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RhoA/ROCK in Metabolic Syndrome

Introduction

Metabolic syndrome (MetS) or insulin resistance syndrome, is a cluster of conditions that raises the risk of heart disease, type II diabetes, and stroke. These conditions include central obesity (i.e., excess body fat at the waist), hypertension, hyperglycemia, high blood triglycerides, and low HDL cholesterol. Those with three or more of these conditions may be diagnosed with MetS. MetS affects approximately one-third of adults in the United States. Pathogenesis of MetS is still not fully understood, though a variety of genetic, environmental, and lifestyle factors are thought to be involved.

Rho GTPases are a family of signaling proteins that play a key role in regulating the cytoskeleton. Activation of RhoA by GTP binding results in the activation of downstream effector proteins that mediate actin polymerization and stabilization and myosin II function[1]. RhoA is involved in essential cellular processes, such as cytokinesis, stress fiber formation, and smooth muscle cell contractility, as well as several pathological processes, including tumor cell migration and invasion and cardiovascular diseases[2-5]. RhoA and its downstream effector proteins, Rho-associated protein kinases (ROCK1 and ROCK2), are also critical in MetS pathogenesis[6]. Elevated ROCK activity has been demonstrated in models of MetS conditions, including obesity, insulin resistance, elevated triglycerides, and hypertension[4,7]. In the PBMCs of patients with type 2 diabetes mellitus, ROCK activity is elevated 420-570% compared to healthy controls[8]. Serum ROCK activity is also elevated in those diagnosed with MetS and correlated with waist circumference, fasting glucose, and triglyceride levels[9]. As a result, RhoA and ROCK have become attractive therapeutic targets for MetS treatment[10,11]. One randomized controlled trial revealed that atorvastatin inhibits ROCK activity, which they suggest contributes to the anti-atherosclerotic benefit of statins[12]. This newsletter will focus on recently identified roles for RhoA and ROCK signaling in the biological processes involved in MetS conditions.

RhoA/ROCK Signaling Mediates Actin Cytoskeleton Dynamics in Adipogenesis

There are three main types of adipocytes. White adipocytes store excess lipids as triglycerides and release free fatty acids during times of high energy expenditure[13]. Brown adipocytes are thermogenic, that is, they convert energy into heat[14]. Brown adipocytes decrease with age, while overall adiposity, particularly white adipocyte accumulation, increases with age. Adipocytes with intermediate characteristics are referred to as beige adipocytes and can switch between the functionality of white and brown adipocytes based on current energy conditions[13,14]. Brown and beige adipocytes are critical for maintaining energy homeostasis and their decline is associated with an increased risk of obesity, diabetes, and other metabolic disorders[15]. For this reason, identifying signaling components that can modulate the adipogenesis program to favor thermogenic adipocytes is a promising therapeutic strategy for MetS treatment.

RhoA and ROCK play a key role in regulating actin cytoskeleton dynamics in all metabolic tissues and during adipogenesis, the process of creating new fat cells[6,16]. RhoA/ROCK activity inhibits the differentiation of white and beige adipocytes as well as the transdifferentiation of white adipocytes to beige[6]. Rho and ROCK activity further promote obesity by shifting away from thermogenic brown and beige adipogenesis in favor of myogenesis and cardiomyocyte differentiation, respectively[6]. Thus, targeting RhoA/ROCK activity represents a means to shift toward brown and beige adipogenesis by affecting multiple points of the adipogenesis program. Indeed, ROCK2 inhibition in mice enhances the thermogenic program in both white and brown adipocytes[17]. Another study demonstrated that mice deficient in the transcription factor MRTFA, which lies downstream of ROCK signaling, were protected from diet-induced obesity and demonstrated "beiging" of white adipose tissue[18].

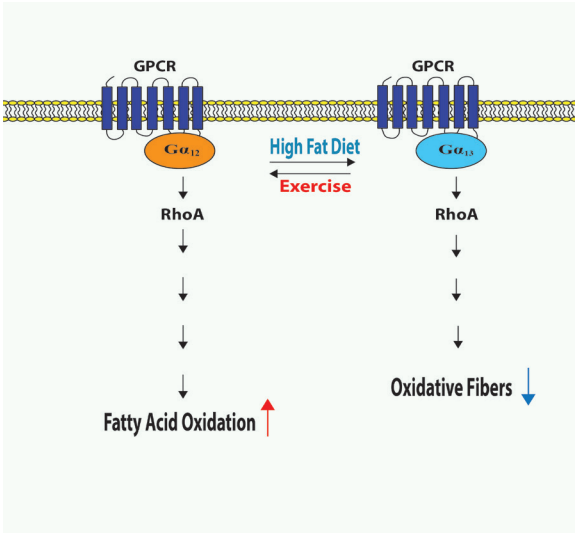

Figure 1. Schematic of how distinct GPCRs induce RhoA activation resulting in opposing metabolic regulatory mechanisms.

