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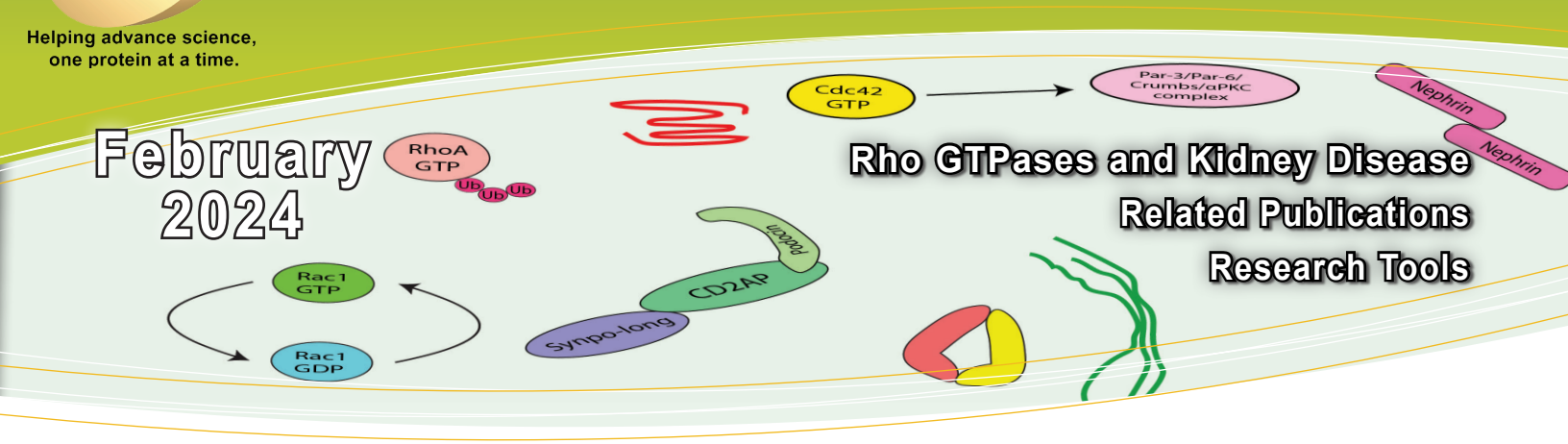
February 2024



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Rho GTPases and Kidney Disease

Introduction

The Rho family of small GTPases are molecular switches with a pivotal role in numerous cellular functions. They regulate the actin cytoskeleton, cellular morphology, motility, adhesion, and proliferation[1,2]. RhoA, Rac1, and Cdc42 are members of the Rho family that play crucial roles in kidney function, particularly in the maintenance of various kidney cell types, such as tubular epithelial cells, mesangial cells, and podocytes[3] (Figure 1). Podocytes are a fundamental component of the glomerular filtration barrier, a structure that prevents the filtration of large proteins and macromolecules into the urine. This glomerular barrier relies on the structural integrity of the actin-based cytoskeleton of the podocytes[4]. As a result, dysregulation of GTPases and their downstream effectors has been implicated in both hereditary and idiopathic kidney diseases. The present newsletter will dive into Rho family-dependent nephropathies and their underlying molecular mechanisms.

Rho GTPases and diabetic nephropathies

Diabetic nephropathy, a progressive kidney disease that arises from glomerular injury, is linked to RhoA activation in mesangial cells under the influence of high glucose levels, ultimately resulting in excessive extracellular matrix production (glomerulosclerosis)[5]. The pathogenesis of diabetic nephropathy is a complex interplay of factors, including hyperglycemia, advanced glycation end products, oxidative stress, and the activation of various cellular pathways. These elements stimulate Rho-GTPase activity, initiating a cascade that culminates in renal damage[6,7]. Additionally, Cdc42 is involved in the regulation of insulin synthesis, insulin granule mobilization, and exocytosis[8], while Rac1 influences oxidative damage and apoptotic activity in mesangial cells[9,10].

