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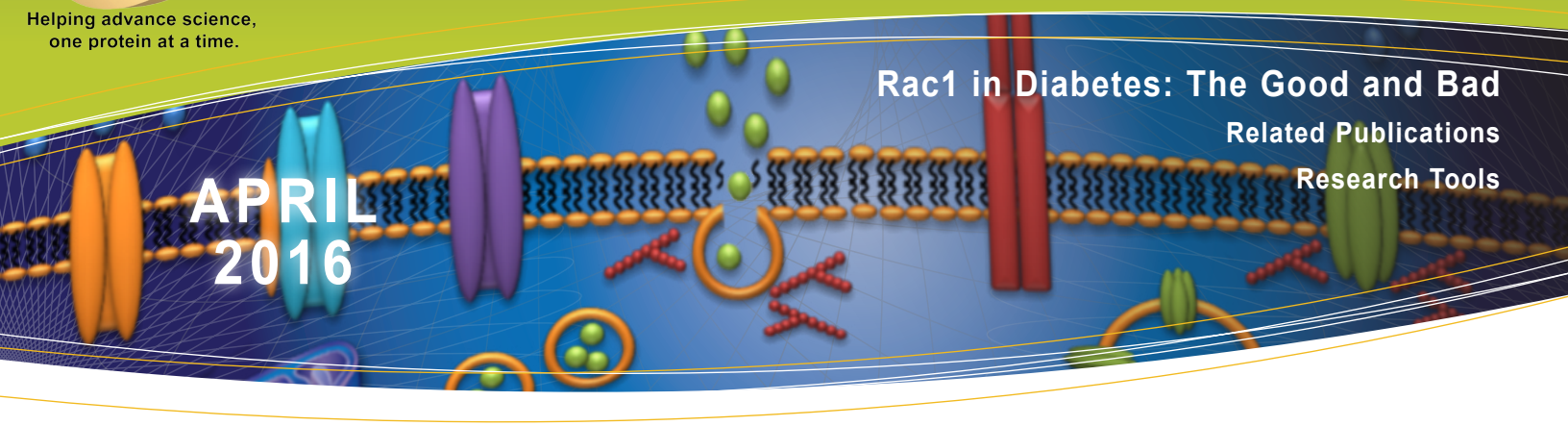

Rac1 in Diabetes: The Good and Bad

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P: 1 (303) 322.2254
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E: cserve@cytoskeleton.com
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Rac1 in Diabetes: The Good and Bad

Introduction

Elevations in blood glucose levels are sensed in pancreatic β-cells, which respond through a complex signaling pathway involving mitochondrial-dependent glucose metabolism[1]. The culmination of this pathway is the mobilization of intracellular insulin-loaded vesicles that fuse with the cell membrane releasing their contents into the bloodstream, which is referred to as glucose-stimulated insulin secretion (GSIS)[1]. Blood glucose normalization is then facilitated by insulin responsive target organs like skeletal muscle, adipose tissue, and the liver. When insulin engages the insulin receptor on the cells in these target tissues it signals the mobilization of the GLUT4 glucose transporter, which is actively shuttled to the plasma membrane thereby allowing the uptake of glucose from the blood[2].

Chronic high blood sugar (hyperglycemia) results in a state of glucose toxicity with concomitant lipid toxicity (dyslipidemia) that can lead to the development of Type 2 Diabetes (T2D). The incidence of diabetes continues to rise and in 2015 there were an estimated 415 million people affected worldwide[3]. The factors leading to persistent hyperglycemia in T2D are typically insulin resistance in the target tissues and impaired GSIS[4].

The positive role of Rac1 in facilitating glucose control

The Rho family small GTPase, Rac1, plays an important role in both GSIS in pancreatic β-cells and GLUT4 translocation to the membrane in skeletal muscle (Fig. 1). The role of Rac1 in both processes appears to center around its ability to facilitate the trafficking of vesicles through changes in the cortical actin structure[5,6]. Loss of Rac1 function either through gene knockout in mice or siRNA mediated reduction in Rac1 levels attenuates GSIS[5,7]. The importance of Rac1 in GSIS has also been validated pharmacologically using small molecules that block Rac1 activation either directly or indirectly through preventing the association of Rac1 with its upstream activators known as guanine exchange factors (GEFs). The indirect inhibitors NSC23766 and EHop-016 inhibit the interaction of Rac1 with the GEFs Tiam1 and Vav2, respectively[8,9]. Using these inhibitors in INS 832/13 cells and primary rat islet cells, glucose-induced Rac1 activation and membrane association was suppressed and GSIS was reduced[10,11].

Additionally, siRNA knockdown of Tiam1 or Vav2 in INS 832/13 cells also blocked glucose-induced Rac1 activation[10,11], confirming a role for each of these GEFs in activating Rac1 in response to glucose. Moreover, the direct Rac1 inhibitor, EHT 1864[12], also blocked glucose-induced Rac1 activation and attenuated GSIS in INS 832/13 cells[13]. It is important to point out that although the focus of this discussion is Rac1 other small G-proteins are known to play a role in GSIS (e.g., Cdc42 and Arf6)[14].

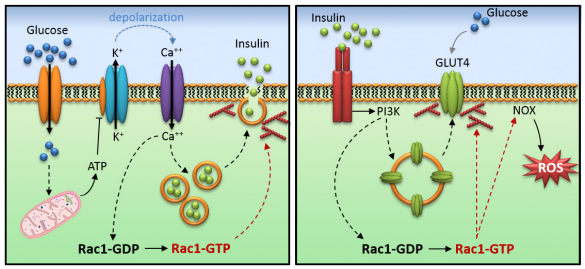

Figure 1. (Left) Schematic representation of GSIS with the role of Rac1 indicated in red. (Right) Insulin signaling in target tissues with the good/bad roles of Rac1 in red.

