Role of NF-κB in Uromodulin-associated kidney disease

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Background:

Uromodulin-associated kidney disease (UAKD) is a heritable renal disease in humans caused by amino acid-changing mutations in the uromodulin (*UMOD*) gene. Clinical symptoms of UAKD can comprise hyperuricemia, gout, and alteration of urine concentrating ability. UAKD is progressive and can lead to end-stage kidney disease. Clinical symptoms of UAKD result from dysfunction of cells of the thick ascending limb of Henle (TALH), which is caused by maturation and trafficking defect of mutant uromodulin protein retained in the hyperplastic endoplasmic reticulum (ER) of TALH cells. UAKD belongs to the ER storage diseases.

Aims:

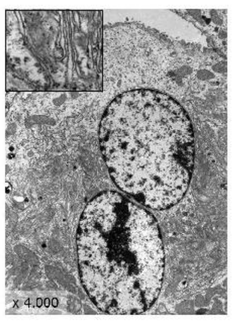
So far, pathways involved in the pathophysiology of UAKD are almost unknown. The recently established mouse models *Umod*^{C93F} and *Umod*^{A227T} harbor different amino acid-changing mutations in the *Umod* gene resulting in UAKD. These mutant mice were used to analyze the role of NF-κB signaling in UAKD.

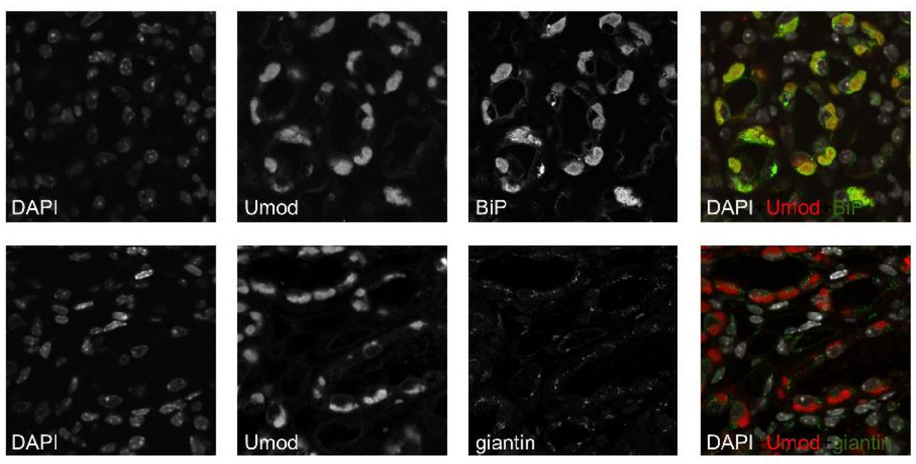
Methods:

The outer medulla of kidneys of homozygous *Umod*^{C93F} mutant, homozygous *Umod*^{A227T} mutant, and wild-type mice was prepared and used for protein isolation. After separation of the lysates on SDS-polyacrylamide gels and electro blotting, immunoblots were performed.

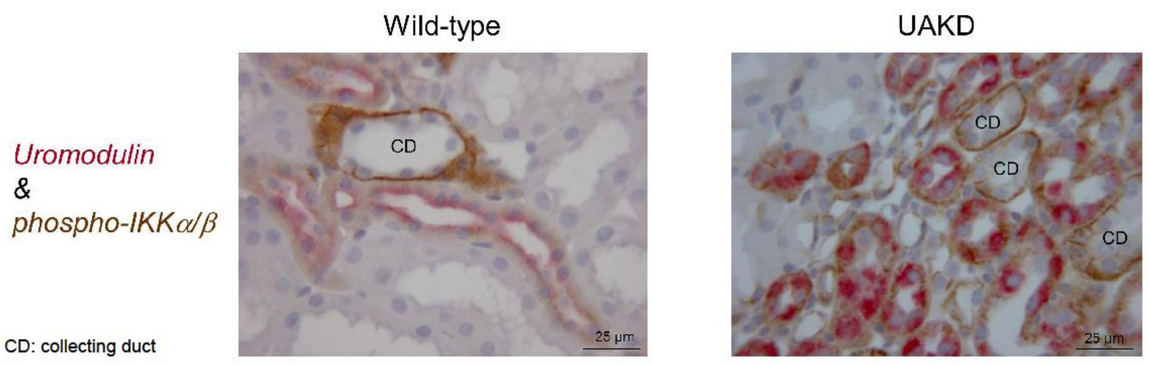
Kidneys of *Umod* mutant mice and wild-type mice were fixed in 4% paraformaldehyde, embedded in paraffin, and used for immunofluorescent and immunohistochemical analyses. For TEM, kidneys were fixed in 3% glutaraldehyde.