Rho GTPases and congenital nephropathies

Rho GTPases play a crucial role in the landscape of congenital nephropathies, notably influencing Focal Segmental Glomerulo-Sclerosis (FSGS), Autosomal Dominant Polycystic Kidney Disease (ADPKD), and Alport syndrome, among others. The nature of these renal conditions and their associations to Rho GTPases will be discussed below.

FSGS describes a histological pattern of disease observed in kidney biopsy, with a phenotype defined by scar tissue in the glomerulus. As such, it can serve as a diagnosis indicator representing various injuries sustained by the kidneys[11]. Initially documented in 1925 among adults exhibiting nephritic proteinuria[12], FSGS can manifest as idiopathic, genetic, autoimmune, or as a response (maladaptive or otherwise) to chronic kidney disease associated with reduced nephron mass, as well as secondary to drugs or infections[11]. In both inherited and sporadic forms of FSGS, mutations in genes encoding regulators of Rho GTPase activities have been identified. These mutations involve the GTPase-activating protein (GAP) Rho-GAP 24 (ARHGAP24), which acts as a GAP for Rac1 and controls the RhoA-Rac1 signaling balance[11,13,14]. Additionally, mutations in Rho GDP Dissociation Inhibitor alpha (ARHGDIA) and intersectin-1 and -2 (ITSN-1 and -2) have been linked to congenital nephrotic syndrome[15–17]. Notably, mutations in ARHGAP24 lead to excessive Rac1 signaling in podocytes, while mutations in ARHGDIA result in the loss of function of RhoGDIα, a negative Rho regulator, causing hyperactivation of Rho-GTPases and impaired cell motility.

ADPKD stands as the most prevalent hereditary kidney ailment and ranks among the leading contributors to end-stage kidney disease. The clinical manifestations of ADPKD involve enlarged kidneys housing proliferating cysts, hypertension, and various extrarenal complications[18,19]. The majority of cases (>90%) are attributed to mutations in two genes, namely PKD1 and PKD2[20]. The cellular phenotype associated with ADPKD is complex and includes changes in proliferation, apoptosis, cell-cell and cell-matrix adhesion, among other manifestations[21]. In this regard, a mutation in PKD1 was recently found to induce substantial alterations in the dynamics of the actin cytoskeleton. Particularly, the study revealed that

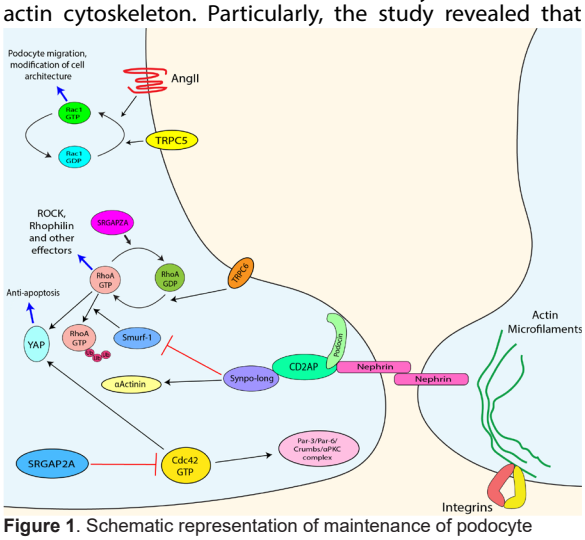

Figure 1. Schematic representation of maintenance of podocyte architecture by Rho GTPases. Adapted from Steichen et al. Small GTPases 2022.

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the dysregulation of compartmentalized centrosomal RhoA signaling, facilitated by a particular Rho GTPase-activating protein (ARHGAP35), resulted in heightened activation of Rho kinase (ROCK) in cells with a PKD1 mutation. In turn, there was an increase in F-actin polymerization and contractility[22]. Furthermore, a crucial signaling pathway involving the Rho guanine nucleotide exchange factor (GEF) LARG, RhoA, its effector ROCK, and myosin light chain (MLC) was identified as a critical mechanism for PKD1 effects in ADPKD[23].

