Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: The role of bioactive lipids in idiopathic nephrotic syndrome – exploring changes on lipid distribution and content in podocytes

Synopsis:

Nephrotic syndrome (NS) is a kidney disease that results from disruption of the glomerular filtration barrier leading to massive loss of proteins in the urine. In the majority of cases a precise etiology cannot be found, and NS is classified as idiopathic. Although in most cases treatment with glucocorticoids leads to remission, in 15-20% the disease follows a chronic relapsing course, significantly increasing the progression to end-stage kidney disease and mortality (1).

Extensive research in the last decades, including studies in hereditary forms of NS, has highlighted the main role of podocytes, epithelial cells crucial for maintaining the permselectivity of the glomerular filtration barrier, in this disease (2). In the majority of idiopathic NS an underlying immune-mediated disease with a circulating permeability factor (CPF) is thought to be leading to podocyte damage. A major feature of NS is a profoundly altered lipid profile in plasma and intra-cellular accumulation of lipids on podocytes and proximal tubular kidney cells (3). Several lipid molecules, particularly sphingolipids (SLs) have long been known by their ability to disturb cell function through intra-cellular mechanisms, in a paracrine way or by changing cell membrane properties. Specific apolipoproteins correlate with idiopathic NS activity (4), and LDL apolipoprotein apheresis can be successfully used to treat idiopathic NS (5). In previous work, we have identified loss of function mutations in SGPL1, encoding sphingosine-1P lyase, a main enzyme of the SL catabolic pathway, in individuals presenting with hereditary NS (6) (7) (8). This was completely novel to the field and established a new link between sphingolipid species deregulation and podocyte dysfunction. It also led us to extrapolate that bioactive lipids, particularly SLs, may be inducers of podocyte toxicity in idiopathic NS. The lack of studies that precisely dissect the modifications on lipid profile, as well as their cellular effects, in idiopathic NS also highlights an unmet research need in this field. The global goal of our team is to elucidate which are the bioactive lipids that may alter podocyte function and that could be implicated as circulating factors leading to idiopathic NS. We are searching for a lipidomic signature in a cohort of patients with idiopathic NS and we will try to identify lipid biomarkers specific of this disease. In the current master thesis proposal, we will address the impact of exposing cultured human podocytes to the idiopathic NS milieu, particularly searching for changes in the intracellular lipid profile by mass spectrometry, and the distribution of specific lipid species on organelles and cell membranes, using well-characterized fluorescent lipid specific probes.

Our team includes people with a strong translational research background and experienced lab staff who manage state-of-the-art cell biology methodologies. We will closely collaborate with Thorsten Hornemann (TH) from the Institute of Clinical Chemistry, University of Zurich, with renowned experience in lipidomics research. Using this methodological approach, we expect to define the main lipid changes occurring in idiopathic NS, that could be implicated in the pathogenesis of this disease.

Aims:

The major goal of our project is to elucidate which are the main modifications in lipid content and distribution in human podocytes, in vitro, when these cells are exposed to patients' plasma. In fact, plasma from patients with idiopathic NS is long known to evoke a deleterious response in podocyte (9) and ultra-structural analysis of podocytes has revealed accumulation of intra-

cellular lipids(10). However, the impact on membrane microdomains, lipid trafficking and intracellular lipid composition has not been thoroughly addressed.

We will use plasma from patients with idiopathic NS, and address its effect on cultured human podocytes, namely on intracellular lipid droplets accumulation, lipid trafficking, membranes microdomains, including lipid rafts, and total lipid composition using lipidomic techniques. Control samples will be used and will include healthy individuals and patients with hereditary forms of NS.

Methods:

We will assess the effect of iNS plasma, when added to cell medium, on the intracellular lipid composition and on membrane microdomains on human podocytes in vitro. We will use plasma from patients with hereditary SRNS and healthy individuals as controls.

Membrane microdomains, cytoskeleton structure, and lipid trafficking will be studied by confocal and wide field fluorescence microscopy. To this end, standard organelle markers, and fluorescent lipid probes displaying selective phase partitioning (e.g., cyanides, NBD-, Rhod (rhodamine)-, BODIPY- lipid analogues), will be used in combination. Short chain BODIPY-SL analogues and mCherry-Theta toxin fragment will be used to study the implication of different SL and cholesterol in domain formation in podocytes. Membrane permeability will be compared using fluorescence microscopy to study the uptake of calcein.

Cell lipid composition analyzed using a comprehensive untargeted liquid chromatography high resolution mass spectrometry (LC/HRMS) based lipidomics workflow with a particular focus on resolving atypical and isomeric (sphingo)lipid species (Thorsten Hornemann, Zurich University).

These studies will be performed using a conditionally immortalized human podocyte cell line, developed by transfection with the temperature-sensitive SV40-T gene, from Moin Saleem. Experiments will be conducted at 37ºC, in differentiated podocytes.

This will be a novel methodological approach in the field of podocyte diseases and expected to provide new mechanistic insights.

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Webpage of the group

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