Function of renal primary cilia in health and disease as revealed by CRISPR/Cas9 technology

Background

The primary cilium is a sensory organelle present on most cells that responds to external mechanical and chemical stimuli. In the kidney nephron tubule, urinary flow causes bending of primary cilia. This mechanical stimulus is translated into cellular function through specific signaling pathways. The specific function elicited by cilia mechanosensation in kidney is currently unknown. As a consequence, how dysfunctional primary cilia in disease affect renal cellular processes and renal reabsorption of electrolytes is poorly understood.

To be able to study the molecular mechanisms characterizing primary cilia function and its physiological relevance in the kidney, we will construct a molecular tool that allows us to knockout the gene *Rpgrip1*, relevant for ciliogenesis. This tool will be applied in a kidney cell line in order to identify the molecular players that connect mechanosensation with cellular electrolyte transport. This novel system will be followed by the generation of a loss-of-function zebrafish model where cilia function is knocked out specifically in the kidney. Studying cilia function in the zebrafish kidney will allow the elucidation of the physiological relevance of primary cilia for renal electrolyte reabsorption. By this from-molecule-to-organism approach, information crucial for the understanding of the mechanisms leading to electrolyte imbalance in ciliopathy will be disclosed.

In detail, knockout of cilia function will be realized by applying state-of-the-art genome editing tools such as the CRISPR/Cas9 system using a mutant Cas9 nickase, and the efficiency will be assessed via quantitative analysis of gene and protein expression as well as with confocal microscopy. Once CRISPR/Cas9 cell and kidney-specific zebrafish models are generated, these platforms will be used to study the molecular mechanisms characterizing primary cilia function and to unravel the physiological relevance of primary cilia in the kidney.

Clinical relevance

Ciliopathies comprise a group of disorders associated with genetic mutations encoding defective proteins, which result in either abnormal formation or function of cilia. As cilia are a component of almost all vertebrate cells, cilia dysfunction can manifest as a constellation of features that include characteristically, retinal degeneration, renal disease and cerebral anomalies. Recent research has revealed the importance of particular cilia genes for kidney function and their role in disease such as polycystic kidney disease (PKD). PKD is an inherited kidney disorder. It causes fluid-filled cyst formation in the kidneys. PKD may impair kidney function and eventually cause kidney failure. One of the main vital functions of the kidney is the reabsorption of electrolytes contained in the pro-urine. In this regard, some ciliopathies are characterized by electrolyte imbalance, i.e. Lowe syndrome.

Aim

In this project we aim to elucidate the function of renal primary cilia in electrolyte transport and its physiological relevance in health and disease, to this end we will create a novel tool (using CRISPR/Cas9) that can be applied *in vitro* and *in vivo*.

In this internship you will be part of an international, highly motivated research team and will accomplish the following goals:

- To create a method that allows for the easy generation of inducible knockout cell lines
- To create an inducible primary cilia knockout (renal) cell line
- To assess the efficiency of an inducible, Cas9 mediated, knockout compared to siRNA knockdown
- To generate a zebrafish model where cilia function in the kidney is knocked out
- To study electrolyte transport in the models generated at cellular and organismal level
- To establish conclusions explaining the mechanisms that lead to electrolyte imbalance in ciliopathy

Techniques

During the internship you will be able to learn and apply various techniques in the fields of molecular, cellular and organismal physiology such as:

- Cellular and zebrafish genome editing by CRISPR/Cas9 technology
- A broad toolbox for isolating, analyzing and manipulating genetic material
- Immunocytochemistry and confocal microscopy
- RNA and protein isolation
- Gene and protein expression analysis using respectively real-time PCR and Western blot
- Techniques to evaluate cellular electrolyte transport
- Techniques to measure renal electrolyte reabsorption in zebrafish
- Presentation and writing skills as well as using a digital laboratory notebook

Contact

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