Results:

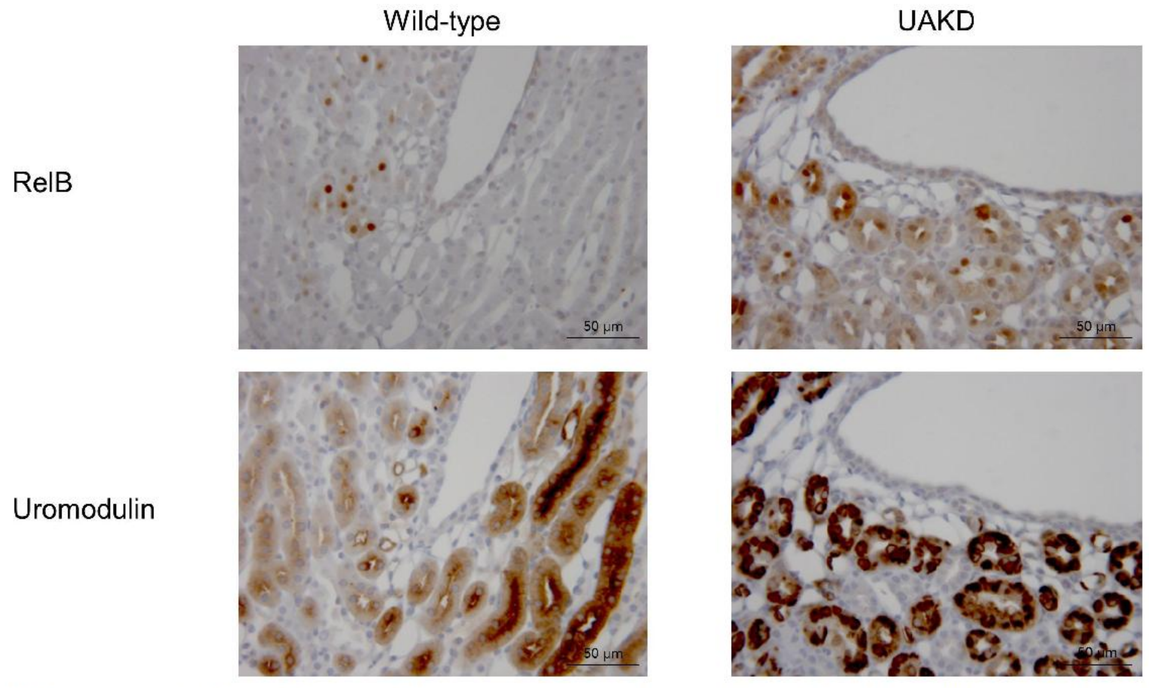




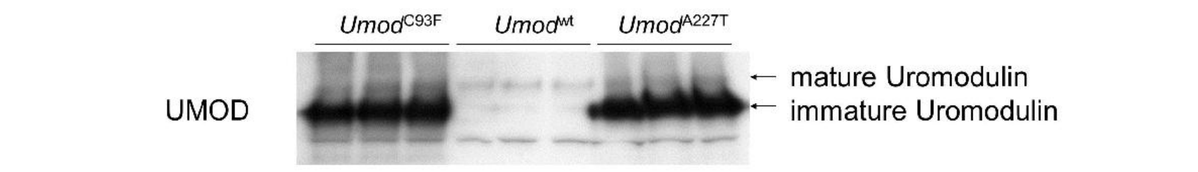
Mutant uromodulin is retained in the hyperplastic endoplasmic reticulum (ER) but not in the Golgi apparatus as demonstrated by co-localization of uromodulin with the ER marker BiP in TALH cells of a homozygous *Umod*^{C93F} mutant mouse.



In contrast to wild-type mice, where immunostaining of phospho-IKKα/β in TALH cells was weak and apical accentuated, a strong cytoplasmic basal and paranuclear staining pattern of phospho-IKKα/β was present in TALH cells of *Umod* mutant mice.

