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CYTOSKELETON NEWS

NEWS FROM CYTOSKELETON INC.

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this issue

Upcoming Meetings

American Association for Cancer Research (AACR)
March 31st - April 4th, 2012
Booth # 4207

Cytoskeleton Products

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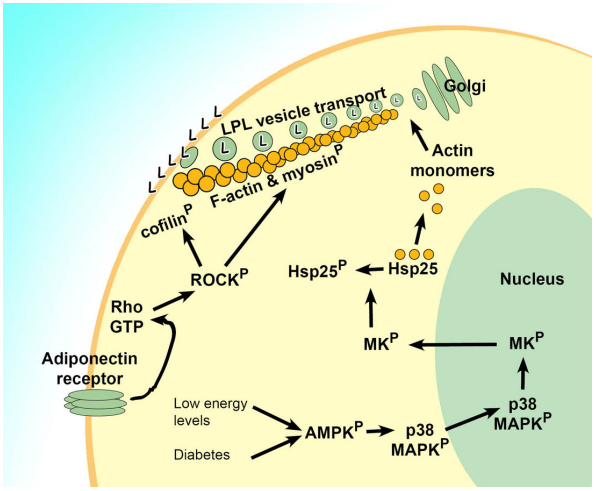
Cardiac research using G-LISA® technology: Studying the Rho pathway in diabetic cardiomyopathy.

The Rho/ROCK pathway is understood to regulate cardiac hypertension in cardiovascular disease[1]. The main manifestation is to regulate the tension of the actomyosin cytoskeleton[2,3,4,5]. It's also well documented that inducing RhoA activation causes rearrangement of the actin cytoskeleton in many cell types. In cardiac tissue, one consequence of this re-organization leads to increased localization of lipoprotein lipase (LPL) to the plasma membrane[6,7], which causes increased reliance on fatty acid metabolism (breakdown of chylomicrons and very low-density lipoproteins) instead of glucose oxidation[8]. Similar physiological effects occur in the hyperglycemic state of type 2 diabetes, which exacerbates the poor metabolic state of cells in this disease[7,8]. Rho activation is a focal point in the pathway from extracellular signals to intracellular mobilization of the cytoskeleton, and hence, re-localization of LPL depends on Rho activation[6]. There are believed to be two pathways that cause LPL mobility to the plasma membrane these are the Adiponectin Receptor - RhoA - Rock pathway and the AMPK - p38 - MK pathway (see Figure 1). The normal physiological cause of LPL re-localization is in response to low energy levels which are detected by AMP-activated protein kinase. This kinase phosphorylates p38 MAPK which translocates to the nucleus and increases transcription of MAPK-activated protein kinase 2 (MK2). MK2 phosphorylates HSP25 which uncouples from actin monomers creating a larger pool of free actin for assembly[8]. In concert with this cascade, circulating in the blood, adiponectin binds to it's extracellular receptor activating the RhoA/ROCK pathway, which causes F-actin fiber formation with an appropriate orientation towards the plasma membrane[6].

The RhoA G-LISA format offered by Cytoskeleton, Inc. has enabled the measurement of Rho activity in cardiology studies despite the small amounts of primary cardiac myocytes or aorta tissues that are available[4,6,9]. The benefits of this format are 1) improved accuracy (cv = 13%) over the traditional Western blot approach[10], 2) rapid processing, 3) small sample size (10-50 µg total protein),

Figure 1. Signalling pathways regulating LPL re-localization.



Legend: Extracellular factors such as adiponectin and intracellular cues such as low energy levels inititate the signalling pathways through AMPK and Rho/Rock to create a cytoskeletal framework to transport LPL from the Golgi to the plasma membrane.

and 4) improved economy per assay. These strengths have allowed researchers to measure Rho activity in smaller pieces of tissue such as mouse aorta, which is 2 mm wide and 8 mm long and too small to be measured by previous methods[4,6,9]. In addition, studying RhoA in aorta homogenates has been a challenge due to high levels of contaminating phosphatases which reduce the GTP-Rho signals to indistinguishable levels in some circumstances. Thus, aorta tissue homogenates require phosphatase inhibitors in order to stabilize the GTP-RhoA signal long enough to measure with G-LISA. Appropriate phosphatase inhibitors are a mixture of false substrates and inhibitors of serine/threonine phosphatases. We recommend 50 mM NaF,

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RHO PROTEIN PRODUCTS

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References

20 mM Na-pyrophosphate, 1 µM microcystin and 1 mM ortho-vanadate. Without these inhibitors, some researchers have found samples produce very similar and low (degraded) GTP-RhoA signals.

Finally, Rho specific inhibitors and activators are an emerging trend in the clinic, such as Bioaxone's BA-210 (Cethrin®) which is indicated for neuro-regeneration. Cytoskeleton offers a Rho inhibitor (Cat. # CT04) and a Rho Activator (Cat. # CN03) which are highly potent cell permeable reagents for Rho pathway research.