As mentioned above, target tissues respond to circulating insulin by mobilizing GLUT4 receptors. This process requires Rac1 and its downstream kinase effector PAK1, except in adipose tissue where Rac1 does not appear to be involved in GLUT4 translocation[15]. It is also interesting to note that Rac1 plays a role in muscle contraction-dependent glucose uptake[16]. Both Rac1 and PAK1 have been shown to be activated in response to insulin in both mouse and human skeletal muscle[17]. A muscle specific knockout of Rac1 in mice results in decreased insulin-dependent PAK1 activation, GLUT4 translocation, and insulin-stimulated skeletal muscle glucose uptake[6]. These mice also display an overall decreased insulin response, reflecting the importance of skeletal muscle in glucose control. Consistent with these findings, the pharmacologic use of the small molecule Rac1 Inhibitor II was shown to reduce glucose uptake in mouse skeletal muscle[18].

The negative role of Rac1 in promoting diabetic complications

Activated Rac1 (i.e., GTP-bound) is a necessary component of the NADPH oxidase (Nox) holoenzyme that plays an important role in

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generating extra-mitochondrial reactive oxygen species (ROS)[19] (Fig. 1). It is known that glucose stimulates Nox-dependent ROS production and that a tonic increase in ROS is important for proper GSIS to occur[20]; however, chronic hyperglycemia/dyslipidemia leads to the overproduction of ROS, in part through Rac1/Nox2, which promotes β-cell dysfunction resulting in a reduction in GSIS[21].

The long term clinical complications of poorly managed T2D are manifold and include diabetic retinopathy, neuropathy, nephropathy, and cardiomyopathy[22]. Growing evidence indicates Rac1 is also involved in many, if not all, of the above pathologies, in large part, as a consequence of the Rac1-depedent overproduction of ROS. A few examples include the involvement of Rac1 in diabetic cardiomyopathy[23] diabetic retinopathy[24] and the endothelial-to-mesenchymal transition (EMT) associated with diabetic nephropathy[25].

Conclusions

The observation that Rac1 has both physiologically and pathologically important roles in T2D implies that therapeutics directly targeting Rac1 to treat diabetic pathologies may experience challenges. In contrast, for Rac1-targeted drugs being developed for use in oncology, there may be an added benefit if the drug alters the glucose uptake of a cancerous cell. At Cytoskeleton Inc., we have the research tools you need to study Rac1 (and other small G-proteins) and actin cytoskeleton dynamics in both live and fixed cells.

Activation Assays

Product	Amount	Cat. #
Rac1 Activation Assay Biochem Kit™	20 assays 50 assays	BK035-S BK035
Rac1 G-LISA® Activation Assay, colorimetric	24 assays 96 assays	BK128-S BK128
Rac1 G-LISA® Activation Assay, luminescence	24 assays 96 assays	BK126-S BK126
RhoA/Rac1/Cdc42 Activation Assay Biochem Kit™	10 assays each	BK030

Antibodies

Antibodies	Host	Type	Reactivity	Amount	Cat. #
Rac1 Specific Antibody Human C-terminal Peptide	Mouse	mAb	Hu, Ms, Rt, other extracts	2 x 200 µl 1 x 50 µl	ARC03 ARC03-S

NEW Signal Seeker™ Kits

Product	Amount	Cat. #
NEW Signal Seeker™ Phosphotyrosine Enrichment Kit	30 Rxs	BK160
NEW Signal Seeker™ Ubiquitin Enrichment Kit	30 Rxs	BK161
Acetyl Lysine Antibody Mouse Monoclonal Validated in WB, IP, IF, ChIP	2 x 100 µl 1 x 25 µl	AAC01 AAC01-S
Phosphotyrosine Antibody Mouse Monoclonal Validated in WB, IP, IF	2 x 100 µl 1 x 25 µl	APY03 APY03-S
Phosphotyrosine Affinity Beads Validated in WB, IP, IF, ELISA	4 x 300 µl	APY03-Beads
Phosphotyrosine-HRP Antibody Mouse Monoclonal Validated in WB	1 x 100 µl 1 x 25 µl	APY03-HRP APY03-HRP-S
SUMO-2/3 Mouse Monoclonal Antibody Validated in WB, IP, IF	2 x 100 µl 1 x 25 µl	ASM23 ASM23-S
SUMO-2/3 Mouse Monoclonal Antibody Validated in IP, IF	2 x 200 µl 1 x 150 µl	ASM24 ASM24-S
SUMO-2/3 Affinity Beads Validated in IP	2 x 400 µl	ASM24-Beads
Ubiquitin Antibody Mouse Monoclonal Validated in WB, IF	2 x 100 µl 1 x 25 µl	AUB01 AUB01-S

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GEF Assay Compound Screening Service

Product	Amount	Cat. #
Tiam1 GEF Screen with Rac1 small G-protein	96 wells	CDS105
Vav1 GEF Screen with Rac1 small G-protein	96 wells	CDS106
Vav2 GEF Screen with Rac1 small G-protein	96 wells	CDS107
SOS1 GEF Screen with H-, K- or N-Ras small G-protein	96 wells	CDS108
Ras GRF GEF Screen with H-, K- or N-Ras small G-protein	96 wells	CDS109
Dbs GEF Screen with RhoA, RhoC or Cdc42 small G-protein	96 wells	CDS110
hSOS1 Protein (Exchange Domain 564-1049)	1 x 100 µg 1 x 1 mg	CS-SOS1-A CS-SOS1-B