) [164; Vyleťal, unpublished data], and its excretion gradually reduces to total absence. The gradual decrease reflects a process of massive intracellular accumulation of UMOD in tubular cells, leading to tubulointerstitial injury probably facilitated by ER stress.

We also identified reduced urinary UMOD excretion resulting from attenuated *UMOD* expression in families presenting with anemia and hyperuricemia caused by mutations in the renin signal sequence [98, 174] (Bleyer et al., unpubl. data).

Similarly, we observed reduced UMOD excretion and expression patterns in a family with features of FJHN associated with diabetes mellitus, in which a mutation in the *HNF1B* gene on chromosome 17q12 was found [175]. *HNF1B* is a critical regulator of *UMOD* transcription [176], so it may be expected that similar UMOD profiles will be present in many sporadic and/or familial cases with renal disease resulting from *HNF1B* mutations [177].

We also observed reduced urinary UMOD excretion in a group of patients with none of the genetic defects described above. Among them, the reduced urinary UMOD excretion reflected either low intracellular UMOD positivity associated with strong presence of UMOD in the hyaline casts or cases with irregular, mosaic-like UMOD staining patterns. Interestingly, in all these cases the UMOD staining patterns were mostly correlated with those of apical membrane marker MUC1 [98], suggesting a general secretory defect.

Mouse Models with UMOD Mutations

To recapitulate the FJHN/MCKD phenotype and to get a tool for studying etiopathogenesis of UAKD, several mouse strains with *Umod* point mutations have been recently generated.

Takiue et al. [178, 179] generated transgenic mice using a chimeric transgene composed of mouse *Umod* promoter and human *UMOD* cDNA harboring mutation c.444T>G (p.C148W). Nevertheless, these mice did not develop any UAKD symptoms except for the cytoplasmic accumulation of UMOD in TALH and DCT epithelial cells. However, accumulated protein was not the product of the transgene, but rather of endogenous wild-type *Umod*.

Kemter et al. [180] reported *N*-ethyl-*N*-nitrosourea (ENU)-induced mutant mice harboring mutation c.871G>A (p.A227T), which has not yet been found in humans and is predicted as tolerated by SIFT analysis (<http://sift.jcvi.org/>). These mice developed some of the features of UAKD, but in a homozygous rather than heterozygous state. Moreover, the mutant animals developed additional urea and bone metabolism alterations never observed in human UAKD patients.

Eventually, in 2010 Bernascone et al. [181] generated a mouse harboring *Umod* mutation c.441T>G (p.C147W), analogous to the well-studied human *UMOD* mutation c444T>G (p.C148W). They used a transgene of exclusively murine origin. Transgenic mice recapitulated most of the biochemical (except for hyperuricemia, most probably due to expression of uricase in mice), clinical and histological features of UAKD in humans, including reduced urinary concentration ability, renal insufficiency with increased serum creatinine concentrations, renal cysts, progressive tubulointerstitial injury and accumulation of mutant UMOD in ER of TALH and DCT epithelial cells.

UMOD Expression

There is a great amount of *UMOD* expression data deposited in gene expression databases (www.nextbio.com,

www.ncbi.nlm.nih.gov/geo) that come from microarray-based studies focused on various renal pathologies. For example, decreased *UMOD* expression was detected in end-stage renal disease [182] and in cysts from autosomal dominant polycystic kidney disease type 1 patients [183]. It has been shown that *UMOD* expression is decreased in all kidney neoplasms studied so far – benign as well as malignant (<https://expo.intgen.org/geo>) [182, 184–195], chronic renal transplant rejections of grades II grade III [196, 197], interstitial fibrosis after renal transplantation [198] and renal grafts with delayed function (gse10419). *UMOD* gene expression was increased, however, in diabetic nephropathic glomeruli [199].

Urinary UMOD Excretion

Several studies [107, 130, 200, 201] attempted to evaluate factors determining physiological UMOD excretion, but provided conflicting results. During the day, there is considerable variation in excretion in adults, but it apparently correlates with urinary volume in individuals in antidiuresis and in those drinking in response to thirst [107]. A positive correlation of the excretion with dietary intake of salt was indicated in humans [200] as well as rats [202].

UMOD excretion seems to increase gradually from birth to adulthood [203–206], where it remains stable until a decline after 60 years of age [207, 208]. The urinary UMOD/creatinine ratio seems to be relatively stable from 4 years of age through the 7th decade of life [97, 204].

