Diabetic nephropathy (DN) constitutes one of the most frequent complications of the diabetes and is characterized by progressive dysfunction of glomerular filtration barrier (GFB) and loss of renal function. Podocyte cells constitute the key layer of GFB and their injury is found in the center of pathogenesis of DN. Mitochondria play a crucial role in the maintaining proper energy, lipid and carbohydrate homeostasis, moreover, mitochondrial dynamics is tightly linked with insulin sensitivity in podocytes. Damaged mitochondria entail detrimental consequence for the cells, including increased oxidative stress, inflammatory response, and apoptosis. Alteration of mitochondrial function can also affect the production and release of extracellular vesicles (EVs), such as exosomes. Mounting evidence indicates that podocytes release the EVs under pathological conditions, and stress-induced exosomes are implicated in the pro-inflammatory responses. However, the involvement of mitochondria in exosomal biogenesis in podocytes during DN has not been elucidated so far.

We hypothesize, that alteration of mitochondrial homeostasis in hyperglycemic environment affects exosome biogenesis and secretion in podocytes, which potentiate deleterious changes in podocytes bioenergetics, function and viability. Thus, **the mail goal of this project is to elucidate the role and molecular mechanisms of mitochondrial-exosomal crosstalk in podocytes in physiological state and under metabolic stress.** In the course of this project we will investigate the potential of inhibitors of exosomal biogenesis and metformin, a popular anti-diabetic drug, to improve mitochondrial efficiency in podocytes and preserve renal function in animal model of diabetes. Additionally, the results of the project may uncover novel mechanism of podocyte injury, involving regulation of mitophagy and exosome secretion by hyperglycemia, which can have an important clinical significance in prevention and treatment of DN.

We have previously shown that podocytes become insulin resistant after 5 days of incubation in high glucose concentration (HG; 30 mM glucose). Our previous studies have also shown that mitochondrial function is impaired in podocytes cultured in HG, which may result from the imbalance of mitochondrial dynamics and mitophagy. Mitochondrial biology is tightly connected with exosome generation, but in podocytes the regulatory mechanisms of exosomes biogenesis, cargo recruitment and secretion are still elusive.

Our preliminary results showed, that hyperglycemia increases the quantity and size of secreted extracellular vesicles in podocyte culture. Moreover, a mitophagy-related marker, PINK1, as well as metabolic stress (e.g., HG) changed the expression levels of proteins involved in exosomal biogenesis and secretion. Therefore, it seems relevant to further explore the role of mitochondrial-exosomal crosstalk in podocytes, especially as a potential mechanism of podocyte injury in the pathogenesis of DN.

In the project we will firstly determine the role of exosomes in podocyte biology, function and mitochondrial bioenergetics by using pharmacological inhibitors of different pathways of exosomes biogenesis (e.g., Manumycin A, GW4869) and genetically modified podocytes (inhibition of exosomal marker CD63). In these systems we will assess the levels of various exosomal markers in cell lysates and in isolated exosomes, podocyte viability, morphology, permeability to albumin and insulin responsiveness. Moreover, we will elucidate the quantity and size of isolated exosomes by Nanoparticle Tracking Analysis (NTA). We will also show the effects of exosomal inhibition on different mitochondrial parameters, e.g., respiratory efficiency, mitochondrial dynamics, mitochondrial DNA (mtDNA) level. In the next steps we will analyze the effects of metabolic stress (e.g., HG) on exosomes biogenesis in podocytes, and elucidate if suppression of exosomal biogenesis can ameliorate immune response in podocytes cultured in HG. One of the goals of the project is characterization of mitochondrial involvement in the exosomes formation and secretion in podocytes, especially, in podocytes cultured in HG. To do this, we will make an attempt to detect mtDNA content (by real-time PCR) and mitochondria-related metabolites (e.g., succinate, fumarate, etc.) in secreted exosomes by liquid chromatography-mass spectrometry (LC-MS). Moreover, using fluorescent dyes and super-resolution microscopy (STED) we will verify whether podocytes secrete functional mitochondria in extracellular vesicles in response to metabolic stress or metformin treatment. Furthermore, in the course of the project we will investigate the novel regulatory mechanism of exosome biogenesis in podocytes, which involves metformin effect on mitochondria.

Taking into account, that podocytes are also vulnerable to harmful effects of exosomes secreted by other stressed cells, we will investigate exosome-mediated intercellular communication in podocytes, including functional and mitochondrial effects exerted by exosomes secreted by stimulated podocyte cells. Finally, we will elucidate the physiological impact and kidney function *in vivo* using rat model of DN treated with inhibitors of exosomal biogenesis and metformin. These studies will define if these compounds can prevent the onset or ameliorate renal disease in the course of diabetes.

All these studies will provide a novel knowledge on the role of exosomes in podocyte biology in physiological conditions and will show the significance of exosomal pathways in the development of DN. Considering that both mitochondria and exosomes play pivotal role in podocytes function in diabetes, it seems of high importance to understand their reciprocal crosstalk and possible regulatory mechanisms in order to develop more effective strategies of prevention or treatment of DN.