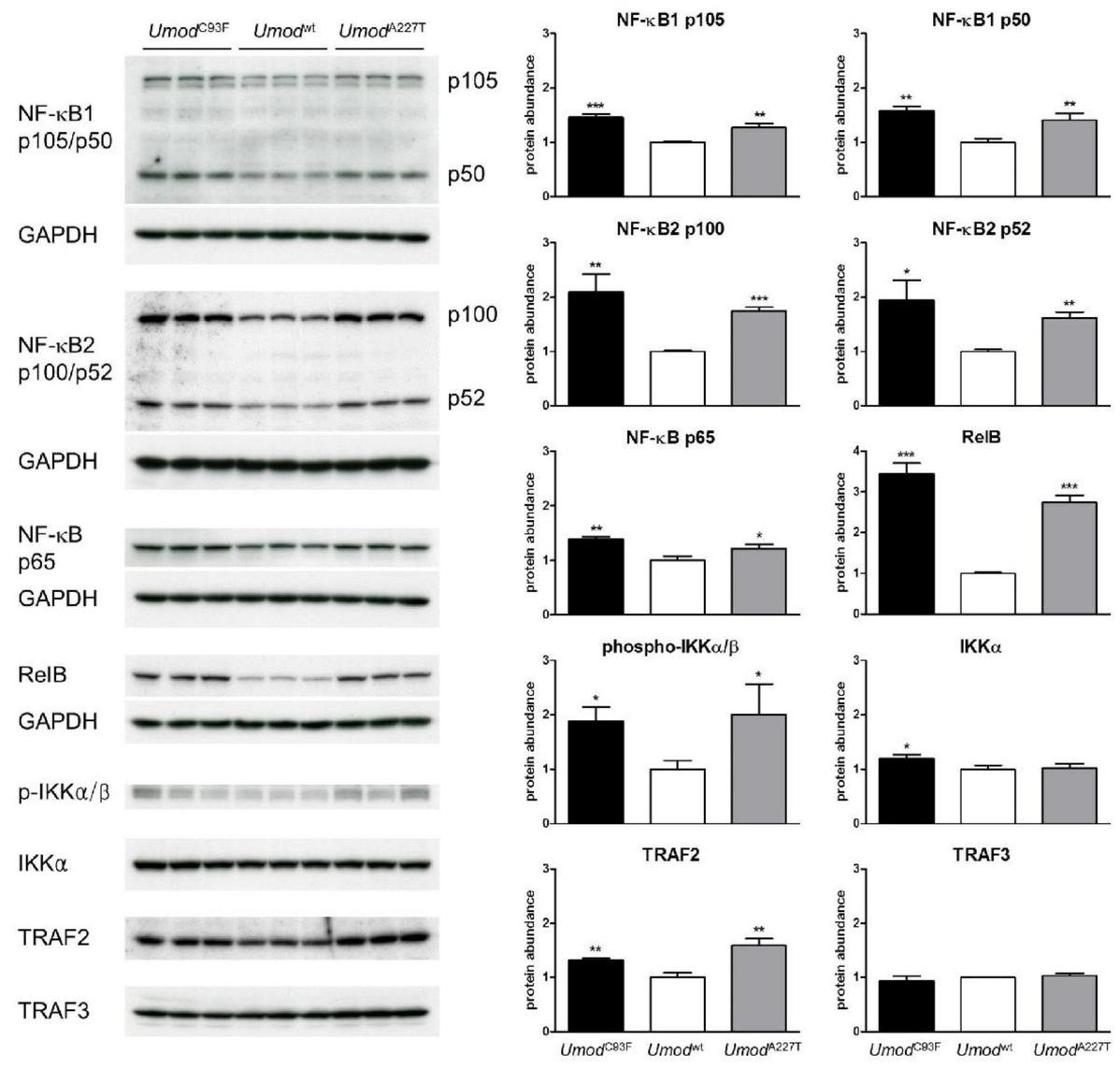


Whereas only few tubular cells were detected containing RelB in wild-type mice, the majority of tubular cells of the outer medulla of *Umod* mutant mice were immunopositive for RelB and the staining intensity was stronger than in the corresponding region of wild-type mice. Identification of TALH segment was enabled by detection of UMOD.



Student's *t*-test with Holm-Bonferroni correction: *p* vs. wild-type, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Increased abundances of BiP, CHOP, phospho-eIF2α and eIF2α in the renal outer medulla of *Umod* mutant mice indicate ER stress.

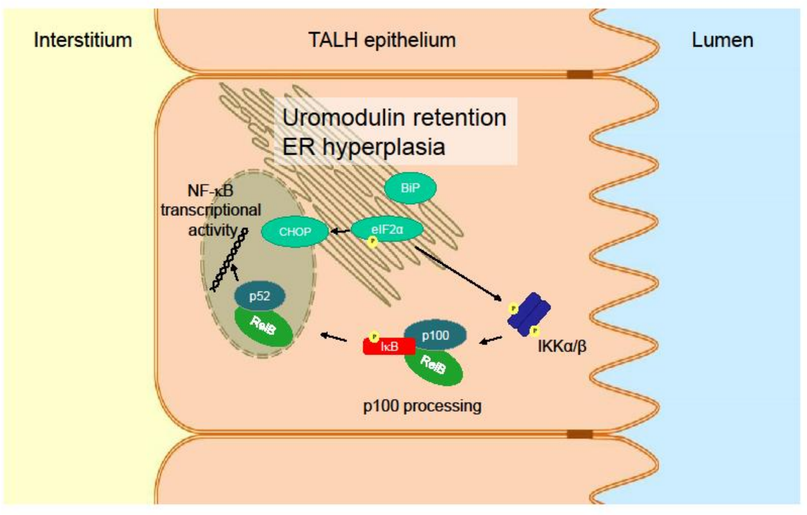


Student's *t*-test with Holm-Bonferroni correction: *p* vs. wild-type, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Homozygous *Umod*^{C93F} mutant and homozygous *Umod*^{A227T} mutant mice had a higher protein abundance of NF-κB1 p105/p50, NF-κB2 p100/p52, NF-κB p65, RelB, phospho-IKKα/β and TRAF2 in the renal outer medulla compared to age-matched wild-type littermates. Protein abundances of IKKα and TRAF3 did not differ between genotypes.

Conclusions:

The results of this study provide evidence for the activation of the non-canonical NF-κB pathway in TALH cells in our two mouse models of UAKD.



References:

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