References:

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4. Matsumoto et al., 2010. Enhancement of mesenteric artery contraction to 5-HT depends on Rho kinase and Src kinase pathways in the ob/ob mouse model of type 2 diabetes. *Br J Pharmacol.*, **160(5)**: 1092–1104.
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7. Punlinilkunnil and Rodrigues, 2006. Cardiac lipoprotein lipase: Metabolic basis for diabetic heart disease. *Cardiovasc. Res.*, **69**, p.329-340.
8. Kim et al., 2007. Acute diabetes moderates trafficking of cardiac lipoprotein lipase through p38 mitogen-activated protein kinase-dependent actin cytoskeleton organization. *Diabetes*, **57**, p.64-76.
9. Seok et al., 2008. Isoflavone Attenuates Vascular Contraction through Inhibition of the RhoA/Rho-Kinase Signaling Pathway. *J. Pharmacol. Exp. Ther.*, **326**, p.991–998.
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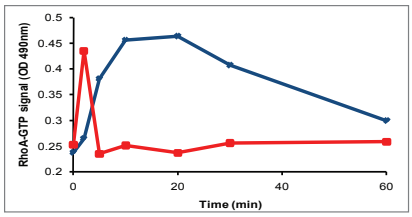
Rho Protein Research Tools

RhoA G-LISA and ELISA



Time course of activation of RhoA in Swiss 3T3 cells by CN01 and LPA. Serum-starved Swiss 3T3 cells were treated with Rho Activator, cat. # CN01 (blue diamonds) or LPA (magenta squares). RhoA activity was measured by reading signals at OD_{490nm}.

Kits	Cat #	Amount
RhoA G-LISA® Activation Assay, colorimetric	BK124	96 assays
RhoA G-LISA® Activation Assay, luminescence	BK121	96 assays
Total RhoA ELISA	BK150	96 assays

GEF and GAP Assays

Products	Cat. #	Amount
RhoGAP Assay Biochem Kit™	BK105	80-160 assays
RhoGEF Exchange Assay Biochem Kit™	BK100	60-300 assays

Rhotekin-RBD Beads

Products	Purity	Cat. #	Amount
Rhotekin-RBD Protein Binds specifically to active (GTP-bound) Rho	>90%	RT01-A RT01-B	1 x 500 µg 3 x 500 µg
Rhotekin-RBD Beads Binds specifically to active (GTP-bound) Rho	>85%	RT02-A RT02-B	2 x 2 mg 6 x 2 mg

RhoA Antibody

Product	Host	Type	Species Reactivity	Cat. #	Amount
RhoA Specific Antibody Human RhoA Peptide (a.a. 120-150)	Mouse	mAb	Hu, Ms, Rt, other extracts	ARH03-A ARH03-B	1 x 100 µg 3 x 100 µg

Rho Activators & Inhibitors

G-protein Modulator	Cell Entry Mechanism	Protein Modulation	Cat. #	Amount
Rho Activator II Deamidation of Rho Gln-63	Cell permeable	Direct	CN03-A CN03-B	3 x 20 µg 9 x 20 µg
Rho Inhibitor I ADP ribosylation of Rho Asn-41	Cell permeable	Direct	CT04-A CT04-B	1 x 20 µg 5 x 20 µg
Rho/Rac/Cdc42 Activator I Deamidation of Rho Gln-63 & Rac/Cdc42 Gln-61	Cell permeable	Direct	CN04-A CN04-B	3 x 20 µg 9 x 20 µg
Rho Pathway Inhibitor I Rho kinase (ROCK) inhibitor Y-27632	Cell permeable	Direct	CN06-A CN06-B	5 x 10 units 20 x 10 units
Rho Activator I SHP-2 phosphatase-mediated Rho activation	Cell permeable	Indirect	CN01-A CN01-B	5 x 10 units 20 x 10 units

Rho Modulator & Effector Proteins

Products	Purity	Cat. #	Amount
C3 Transferase Protein Specific inhibitor of Rho activity (not cell permeable, but also see CT04 above).	>90%	CT03-A CT03-B CT03-C	1 x 25 µg 2 x 25 µg 4 x 25 µg
Dbs His Protein, RhoGEF domain (DH/PH) GEF for Cdc42 and RhoA	>80%	GE01-A GE01-B	2 x 50 µg 8 x 50 µg
p50RhoGAP GST Protein, full length GAP for Cdc42, Rac and Rho	>90%	GAP01-A GAP01-B	1 x 50 µg 4 x 50 µg
p50RhoGAP GST Protein, GAP domain GAP for Cdc42, Rac and Rho	>90%	GAS01-A GAS01-B	1 x 50 µg 4 x 50 µg
RhoGDI GST Protein Inhibitor of Cdc42, Rac and Rho	>90%	GDI01-A GDI01-B	1 x 25 µg 4 x 25 µg

Rho Purified Proteins

Purified G-proteins	Purity	Cat. #	Amount
RhoA His Protein, constitutively-active (Q63L)	>90%	R6301-A R6301-C	1 x 10 µg 4 x 10 µg
RhoA GST Protein, wild-type	>90%	RHG01-C	8 x 25 µg
RhoA His Protein, wild-type	>80%	RH01-A RH01-C RH01-XL	1 x 100 µg 3 x 100 µg 1 x 1 mg
RhoC His Protein, wild-type	>90%	RH03-A RH03-C	1 x 100 µg 3 x 100 µg