Alport syndrome is a rare hereditary glomerulonephritis associated with progressive loss of kidney function, hearing loss and eye abnormalities[24]. A mechanistic connection between Rho GTPases and the development of Alport syndrome has been previously established. The stretch-induced activation of Rac1 and Cdc42 serves as a trigger for mesangial filopodial invasion within the glomerular capillary loops[25]. Recently, a further indirect relationship between this syndrome and Rac1 and Cdc42 was found through the involvement of the Rac1/Cdc42 guanine nucleotide exchange factor β1Pix[26]. According to this study, β1Pix plays a role in regulating LDL receptor stability and function in renal cells affected by this condition, with implications in its pathogenesis.

Summary and future perspectives

Efficient kidney barrier function necessitates the dynamic regulation of the podocyte cytoskeleton. Rho GTPases, as master regulators of actin cytoskeletal dynamics, are thus critically important for maintaining the sustained function of the kidney barrier. These proteins play crucial roles in congenital nephropathies like FSGS, ADPKD, and Alport syndrome.

Additionally, Rho GTPases are known for their role in HIV-associated nephropathy, an ailment characterized by collapsing focal segmental glomerulosclerosis (cFSGS)[27]. In this context, Nef, the HIV-1 accessory and determinant protein, influences podocyte morphology by inhibiting RhoA activity and activating Rac1. Moreover, HIV-1 transactivator Tat, combined with FGF-2, induces podocyte dedifferentiation and proliferation through the stimulation of the RhoA/phospho-Myosin Light Chain 2 pathways[28,29]. Further research has identified host proteins interacting with Nef, among which is TNK2, also known as ACK, closely involved with Cdc42 activity[30]. Overall, Rho GTPases are master regulators of the actin cytoskeleton and their dysregulation is implicated in the pathogenesis of glomerular diseases. However, given the diverse biological functions associated with Rho GTPases, it is imperative to thoroughly investigate potential therapeutic targets while also identifying signaling pathways that minimize 'off-target' effects.

G-LISA Activation Assay Kits

Product	Assays	Cat. #
Cdc42 G-LISA™ Activation Assay Kit (Colorimetric format)	96	BK127
Rac1 G-LISA™ Activation Assay Kit (Luminescence format)	96	BK126
Rac1 G-LISA™ Activation Assay Kit (Colorimetric format)	96	BK128
RalA G-LISA™ Activation Assay Kit (Colorimetric format)	96	BK129
RhoA G-LISA™ Activation Assay (Luminescence format)	96	BK121
RhoA G-LISA™ Activation Assay Kit (Colorimetric format)	96	BK124
RhoA / Rac1/ Cdc42 G-LISA™ Activation Assay Bundle 3 kits (24 assays per kit)	96	BK135
Rac1,2,3 G-LISA™ Activation Assay (Colorimetric format)	96	BK125
Rac1 G-LISA™ Activation Assay (Luminescence format)	96	BK126
Rac1 G-LISA™ Activation Assay Kit (Colorimetric Based)	96	BK128
Ras G-LISA™ Activation Assay Kit (Colorimetric Based)	96	BK131
Total RhoA ELISA	96	BK150

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RhoA Small G-Proteins, Activators and Inhibitors

Labeled Actin	Amount	Cat. #
RhoA Protein: His Tagged Human Wild Type	1 x 100 µg 3 x 100 µg 1 x1 mg	RH01-A RH01-C RH01-XL
RhoA Protein: His Tagged: Human Constitutively Active	1 x 10 µg	R6301-A
Rho Activator I	5 x 10 units 20 x 10 units	CN01-A CN01-B
Rho Activator II	3 x 20 µg 9 x 20 µg	CN03-A CN03-B
Rho Inhibitor I	1 x 20 µg 5 x 20 µg 20 x 20 µg	CT04-A CT04-B CT04-C