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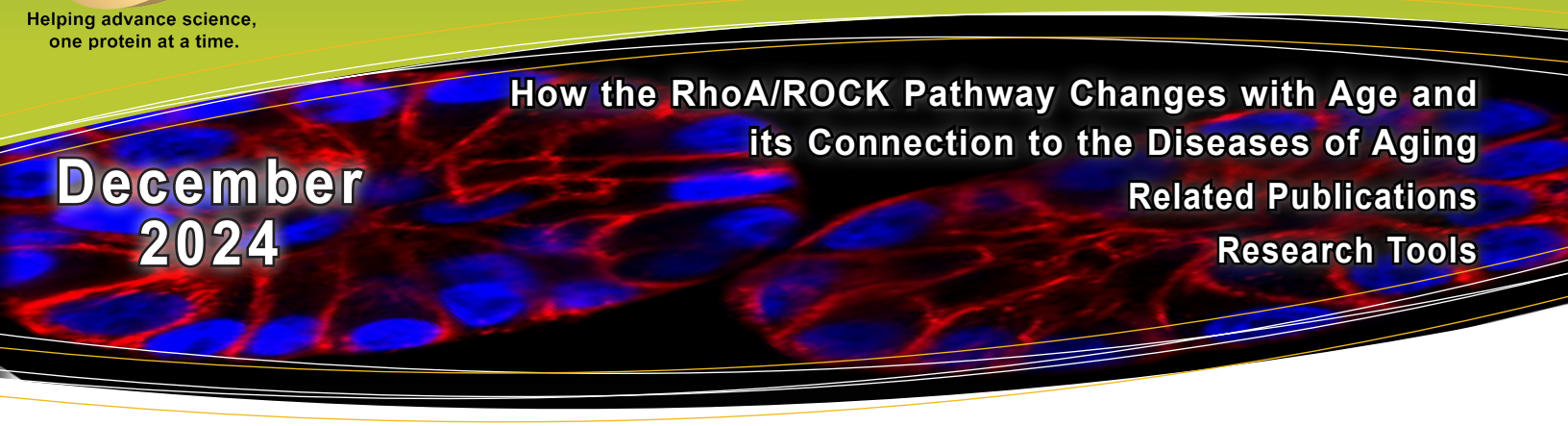

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How the RhoA/ROCK Pathway Changes with Age and its Connection to the Diseases of Aging

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How the RhoA/ROCK Pathway Changes with Age and its Connection to the Diseases of Aging

Introduction

The Rho GTPases form a family of 20 closely related proteins with essential roles spanning diverse cellular processes. First known for their regulation of actin organization and dynamics, they are now also recognized as key mediators of gene expression and cell cycle progression[1].

Transforming protein RhoA, also known as Ras homolog family member A , was the first example to be discovered. Like other small GTPases, it serves as a "molecular switch" based on GTP/GDP binding status and is regulated by many other factors including guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs), through mechanisms that are reviewed in depth elsewhere[2].

RhoA signaling is propagated by an array of downstream effectors (Figure 1), the best characterized of which are the Rho-associated protein kinases, ROCK1 and ROCK2 . In common with all Rho GTPases, RhoA contains a unique "insert domain" enabling direct interaction with these proteins[3]. When activated, ROCKs phosphorylate downstream targets including the PPP1R12A/MYPT1 subunit of myosin light chain phosphatase (MLCP), resulting in activation of MYL2/MLC2, the regulatory light chain of myosin II[4]. This effects actomyosin contractility, an underlying mechanism of force generation in cell motility and muscle contraction[5]. In addition, ROCKs can phosphorylate PPP1R14A/CPI-17, an inhibitor of MLCP[6], and activate LIM domain kinases (LIMK1/2) toward cofilin phosphorylation to stabilize actin filaments[7].

Ever since RhoA was proposed as an oncogene in the 1980s[8] RhoA/ROCK signaling has been extensively studied in cancer and has been found to be dysregulated in many cancer types[9,10]. However, this newsletter will focus on age-related changes that have also been identified across a range of studies, suggesting a role in other diseases of aging.