UMOD half-life turnover was estimated to be 16 h in humans [209], but ranges as wide as 3–7 to 168 h have been proposed [107].

It was also shown that urinary excretion is correlated with (estimated) glomerular filtration rate [201, 204, 210] and hence corresponds to declining kidney function in virtually all chronic kidney diseases.

Numerous studies conducted mainly in the 1980s/1990s attempted to correlate urinary UMOD excretion with various clinical conditions. In these studies, UMOD was quantified in urine samples mainly by different ELISA, radioimmunoassay or electroimmunoassay analyses. Table 1 presents a comprehensive overview of urinary UMOD excretion in various pathological conditions. As can be seen, major attention was given to diabetes and nephrolithiasis.

In diabetes type I, it was shown that decreased UMOD excretion is predictive of renal failure and cardiovascular disease in adults [211]. In affected children, however, other tubular markers seem to be more informative [212]. In nephrolithiasis, results from different studies

Table 1. Urinary UMOD excretion in various pathological conditions

Condition	Method	Trend	Ref.
Acute renal failure			
Oliguric phase	RIA	↓	221
Polyuric phase		↑	
Asthma	RIA	↑	222
Balkan endemic nephropathy	EIA	↑ (all groups)	223
	EIA	↑ (all groups)	200
	SDS-PAGE+D	0	224
	EIA	↑ (at risk)	225
Tubular proteinuria	EIA	↑	226
Burns			
Moderate to severe	RIA	↑	227
Very large or renal failure		↓	
Cadmium nephropathy	RIA	↑	228
	SSA/CBBc [†]	↑	229
Chemotherapy			
Cisplatin	ELISAh	↑	230
	ELISAh	↑	231
Ifosfamide	SDS-PAGE+D	↓ (60%)	232
Cyclosporin A	RIA	↓	233
Chronic nephropathy	RIA	↓	201
	ELISAh	↓ (78%)	210
Glomerulonephritis	ELISAh	↓	234
Diabetic nephropathy		↓	
PKD		↓	
Tubulointerstitial nephropathy		↓	
Chronic renal failure	EIA	↓	112
Diabetes (type I)			
Children 4–15 years	ELISAh	↓ (25%)	235
	ELISAh	0	212
Children during ketoacidosis	ELISAh	↓	236
Renal failure and cardiovascular death	ELISAh	↓	211
Retinopathy	ELISAh	0	237
Diabetic nephropathy	ELISAh	↓	238
	ELISAh	↓	239
	RIA	↓	108
+macroalbuminuria	ELISAh	↓	240
+microalbuminuria	ELISAh	0	241
		0	230
w/o nephropathy		0	238
+microalbuminuria	ELISAh	0	242
w/o any complications		↓	239
Normotensive, normoalbuminuria	ELISAh	↑ (DM >10 years)	99
		↓ (DM >15 years)	
	ELISAh	0 (DM <9 years; >15 years)	243
		↑ (DM 10–14 years)	
Overnight	ELISAh	0	244
Orthostasis		0	
Acute euglycemia + water load		↑	
Women (postmenopausal)	ELISAh	↓	245
Diabetes (type II)			
Women (postmenopausal)		0	245
Renal failure and cardiovascular death		0	211
Diabetes (nonselected)	LIA	↓	246

Table 1 (continued)

Condition	Method	Trend	Ref.
Elderly	ELISAh	↓	208
Women (disaggregated)	ELISAh	↓	247
Women (aggregated)		↑	
Normotension	RIA	↓	207
Fanconi syndrome			
Cystinosis		↑	228
Dent's disease	1-DE, 2-DE+MS	↓	248
	2-DE+MS	↓	249
Lowe's disease		↓	
AD idiopathic		↓	
Hyperprostaglandin E-syndrome	ELISAh	↓	250
Hypertension			
Essential		0	238
	RIA	0	251
Elders		0	207
Interstitial cystitis	ELISAh	↑	252
	2-DE+MS	↓	253
Intravenous urography			
Total	RIA	0	254
Aggregated		↓	
Lead exposure	ELISAc	0	255
	LIA	↑ (30%)	256
Lithiasis			
Nonselected	SDS-PAGE(+WB)+D	↓	257
	ELISAc	↓	130
		0	201
	SP+F-C+Ph	0	258
	SDS-PAGE+D	↓	115
	ELISAh	↓	218
	RIA	↑	259
Children	EIA	↑	260
First time	SDS-PAGE+D	↓	261
Recurrent		0	
Ca	FE+Sch+P	↑	262
		0	112
	ELISAc	↓	263
	ELISAc	0	264
	EIA	0	87
	EIA	0	111
Idiopathic	CTAB/Asbestos-BaBr ₂ +HL+Ph	↓	265
Children	EIA	0	266
Potential formers (boys)	RID	0	267
+renal tubular acidosis	EIA	↓	268
		↓	87
Recurrent	SDS-PAGE+D	0	116
	ELISAh	↓	269
Familial	ELISAc	↑	270
Idiopathic	RID	↓	109
+acute UTI		↑	116
Urate	ELISAc	↓	271
		↓	87
		↓	111
		↓	130
Cystine		0	87
Staghorn calculi		↓	111