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$G\alpha_{12,13}$/RhoA Affect Obesity, Whole-Body Energy Metabolism, Insulin Sensitivity, and Liver Steatosis

Upstream of RhoA, heterotrimeric G-proteins containing $G\alpha_{12/13}$ subunits transduce signals from a wide range of G protein-coupled receptors (GPCRs), including sphingosine 1-phosphate (S1P) receptors, thrombin receptors, and purinergic receptors[19]. Significant evidence indicates that GPCR signaling through $G\alpha_{12/13}$ influences a wide-range of metabolic disorders[19-21]. Though both $G\alpha_{12}$ and $G\alpha_{13}$ lead to the activation of RhoA, they appear to exert their effects in a tissue-specific manner and serve opposing roles in the regulation of pathways involved in energy homeostasis in certain tissues[19] (Figure 1).

In mice, exercise reduces $G\alpha_{13}$ levels in skeletal muscle while high-fat diet (HFD)-fed mice have elevated $G\alpha_{13}$ levels. Similarly, $G\alpha_{13}$ is elevated in the skeletal muscle of patients with type II diabetes mellitus[19]. Skeletal muscle-specific ablation of $G\alpha_{13}$ in mice leads to higher levels of whole-body metabolism and increased insulin sensitivity[22]. This effect is dependent on $G\alpha_{13}$/RhoA/ROCK2-mediated suppression of nuclear factor of activated T cells 1 (NFATc1) by phosphorylation at Ser243[22]. Phosphorylation of NFATc1 at this site results in its inactivation and is elevated in obese mice and reduced following exercise[23]. While $G\alpha_{13}$-mediated activation of RhoA in skeletal muscle leads to ROCK-mediated diet-induced adiposity, $G\alpha_{12}$-mediated activation of RhoA leads to HIF-1α-dependent fatty acid oxidation[19,22,23]. Mice fed a HFD and patients with liver steatosis have lower levels of liver $G\alpha_{12}$ while expression is upregulated under fasting conditions[23]. Ablation of $G\alpha_{12}$ in mice leads to higher rates of lipid accumulation[23]. Mechanistically, this role for $G\alpha_{12}$ in the regulation of fatty acid oxidation and mitochondrial respiration is mediated by the SIRT1/PPARα pathway[23,24]. Thus, in skeletal muscle, $G\alpha_{12}$/RhoA and $G\alpha_{13}$/RhoA serve opposing roles and together maintain energy homeostasis.

This dual regulatory program is absent in the liver where NFATc1 is not expressed. In contrast to skeletal muscle expression levels, hepatic $G\alpha_{13}$ levels are lower in HFD-fed or genetically obese mice and patients with diabetes[19]. Additionally, hyperglycemia in mice leads to decreased hepatic $G\alpha_{13}$ levels, contributing to glucose intolerance and insulin resistance[25]. In the liver, $G\alpha_{12}$ contributes to hepatic fibrosis by promoting JNK-dependent autophagy in hepatic stellate cells (HSCs)[19].

Summary and Future Perspective: $G\alpha_{12/13}$/RhoA/ROCK as Therapeutic Targets in MetS

Though clinical trials to evaluate the pharmacological modulation of these signaling components for MetS are not yet underway, many preclinical animal studies have underscored the opportunities that exist. In one such example, the inhibition of ROCK suppressed obesity, hypercholesterolemia, and glucose intolerance in mice through the activation of the LKB1/AMPK pathway[26]. Because RhoA downstream effectors and subsequent cellular processes are tissue-specific, targeted therapeutic approaches such as liver-specific ROCK1 inhibition, have been evaluated in preclinical mouse models, resulting in increased energy expenditure, greater insulin sensitivity, and reduced lipid accumulation[27]. The multifaceted roles of $G\alpha_{12/13}$/RhoA/ROCK signaling in metabolic syndrome continue to be uncovered. Recent evidence suggests this signaling axis represents a novel therapeutic target for MetS treatment based on several preclinical studies that have investigated RhoA signaling components as therapeutic targets.

RhoA Small G-Proteins

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

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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