Age-Associated Variations in the RhoA/ROCK Pathway

These changes have mostly been studied in the cardiovascular system, as RhoA/ROCK signaling is one of two canonical pathways of muscle contraction (the other being myosin light chain kinase activation by calmodulin) and ROCKs are known to control vascular tone[11].

Arterial RhoA levels were first observed to increase with age[12], then a role was established for ROCK activation in declining contractile function[13]. In mice, age-dependent increases in arterial ROCK2 expression were functionally associated with increased myogenic tone (i.e., reduced capacity of blood vessel smooth muscle cells to contract/constrict in response to pressure changes)[14]. Thus, overactivation of RhoA and/or ROCKs causes prolonged contraction, potentially leading to vascular dysfunction that manifests as age-associated hypertension or arterial stiffening[15,16]. Recent experiments with heterozygous knockout mice revealed a role for both ROCK isoforms in age-related aortic stiffening, with an apparently greater role for ROCK2[16].

Increased expression of ROCK2 with age has also been found in the CNS[17], where similarly, hippocampal RhoA protein levels and GTPase activity are higher in old mice[18]. Another study reported both elevated RhoA expression and ROCK activity in the substantia nigra of older versus younger rats, and interestingly, these effects were attenuated by regular physical exercise[19]. The RhoA/ROCK pathway also affects neurogenesis, since progenitors derived from neural stem cells move through the ventricular-subventricular zone during lineage progression. Motility declines with age, impacting regenerative ability. One recent study used single-cell RNA-Seq to identify RhoA/ROCK signaling as a major contributing factor and found that pharmacological ROCK inhibition restored "young" progenitor cell dynamics[20].

With the above in mind, we will now briefly summarize current knowledge on RhoA/ROCK activation in some of the major diseases of aging: cardiovascular diseases, neurodegenerative diseases, and diabetes.

RhoA/ROCK Overactivation in Cardiovascular Diseases

As alluded to earlier, abnormal activation of RhoA/ROCK signaling has long been known in various cardiovascular conditions including hypertension, atherosclerosis, restenosis, and cardiac hypertrophy[21]. Higher pathway activity has also been observed in heart failure patients[15]. Several studies have linked age-related hypertension to RhoA/ROCK upregulation[15,16,22], and this has been further attributed to increased signaling via Gαq/11 proteins acting upstream of RhoA[23]. A notable study in mice identified age-associated increases in arterial ROCK2 expression and basal myogenic tone. The latter could be markedly reduced by treatment with belumosudil, an FDA-approved ROCK2 inhibitor[14].

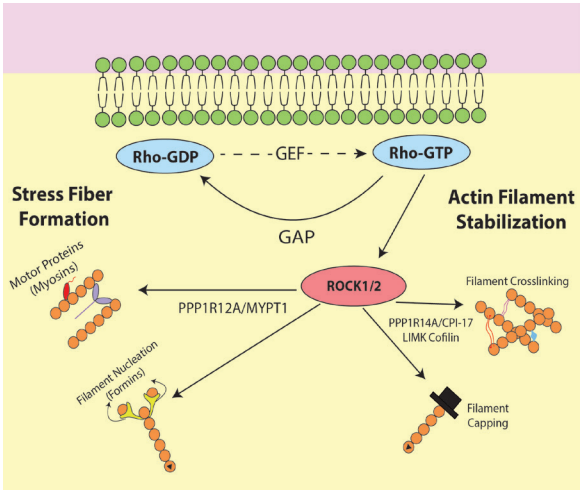

Figure Legend: Prenylated RhoA protein is closely associated with the plasma membrane, where GEFs and GAPs modulate the nucleotide status of RhoA. The GTP-form of RhoA activates ROCK1 and ROCK2 to modulate down stream effectors which result in stress fiber formation and actin filament growth and stabilization.

