Milena Traykova-Brauch
Dr. med.

Glycosphingolipids of renal tubular epithelia influence excretory kidney function

Geboren am 29.08.1976 in Sevlievo, Bulgarien
(Staats-)Examen am 16.11.2001 an der Medizinische Universität Sofia, Sofia, Bulgarien

Promotionsfach: DKFZ (Deutsches Krebsforschungszentrum)
Doktorvater: Prof. Dr. med. H.-J. Gröne

Glycosphingolipids (GSLs) are amphipathic molecules of the outer leaflet of the plasma membrane of eukaryotic cells. GSLs may be organized in cholesterol/glycosphingolipid enriched membrane microdomains and by that participate in processes like membrane trafficking, signal transduction, cell adhesion and differentiation. It has been shown that the absence of glucosylceramide (GlcCer)-based glycosphingolipids is embryonically lethal in mice. Their deficiency was achieved by thorough systemic deletion of the enzyme glucosylceramide synthase (GCS, e.g. UDP-glucose:ceramide glucosyltransferase, Ugcg).

Kidneys contain various GlcCer-based GSLs. We hypothesised that the absence of GlcCer-based glycosphingolipids in the kidney might be important for the development and excretory function of the kidney. Our aim was to generate a constitutive and an inducible renal tubular epithelium-specific Ugcg deletion mouse models and to investigate *in vivo* kidney development and its excretory function under different physiological conditions.

Mice depleted of glycosphingolipids in the tubular epithelium were generated, using the Cre-loxP recombination system. Pax8 was chosen as a promoter driving expression of Cre recombinase specifically to renal epithelial cells. Two existing strains were interbred, e.g. $Pax8^{Cre}$ and $Ugcg^{flox/flox}$. To overcome potential postnatal lethality and compensatory mechanisms during embryogenesis an inducible model for glycosphingolipids deficiency in renal epithelial cells was generated. This model represented a triple transgenic mouse, obtained by crossbreeding of a $Pax8^{rtTA/LC-1}$ line with $Ugcg^{flox/flox}$ mice. The excretory functions of the kidneys of GSL-deficient animals and their controls were investigated under different physiological conditions and pharmacologic interventions: regular water intake, acute water overload, thirst for 24 h and diuresis after amiloride and furosemide.

$Ugcg^{flox/flox//Pax8Cre}$ animals were viable, fertile and showed no obvious phenotype compared to their control littermates. Specificity of the *Ugcg* gene deletion in the kidney was proven with Southern blot analysis. Lipid extracts from kidneys of $Ugcg^{flox/flox//Pax8Cre}$ mice

showed absence of complex glucosylceramide-based glycosphingolipids. These results were confirmed by mass spectrometry. Light microscopy and electron microscopy revealed no obvious morphological changes in the kidneys of GSL-deficient animals compared to controls. However there was a constant statistically significant difference in the urine pH between the two groups under all investigated physiological conditions. The urinary pH of mutant mice ($Ugcg^{flox/flox//Pax8Cre}$) was always about one pH unit lower than in that in controls. Furthermore mutant mice showed repeatably a tendency of higher excretion of phosphate and calcium in the urine under different physiological conditions. Ammonia excretion was significantly lower in $Ugcg^{flox/flox//Pax8Cre}$ mice, as compared to controls. Analysis of blood gases and electrolytes showed no signs of systemic acid-base dysbalance and did not differ between the two groups. Semiquantitative evaluation of the expression of electrolyte transporters (e.g pendrin, calbindin), ammonia transporters (e.g Rhbg and Rhcg) and a proton pump (V H^+-ATPase) was performed. The ammonia transporters and the proton pump had no differential cortical expression but a pronounced medullary downregulation in the GSL-depleted mice. There was no statistically significant difference in the expression of pendrin, calbindin and aquaporin 2 (APQ2). No difference in the subcellular, e.g apical to basolateral localization of any of the transporters in the kidneys of $Ugcg^{flox/flox//Pax8Cre}$ and control mice was found.

Induced deficiency of the GlcCer-based lipids in the kidneys of $Ugcg^{flox/flox//Pax8rtTA//LC-1}$ mice was achieved upon doxycycline administration via the drinking water. Induction of gene deficiency was possible at any given time, e.g. embryonically or in adulthood. Embryonic GSL-depletion was nearly identical to the constitutive model, whereas in adult induction the reduction of globotriaosylceramide (Gb_3Cer) was significantly smaller. The inducible model opened the possibility to study the kinetics of glycosphingolipid depletion in the kidneys. We could show that it took one month of *Ugcg* gene deletion to obtain a stable residual low level of GlcCer-based GSLs. Parallel investigations of the kinetics of urinary pH change in $Ugcg^{flox/flox//Pax8rtTA//LC-1}$ mice resulted in a high temporal relationship between the fall of GSL content and urinary pH.

This is the first report on *in vivo* functional defects of GlcCer-based GSL-depletion in the kidney. This investigation describes a phenomenon of urinary pH acidification in GlcCer-based GSL-deficient animals under different physiological conditions. The significant increase in proton, phosphate and calcium excretion in GSL-deficient mice reveals an important modulatory function of GlcCer-based GSLs on functions of renal tubular cell membranes. Ammonia transporters might be involved in the molecular mechanism. Further

efforts will be necessary to elucidate directly the interaction between renal GSLs and transport molecules.