Table 1 (continued)

Condition	Method	Trend	Ref.
Lupus nephritis	ELISAh	↓	272
Mercury vapors exposure	LIA	↑	273
Nephrotic syndrome	SP+F-C+Ph	↑	205
Newborns (1st week)			
Prematurity (uncomplicated)	RID	0	274
Asphyxiation		0	
Respiratory distress syndrome (idiopathic)		0	
Organic solvents exposure			
Various	NA	0	275
Dinitrotoluene	SDS-PAGE+D	↑ (36%)	276
Perchloroethylene	LIA	↑	277
Polycystic ovary syndrome	SP+TM	↓	278
Pregnancy	ELISAh	↑	279
+Hypertension	SDS-PAGE+D	0	280
Postpartum		↓ ↓ (83%) ↑ (transiently) 0 (92%)	
Proximal renal cyst (simple)	RIA	↑	281
Renal disease (various)	RIA	↓	282
	EIA	↓	283
	SDS-PAGE+D	↓	284
		↓	201
Surgery			
Children 3–13 years	ELISAh	0	285
Acute kidney failure postoperation	ELISAc	↓	286
CPB			
w and w/o AKI postoperation	2D-DIGE	↓	287
w renal dysfunction postoperation	ELISAc	↓	288
w/o renal dysfunction postoperation		0	
Elders	ELISAc	0	289
Stomach carcinoma post operation	SDS-PAGE+D	↓	114
Esophagus carcinoma post operation		↓	
Transplantation			
Kidney (recipients)	ELISAh	0	290
	ELISAc	0	291
Acute allograft rejection (4–14 days prior to)	SP+F-C+Ph	↑ (transiently)	206
Graft failure (acute tubular necrosis)	ELISAh	↓	292
Graft failure (acute immune rejection)		0	
Kidney (donors)	ELISAh	↑	290
Bone marrow (pretransplant conditioning regimen)	ELISAc	0	293
	RIA	0/↑ (during)	294
Hematological stem cells	ELISAc	0	295
Liver (preoperation)			
w renal insufficiency post operation	ELISAh	↓	296
w/o renal insufficiency post operation		0	

Table 1 (continued)

Condition	Method	Trend	Ref.
Urinary tract infection, recurrent			
Girls (median 9 years)	RID	0	297
(aggregated and disaggregated)	ELISAh	0	298
Acute phase (aggregated)		↓	
Women	ELISAh	0	299
Elderly, acute phase (disaggregated)		0	247
Elderly, acute phase (aggregated)		↓	
Adults		↑	116
Children	RIA	↑	300
Infants (5 days to 6 months)	ELISAh	↓	301
Vesicoureteral reflux (primary)	ELISAh	↑ (42%)	100

An increase, decrease or no change in urinary UMOD concentration is indicated by an upward arrow, downward arrow or zero, respectively. Additional information about study design or results is given in parentheses. By repeating references, urinary UMOD determination method is indicated only at first appearance. PKD = Polycystic kidney disease, DM = diabetes mellitus, CPB = cardiopulmonary bypass, AKI = acute kidney injury, UTI = urinary tract infection; ELISA = enzyme-linked immunosorbent assay, RIA = radioimmunoassay, EIA = electroimmunoassay, IE = immunoelectrophoresis, RID = radial immunodiffusion, LIA = latex immunoassay, SDS-PAGE = sodium dodecylsulphate-polyacrylamide gel

electrophoresis, 1-DE = one-dimensional electrophoresis, 2-DE = two-dimensional electrophoresis, 2D-DIGE = two-dimensional differential gel electrophoresis, D = densitometry, MS = mass spectrometry, FE = free electrophoresis, Sch = Schlieren imaging, P = planimetry, SP = salt precipitation, TM = turbidimetry, SSA = sulfosalicylic acid, CBB = Coomassie brilliant blue, CTAB = cetyltrimethylammonium bromide, F-C = Folin-Ciocalteu reagent, Ph = photometry, HL = heated Lowry assay, h = home-made, c = commercial; NA = not applicable.

