

Diabetic kidney disease (diabetic nephropathy, DN) is the most common complication of diabetes mellitus type 2 characterizing by morphological and physiological alternations in the kidney DN is directly caused by hyperglycemia. The first clinical symptom of DN is albuminuria, which is a consequence of the glomerular filtration barrier injury. Glomerular filtration barrier consists of fenestrated endothelia, glomerular basement membrane, and podocytes. Podocytes are highly specialized, terminally differentiated epithelial cells that are not able to regeneration. Disturbances in proper functioning of podocytes are the main cause of increased permeability of glomerular filtration barrier and their loss may be irreversible to the kidney.

Podocytes are insulin sensitive cells, which plays a key role in regulation of podocyte morphology and function. Insulin regulates glomerular filtration barrier permeability and positively influencing cellular glucose uptake through enhancement of glucose transporters 4 (GLUT4) translocation from cytoplasm to the plasma membrane.

Lysosomes are important podocytes organelles involved in the regulation of the podocyte functioning, including regulation of insulin signaling pathway. Prolonged exposition of podocytes to high concentration of insulin leads to lysosome-dependent degradation of insulin receptor and development of insulin resistance. Lysosomes also mediate degradation of endocytosed albumin to prevent the clogging the glomerular filtration barrier. Moreover, near the lysosome surface, under glucose-deprived conditions, AMP-activated protein kinase (AMPK) is activated by the lysosome complex, including Ragulator, v-ATPase, axin oraz LKB1 kinase.

AMPK is a serine/threonine protein kinase that is responsible for maintaining cellular energy homeostasis. The classical AMPK activation pathway is based on changes in the energy status of the cell. Decreased ATP concentration during cellular energy and nutrient depletion leads to AMPK activation, which inhibits anabolic pathways and activates catabolic processes.

AMPK is involved in insulin-dependent regulation of glucose uptake like regulation of cellular glucose uptake and mediates regulation of glomerular filtration barrier permeability. In insulin-resistant states both AMPK activity and insulin-dependent glucose uptake are inhibited.

Despite the intensive research on renal AMPK, there are only a few papers that indicate an importance of lysosomes in regulation of AMPK activity. Furthermore, it seems that the insulin influence on AMPK activity through the lysosome complex have not yet been investigated, although it may play an important role in the pathogenesis of insulin resistance.

The main aim of this project is to investigate the role of v-ATPase-Ragulator-axin/LKB1 complex in insulin-dependent regulation of AMPK activity and expression, and the effect of lysosomes on glomerular filtration barrier permeability.

In this project, we will use podocytes isolated from Wistar rats and cells will be incubated in medium with standard (SG, 11 mM) and high glucose (HG, 30 mM) concentration in the presence or absence of insulin. Additionally, we will analyse the lysosomal complex activity in kidney glomeruli from Zucker rats. To explain this scientific problem, the following issues will be investigated: a) the effect of insulin and HG on the lysosomal complex activity, b) the role of lysosomes in regulation of cellular glucose uptake and c) the role of lysosomes in regulation of glomerular filtration barrier permeability.

We believe that our experiments will provide insight into the alternative molecular mechanisms responsible for activation of AMPK and they allow partially explain the role of lysosomes in diabetes pathogenesis. Moreover, study of this issue may allow developing innovative pharmacological strategies against diabetic nephropathy.