Ubiquitin C-Terminal Hydrolase L1 is required for regulated protein degradation through the ubiquitin proteasome system in kidney

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Ubiquitin C-terminal hydrolase L1 (UCH-L1) is a major deubiquitinating enzyme of the nervous system and associated with the development of neurodegenerative diseases. We have previously shown that UCH-L1 is found in tubular and parietal cells of the kidney and is expressed de novo in injured podocytes. Since the role of UCH-L1 in the kidney is unknown we generated mice with a constitutive UCH-L1-deficiency to determine its role in renal health and disease. UCH-L1–deficient mice developed proteinuria, without gross changes in glomerular morphology. Tubular cells, endothelial cells, and podocytes showed signs of stress with an accumulation of oxidative-modified and polyubiquitinated proteins. Mechanistically, abnormal protein accumulation resulted from an altered proteasome abundance leading to decreased proteasomal activity, a finding exaggerated after induction of anti-podocyte nephritis. UCH-L1–deficient mice exhibited an exacerbated course of disease with increased tubulointerstitial and glomerular damage, acute renal failure, and death, the latter most likely a result of general neurologic impairment. Thus, UCH-L1 is required for regulated protein degradation in the kidney by controlling proteasome abundance. Altered proteasome abundance renders renal cells, particularly podocytes and endothelial cells, susceptible to injury.

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Membranous nephropathy is the most common cause of nephrotic syndrome in adults, and one-third of patients progress to dialysis-necessitating terminal renal insufficiency. Membranous nephropathy (MN) is caused by autoantibodies directed against podocyte antigens such as the PLA2R[1] and the recently identified THSD7A.[2] We could recently demonstrate that altered protein degradation through the ubiquitin proteasomal system is a feature of irreversibly injured podocytes in MN.[3] Hence, understanding the role of protein homeostasis in the kidney is of great importance, and restoring altered protein degradation in disease could potentially prevent irreversible renal injury.

The ubiquitin proteasome system (UPS) is one of the most prominent systems for intracellular protein degradation and is mainly responsible for the removal of short-lived proteins. The UPS comprises enzymes that ubiquitinate or deubiquitinate target proteins, and the 26S proteasome system, which degrades ubiquitinated proteins.[4] Ubiquitin is covalently attached to target proteins either as monomers (mono-ubiquitination) or as di-, oligo-, and polyubiquitin chains by a multi-enzymatic system consisting of E1 (ubiquitin-activating), E2 (ubiquitin-conjugating), and E3 (ubiquitin-ligating) enzymes. The mode of conjugation determines the fate of ubiquitinated proteins. The conjugation of ubiquitin to proteins is a reversible process that is tightly controlled by deubiquitinating enzymes. Deubiquitinating enzymes cleave monoubiquitin from proteins and disassemble polyubiquitin chains that are released from substrates before degradation in the multimeric protein complex called 26S proteasome, or in a more proteolytic effective form, the i26S immunoproteasome.[5]

UCH-L1 is a small 27 kDa soluble protease,[6] mainly expressed in neuronal tissues,[7] but also found in the testis[8] and in tumors.[9,10] Neural functions ascribed to UCH-L1 are in neuronal differentiation, regulation of synaptic function[11] and structure,[12] and cell process formation. UCH-L1 is a member of the family of deubiquitinating cysteine proteases, which regulate monoubiquitin levels and thereby play an important role in ubiquitin protein modification. A role for UCH-L1 in the regulation of the intracellular monoubiquitin pool[13] and on K63-alternate ubiquitin linkage[14] has been shown. Additionally, UCH-L1 function appears to be not

solely dependent on its hydrolase activity but also on its ability to bind to and stabilize ubiquitin.[15] Mouse models with loss of UCH-L1 function have been described, whereby the *gad* mice (del. ex7/8)[16] and *nm3419* mice (del. ex6 and partial incl. in6)[17] both exhibit decreased monoubiquitin levels and a similar neurologic phenotype with early sensory ataxia followed by motor ataxia.[18]

In the human and rat kidney, UCH-L1 is found in tubular cells[19] and in rat parietal epithelial cells[20,21] and *de novo* in injured podocytes.[22–24] We have previously shown that UCH-L1 is *de novo* expressed in injured podocytes in human MN[22] and in a rat model of MN.[25] However, it is unclear how UCH-L1 is functioning at the cellular and molecular level in kidney.

