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Executive report - 6 Months (Feb 28, 2013)

Defective Transport and Epithelial Dedifferentiation:

Genesis of Key Events in Nephropathic Cystinosis

The overall goal of this project is to take advantage of a **detailed characterization of the C57BL/6 *Ctns* mouse** and the availability of cutting-edge methods to analyze **cellular mechanisms of proximal tubule (PT) transport defects** in the **early stage of cystinosis** (i.e. before the onset of renal failure). These transport defects play an essential role in the burden of disease (renal Fanconi syndrome) and are probably instrumental for renal disease progression.

The project started officially on **Sept. 1st, 2012**.

Our first objective was to characterize PT transport defects *in vivo*, using the C57BL/6 *Ctns* mouse model. We have established a pure colony of C57BL/6 *Ctns* mice (a gift of Dr. Antignac) in Brussels and Zurich, in order to analyze the time-course and markers of PT dysfunction. The *Ctns* KO mice show a growth retardation from birth and progressive manifestations of renal Fanconi syndrome: low-molecular-weight proteinuria, followed by polyuria, phosphaturia and glucosuria, *before the apparition of manifestations of renal failure.* The urinary loss of solutes is associated with decreased expression of specific transporters (incl. NaPi-IIa and SGLT2) and receptors (megalin/cubilin) in *Ctns* KO kidneys, which contain elevated cystin levels in PT cells. The changes in the expression of receptors and transporters are not associated with histological lesions of these PT segments (less than 5% of nephrons show swan-neck lesions in the S1 segment at this stage).

The second step was to further analyze these modifications in primary cultures of PT cells (mPTC) obtained from microdissected PT segments of *Ctns* KO mice. Our functional studies showed that *Ctns* KO mPTC show a decreased uptake of albumin, caused by a decreased expression of megalin and cubilin receptors. A decreased expression of NaPi-IIa and SGLT2 was also observed in mPTC. These changes, which are observed in absence of apoptosis, reveal that kidney PT cells lacking cystinosin show a functional defect caused by a decreased expression of apical receptors and transporters.

Over the last months, we have started to investigate potential mechanisms for the loss of receptor and transporter expression. By analogy with other congenital or acquired disorders of the PT cells (e.g. Dent disease, light chain disease), we observed that *Ctns* KO kidneys and mPTC show an increased expression of carbonic anhydrase type 3 (CA3), a marker of dedifferentiation and oxidative stress, together with a nuclear translocation of the Y-box transcription factor ZONAB and an increased expression of its targets PCNA and cyclin-D1. Since ZONAB is known to be a direct repressor of megalin and cubilin, these data suggest that the early phase of nephropathic cystinosis is characterized by specific transcriptional events modifying the expression of key transporters/receptors in PT cells, with a phenotype of dedifferentation and proliferation. These changes are observed both *in vivo* and *in vitro,* and occur before any visible histological lesion of PT cells.

Our objectives for the next 12 months are to take advantage of the resources available in Zurich and Brussels to investigate in more details the mechanisms of altered differentiation of PT cells. These studies will combine three distinct approaches, as follows.

1. Based on our experience with mouse models of PT dysfunction, we will document the transport defects of *Ctns* mice *in vivo* using single photon emission computed tomography (SPECT) for measurements of apical and basolateral PT transport pathways and micro-perfusion studies to evaluate apical ligand uptake, internalization of receptors and transporters, and transepithelial secretion. These studies will decipher the defective handling of phosphate and LMW ligands that is associated with early cystinosis.

2. Micro-perfusion studies of PT segments isolated from *Ctns* kidneys will be used to characterize the mechanisms primarily involved in the PT segments *ex vivo*. At this stage of established RFS, micro-perfusion will be used to evaluate with high-resolution the kinetics of internalization and processing of fluorescent albumin/transferrin (endocytic ligands) and that of NaPi-IIa from the brush border membrane to lysosomes (immunostaining and immunoblotting) in *Ctns* WT vs. KO PT segments. The effect of apical or basolateral exposure to (1-34) PTH (10^{-8}M) on internalization of NaPi-IIa will be compared in the two genotypes, and related to potential changes in the expression and localization of PTHR and the PDZ protein NHE3 regulatory factor (NHERF1), which is instrumental to couple the apical PTHR to NaPi-IIa internalization.

3. We will investigate the link between proliferation/differentiation and transport processes in cystinosis by using mPTC obtained from *Ctns* mouse kidneys. When grown on filters, these cells polarize and keep their differentiation as shown by morphological features and sodium-dependent apical transport processes and receptor-mediated endocytosis. The mPTC are obtained from defined PT segments, at very precise stages of disease and with perfectly matched control cells from littermates. The mPTC are well-suited to investigate sodium-dependent transport of phosphate and receptor-mediated endocytosis.

These studies should provide critical insights into the early chain of events leading to PT transport defects, before structural damage. In turn, these insights may provide new targets for interventions which could alleviate the burden caused by the urinary loss of vital metabolites in patients with nephropathic cystinosis.

Publication:

Raggi C. et al. *Defective transport and epithelial dedifferentiation: Genesis of key events in nephropathic cystinosis*. Manuscript in final preparation

Communications:

O. Devuyst (Plenary lecture): *The proximal tubule in renal diseases*. 45th Annual Meeting, European Society of Pediatric Nephrology, Krakow, Sept. 7, 2012

O. Devuyst: *GWAS studies, from rare diseases to common disorders*. Symposium on Inherited Kidney Disorders, Shanghai, Nov. 16, 2012

O. Devuyst: *Renal Fanconi syndrome and body fluid homeostasis*. Symposium for the Rare Disease Day and Inauguration of Rare Disease Initiative in Zurich, Zurich, Feb. 22, 2013.

Budget: Attached. Please note that salary costs have started only in Feb 2013, as most of the work has been performed thus far by a PhD student and a Post-Doc paid by another budget. Costs of training technicians and PhD have been included.