[†] = U-MP kit (Otsuka Assay Co., Tokushima, Japan).

are contradictory and, hence, inconclusive. The UMOD excretion rate itself is not sufficient to predict and/or to explain susceptibility to crystal formation (see also next section).

Recently, we reported the results of UMOD urinary excretion in Fabry disease [80]. Interestingly, in about half of the examined hemizygous patients, we observed abnormal UMOD excretion patterns on Western blots, i.e. the presence of shorter aberrant protein forms. Subsequently, we observed identical patterns in several other thesaurismoses, namely mucopolidosis type II, GM1 gangliosidosis, metachromatic leukodystrophy and Schindler disease (Vyletal, unpubl. data).

Subjects and Perspectives of Further Research

UMOD has been known since 1950. Despite this, there are many questions for which satisfactory answers are still missing. In the past, interest in this remarkable molecule waxed and waned. We believe that we are currently experiencing a UMOD research renaissance due to its well-established role in UAKD, association of common UMOD variants with chronic kidney disease [13, 14, 213–215] and

the availability of sensitive and quantitative urinary proteome analysis techniques [15–17, 19–22, 216, 217].

In UAKD, the pathogenetic mechanisms of aberrant UMOD trafficking must be better defined by studying responses and consequences of ER stress, apoptotic and ER-associated protein degradation (ERAD) pathways before any specific therapeutic targets may be devised.

Regarding continuous massive UMOD biosynthesis, it can be assumed that even subtle changes in UMOD transcription, expression and intracellular trafficking may affect secretory pathway efficiency, membrane organization and, thus, UMOD expressing cell functionality. In this context, true biological consequences of identified common UMOD variants must be studied through in silico and in vitro analysis of the UMOD promoter and further assessed in relevant cellular and/or transgenic animal models.

There is still no consensus on the role of UMOD in nephrolithiasis. While some authors believe that it acts only as an inhibitor of aggregation and growth of calcium oxalate crystals, others state that it can function as an inhibitor or promoter, depending on a number of physicochemical properties of urine and (secondarily) on the activity of tubular cells [218–220].

Finally, as it has emerged that UMOD urinary excretion is altered, either primarily or secondarily, in numerous disease conditions, qualitative and quantitative UMOD analysis using urinary proteome analysis techniques deserves attention in all clinical states in which apparent or potential renal involvement comes into question.

Conclusion

UMOD is a protein expressed solely in the mammalian kidney, namely in TALH and early DCT epithelial cells in humans. It is extensively glycosylated, glypiated and GPI-anchored in the luminal side of the apical plasma membrane. Here, UMOD forms organized structures, probably ensuring water impermeability and countercurrent gradient. Besides this, it might contribute to various biological processes such as receptor-mediated endocytosis, mechanosensation of urinary flow, Wnt signaling, cell cycle regulation and planar cell polarity.

A specific, but as yet unidentified, protease(s) cleaves off and releases UMOD into urine, where it can be found in the highest concentrations compared to other urinary proteins. In urine, UMOD might contribute to the colloid osmotic pressure, retard passage of positively charged electrolytes and prevent a number of bacteria strains

from attaching to tubular and bladder epithelia, therefore helping to prevent urinary tract infections. It modulates aggregation and growth of supersaturated salts and their crystals, respectively.

Urinary excretion of UMOD related to urinary creatinine is stable from 4 years of age in humans. *UMOD* gene expression, biosynthesis and excretion are regulated in a complex manner and reflect a wide range of pathological conditions.

UMOD excretion is strongly reduced or absent in cases with mutations in *UMOD*, *REN* and *HNF1B* genes, as well as other genetic disorders causing autosomal dominant hyperuricemic nephropathy (UAKD). In contrast, elevated UMOD excretion may be associated and, thus, predictive of chronic kidney disease.

Qualitative and quantitative analysis of UMOD urinary excretion is therefore important in all conditions with renal involvement and may be useful in the proper classification of renal disease based on analyses of urinary proteome.

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