Continued from Page 1

Multiple Mechanisms in Neurodegeneration

While RhoA has normal biological roles in controlling axonal elongation and synaptic plasticity[24], dysregulation of the RhoA/ROCK pathway has been directly implicated in neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis. Several pathogenic mechanisms have been proposed in the case of the former[25]. First, heightened ROCK expression in AD brain can directly contribute to hyperphosphorylation of tau, potentiating the formation of neurofibrillary tangles that have also been shown to colocalize with RhoA[24]. Second, ROCK activation and Aβ1–40 secretion appear to amplify each other in a positive feedback loop. Additionally, increased actin contractility mediated by ROCK2 leads to neuronal dendritic spine loss, known as the major correlate of cognitive decline in AD[26].

Emerging Roles for RhoA/ROCK Signaling in Diabetes

Early studies[27] noted pathogenic increases in RhoA activity and phosphorylated CPI-17 (a direct ROCK substrate, as noted above) in aortas and arteries of db/db mice, a widely used animal model of type 2 diabetes (T2D). It is now established that RhoA mediates the translocation of SLC2A4/GLUT4, an essential transporter for glucose uptake into skeletal muscle[28], and that RhoA/ROCK1 regulate glucose transport in muscle cells and adipocytes through actin cytoskeleton remodeling[29]. Thus, circulating insulin activates RhoA in muscle[30] while in pancreatic β cells, hyperglycemia upregulates RhoA/ROCK signaling to cause enhanced growth of stress fibers and reduced glucose-stimulated insulin secretion[29]. Hyperactivation of RhoA therefore seems to contribute to diabetic pathology to the extent that RhoA/ROCK signaling was recently proposed as a novel therapeutic target in T2D, based on the available evidence[29].

Outstanding Questions and Future Prospects

Despite three decades of intensive study, several aspects of RhoA/ROCK signaling remain unclear. The precise functional roles of RhoA are still debated[31] and some of the relevant mechanisms are not well understood due to inconclusive or contradictory results[32]. For example, while ROCK activation has been observed in heart failure patients[15], cardiomyocyte-specific knockout of RhoA in mice led to accelerated heart aging and fibrosis[33].

In a similar vein, studies in cancer suggest that RhoA can exert either pro- or anti-tumorigenic effects depending on the context[10], highlighting how RhoA signaling networks may be tissue or cell type specific. Failure to consider different proteoforms could also be a confounding factor, given the many known posttranslational modification sites in RhoA that can modulate its activity and subcellular localization[34,35]. Also, intriguing recent studies suggest RhoA is not predominantly membrane-bound as traditionally assumed and that it cycles much more rapidly between the membrane and cytoplasm than other Rho GTPases[31].

Nevertheless, the recent approval of belumosudil (a first-in-class selective ROCK2 inhibitor) for treatment of chronic graft-versus-hose disease represents a notable success story, and the ongoing development of more potent and selective ROCK inhibitors offers new therapeutic potential for successfully targeting the RhoA/ROCK pathway in cancer[9].

Rho, Rac, and Cdc42 Activation Assays

Product	Amount	Cat #
RhoA / Rac1 / Cdc42 Activation Assay Combo Biochem Kit	3 x 10 Assays	BK030
RhoA Pull-down Activation Assay Biochem Kit	80 Assays 20 Assays	BK036 BK036-S
RhoA G-LISA™ Activation Assay (Luminescence format)	96 Assays	BK121
RhoA G-LISA™ Activation Assay Kit (Colorimetric format)	96 Assays 24 Assays	BK124 BK124-S
Total RhoA ELISA	96 Assays	BK150
Rac1,2,3 G-LISA™ Activation Assay (Colorimetric format)	96 Assays	BK125
Rac1 G-LISA™ Activation Assay (Luminescence format)	96 Assays	BK126
Rac1 G-LISA™ Activation Assay Kit (Colorimetric Based)	96 Assays 24 Assays	BK128 BK128-S
Ras Pull-Down Activation Assay Biochem Kit	50 Assays	BK008
Ras G-LISA™ Activation Assay Kit (Colorimetric Based)	96 Assays	BK131

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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