Nephron is the basic structural and functional unit of the kidney. Each nephron is composed of an initial filtering component and a tubule specialized for reabsorption and secretion (proximal, distal convoluted tubules). Glomerular capillary pressure, and thus glomerular filtration rate, can be influenced by constriction or relaxation of the afferent arteriole, resulting in decreases or increases in pressure. The glomerular filtration barrier is composed of the fenestrated endothelium of the glomerular capillaries, the fused basal lamina of the endothelial cells and podocytes, and the filtration slits of the podocytes. The structures of the layers determine their permeability.

Podocytes are highly specialized cells that cover the glomerular capillaries and are a key part of the glomerular filtration barrier. Podocytes are continually exposed to transmural hydrostatic pressure, which favors glomerular filtration. Podocytes confer the specific size and charge characteristics of the glomerular filtration barrier; podocyte damage leads to a retraction of the foot processes and proteinuria ensues. These cells express some proteins that are characteristic of the smooth muscle cell contractile system. The arrangement of F-actin, myosin, and α-actinin in foot processes has been proposed to facilitate glomerulus adaptation to changes in pressure gradients by modifying the surface area for filtration. Podocytes also express receptors for factors that regulate contraction and relaxation; this suggested that podocyte function may be regulated by vasoactive hormones and autocrine/paracrine factors. Hence, the hormonal regulation of podocytes affects the size-selectivity of the filtration barrier, and this regulation may be similar to that of smooth muscle cells. Moreover, the size-selective barrier properties of podocytes are regulated by cGMP-dependent changes in proteins, like protein kinase G type I alfa (PKGIα), that may regulate the cytoskeleton and slit diaphragm. However, the means by which these proteins regulate actin dynamics in podocyte foot processes is only partially understood, especially in diabetes. It has long been know that the podocyte foot processes actin cytoskeleton is highly dynamic, although the underlying mechanisms remained ill defined. In many tape of glomerular diseases, the integrity of actin cytoskeleton in podocytes is altered, indicating that proper organization and regulation of the actin cytoskeleton are essential for podocyte structure and function. This raises great interest in proteins that function in the regulation of dynamic actin organization as potential therapeutic targets in the treatment of glomerular diseases, including diabetic nephropathy.

Rho family GTPases are molecular switches best known for their pivotal role in dynamic regulation of the actin cytoskeleton. The mammalian Rho family consist of at last 20 distinct members, among which, RhoA, Rac1 and Cdc42 proteins have been most extensively studied. RhoA regulates stress fiber assembly as well as focal adhesion formation. Moreover, in the classical view, Rac1 and Cdc42 promote cell motility at the leading edge through the formation of lamellipodia and filopodia, respectively. Rho-dependent signaling cascades modulate cellular morphology and actin polymerization, adhesion, migration, proliferation and apoptosis as well as participate in contractile responses.

Rho family GTPase are expressed in cells forming glomerular filtration barrier. Thus changes of Rho GTPase activity may affect glomerular permeability for albumin leading to albuminuria/proteinuria, a sign of kidney disease and independent risk factor for the progression of renal failure. Early stage type 2 diabetes is characterized by the generation of excess free oxygen radicals, insulin resistance, and hiperglicemia. Recently, we demonstrated a relationship between oxidative stress, protein kinase G type I alpha (PKGIα) activation, actin reorganization, and changes in the permeability of the podocyte barrier. Moreover, PKGIα opposes the inhibitory effect of Rho/ROCK on MLCP activity. PKGIα directly inhibits RhoA by phosphorylation at Ser188. To date, there is very limited information about the role of ROCK and their substrates on podocytes permeability. It has been shown that Rho-kinase inhibition (by Y-27632 inhibitor) leads to significant increase flux across podocyte monolayers. We therefore hypothesized that PKGIα regulates the balance between contractility and relaxation (permeability) of the podocyte barrier by the regulation of actin cytoskeleton and that this mechanism could be disrupted in diabetes.

In this project, we will investigate the effect of Rho family GTPases on podocyte migration and permeability and the role of PKGIα-dependent signaling on this process under normal and high glucose condition and in the present of insulin. We also will investigate these mechanisms in an obese, insulin-resistant animal model (Zucker rats). These studies might help to understand the molecular mechanism of proteinuria and to further develop new targets for dealing with proteinuria. Nevertheless, it is desirable to explore the possibility that signaling cascades involving Rho family GTPases may become novel therapeutic targets for type 2 diabetes.