To assess the role of UCH-L1 in kidney, we generated a unique mouse model of UCH-L1 deficiency allowing the complete and tissue-specific knockout of UCH-L1 by Cre-lox technology. Here, we have evaluated the effect of complete UCH-L1-deficiency on murine kidney function and following the induction of an immune-complex nephritis, termed antipodocyte nephritis (APN). APN is induced by injection of polyclonal rabbit[26] or sheep antibody[27] directed against cultured murine podocytes. Mice exhibit linear glomerular binding of the injected anti-podocyte antibodies and develop severe nephrotic syndrome with subepithelial immune deposits.[27]

RESULTS

Constitutive UCH-L1 expression in tubulointerstitial and glomerular cells of the murine kidney

UCH-L1 was knocked out by crossing UCH-L1$^{fl/fl}$ mice with constitutive Cre-deleter mice. Analysis of UCH-L1 transcript levels in total kidney lysates confirmed complete loss of UCH-L1 mRNA and protein expression in UCH-L1-deficient mice in comparison with control littermates (Figure 1a and b). UCH-L1 protein levels were comparable between UCH-L1–deficient mice missing one *Uchl1* allele (heterozygous mice) and wild-type littermates (Figure 1b). Therefore, both genotypes were used as control littermates for subsequent analyses. Immunofluorescence confirmed complete loss of renal UCH-L1 expression in UCH-L1–deficient mice. Wild-type mice demonstrated a constitutive UCH-L1 expression in tubulointerstitial and glomerular cells (Figure 1c). Specifically, UCH-L1 was expressed in nerve endings of renal arterioles (Figure 1d) and glomerular and tubulointerstitial endothelial cells (Figure 1e and f) and tubulointerstitial cells of nonvascular origin (Figure 1f).

UCH-L1 deficiency results in proteinuria, urine retention, and hypotension

UCH-L1–deficient mice exhibit a profound neurodegenerative phenotype as published for other spontaneous UCH-L1–deficient knockout models.[16,17] We therefore analyzed blood pressure levels of UCH-L1–deficient mice at 25 weeks of age, when neurologic impairment was established. We noted a decrease of systolic blood pressure by around 35 mm Hg in comparison with control littermates (Figure 2a). Measurement of renin mRNA levels demonstrated a significant upregulation of renin in UCH-L1–deficient kidneys (Figure 2b). Furthermore, morphological examination demonstrated a significantly hypertrophied juxta-glomerular apparatus by periodic acid–Schiff staining and by specific labeling with lysosomal integral membrane protein (Limp)-2 (Figure 2c). These findings suggest a profound systemic hypotension secondary to neurologic impairment with renal hypoperfusion, leading to a compensatory increase of renin expression. Besides hypotension, we noted a neurogenic urinary retention in UCH-L1–deficient mice starting around 16 weeks of age with bladders filled with up to 2 ml of urine at the time of killing (Figure 3a and b). Of note, no signs of obstructive uropathy were seen macroscopically. The renal pelvis was not dilated. Resolving creatinine-adapted urinary proteins collected from 25-week-old mice by a silver gel demonstrated a preferential loss of albumin and of low molecular weight proteins (Figure 3c). Quantification of urinary albumin levels by enzyme-linked immunosorbent assay (ELISA) confirmed the finding that UCH-L1–deficient mice developed significant albuminuria with age (Figure 3d).

Accumulation of oxidative-modified proteins in kidneys of UCH-L1–deficient mice

Due to the fact that UCH-L1 deficiency resulted in the development of proteinuria, we evaluated the integrity of the glomerular filtration barrier. However, we noted no significant morphologic changes. Synaptopodin (Figure 4a) and nephrin immunofluorescence (not shown) showed no major alterations of staining pattern. Electron microscopic evaluations exhibited a normal architecture of the glomerular filtration barrier including an intact fenestrated endothelium, regular glomerular basement membrane, and normal podocyte foot processes (Figure 4b). We searched for signs of glomerular cell stress by Western blotting and by immunofluorescence for oxidative-modified proteins. We noted increased levels of oxidative (carbonyl)-modified proteins in glomerular lysates of 25-week-old mice (Figure 4c) following derivatization 2,4-dinitrophenylhydrazine (DNPH) and Western blotting for 2,4-dinitrophenyl (DNP). Immunofluorescence for DNP (Figure 4c) or for 8-hydroxyguanosine (Figure 4d) (a DNA base modified by hydroxyl radicals) demonstrated that oxidative stress was most apparent in podocytes, glomerular and tubulointerstitial endothelial cells, and in tubuli of UCH-L1–deficient mice. In line with enhanced accumulation of oxidative modified proteins in glomerular cells, staining for superoxide dismutase (SOD)-2, a mitochondrial enzyme involved in detoxification of reactive oxygen species, demonstrated an enhanced expression of SOD2 in podocytes but not in glomerular endothelial cells of UCH-L1–deficient mice (Figure 4e).

Taken together our data demonstrate that UCH-L1–deficient mice develop hypotension, neurogenic urine retention, and proteinuria without major morphologic alterations but with a significant accumulation of oxidative-modified proteins and DNA.