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CYTOSKELETON NEWS

NEWS FROM CYTOSKELETON INC.

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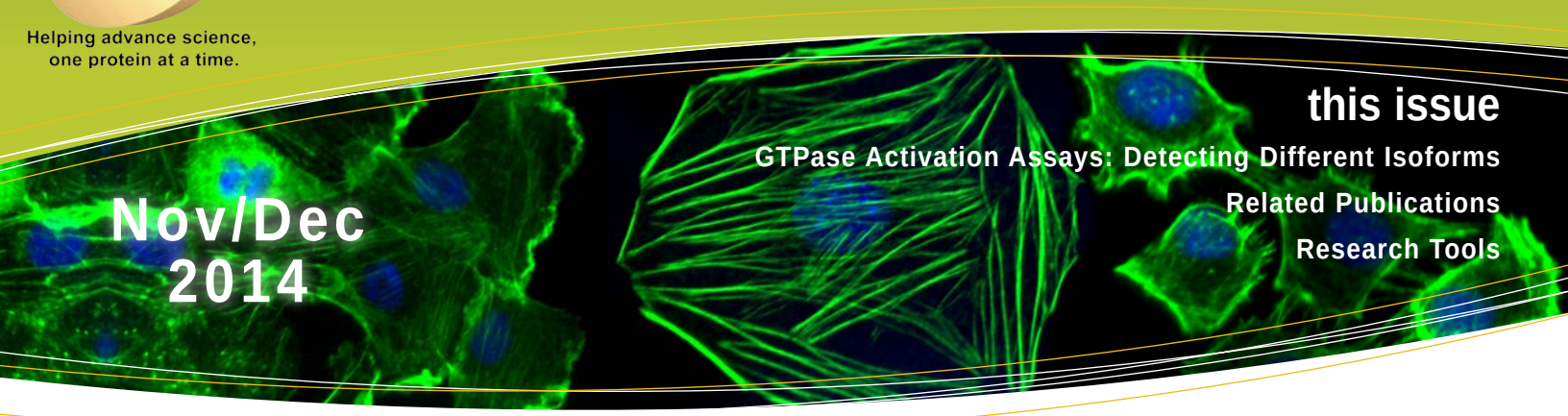

this issue

Meetings

Society for Neuroscience 2014
November 15-19,
Washingon, D.C.
Booth #1917

ASCB/IFCB 2014
December 6-10,
Philadelphia, PA
Booth #818

Cytoskeleton Products

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GTPase Activation Assays: Detecting Different Isoforms

Ras and Rho family GTPases are cytoskeletal small G-proteins that critically regulate multiple actin-dependent cell processes, including development, growth, motility, and intracellular trafficking[1,2]. Moreover, dysfunction of Ras and Rho family GTPases are correlated with several human diseases (e.g., cancer, neurodegeneration) and these GTPases are targeted by multiple pathogenic bacteria[3-5]. The GTPase families are comprised of multiple isoforms, including Cdc42 and RhoJ; RhoA, RhoB, and RhoC; and Rac1, Rac2, and Rac3 for the Rho family. The Ras family contains N-, K-, and H-Ras. Given the important role that these GTPases have in physiological and pathological processes, the ability to measure the activity of specific Rho and Ras isoforms is paramount (Tables 1 and 2).

Rho and Ras family GTPases cycle between an inactive GDP-bound state and an active GTP-bound state. Traditional small GTPase activation assays utilize an effector protein conjugated to agarose beads to isolate or "pull-down" the activated respective GTPase which is then quantitated by western blotting. G-LISAs are a second type of activation assay based on ELISA technology which provide a more quantitative, quicker, and sensitive alternative to pull-down assays. Both formats are offered by Cytoskeleton, Inc. and both kit types can be modified to measure the activity of specific Ras or Rho family isoforms (Tables 1 and 2). Importantly, some isoforms (e.g., H-Ras vs K- and N-Ras; RhoC vs RhoA) are expressed at a much lower level than their related isoforms[6,7]. Because there is substantial variability in expression, detection of the less abundant, activated isoforms can be more difficult. Potential assay variables that might require modification include: lysate concentration, lysis buffer, and antibody concentration and/or dilution, to name just a few. In the examples discussed below, the main modifications were titrating the lysate and isoform-specific antibody.

RhoB and RhoC activity with RhoA G-LISA kit

Rho GTPases mediate a variety of physiological and pathological cell functions. Cytoskeleton's RhoA G-LISA was used to study the activity of RhoB in angiogenesis, the formation of new blood vessels from existing vasculature. Angiogenesis requires activation of endothelial cells by growth factors and because RhoB has been shown to regulate the trafficking and function of growth factors[8,9], this GTPase was investigated for its role in growth factor-mediated angiogenesis in endothelial cells[10]. RhoB expression and activity were examined after vascular endothelial cell growth factor (VEGF) stimulation. To measure activity, the RhoA G-LISA kit was modified by substituting a RhoB antibody for the normal RhoA antibody. The authors concluded that VEGF-mediated endothelial cell morphogenesis is dependent upon RhoB and RhoB-mediated inhibition of RhoA activity[10].

Table 1. Reagent Details For Modified GTPase Assays

GTPase	Cytoskeleton Kit	Citation	Antibody & Supplier	Antibody Dilution
RhoB	BK124	10	SC-8048, Clone C-5 Santa Cruz	NA
RhoC	BK124	10	SC-130339,Clone 37 Santa Cruz	NA
RhoC	BK124	14	NA Home-made	1:50
RhoC	BK124	15, 16	NA Cell Signaling	NA
RhoJ	BK127	18	M01, Clone 1E4 Abnova	NA
RhoJ (HA-tag)	PAK02 or BK034	19	Clone 3F10 for HA Roche	NA
N-Ras	BK008	17	NA Santa Cruz	NA

In addition to RhoB, the RhoA G-LISA can also be used to measure RhoC activity. Because RhoC's constitutive activity is correlated with tumor progression, invasion, and metastasis in many cancers, this GTPase is believed to be involved in cancer cell motility[11-13]. The role of RhoC in the metastasis of prostate cancer to bone was examined using the RhoA G-LISA activation assay kit with the substitution of the RhoA antibody with a RhoC antibody[14]. Regulators of RhoC activity have also been examined with a report that atorvastatin, an inhibitor of cholesterol biosynthesis, reduces RhoC activity in multiple head and neck squamous cell carcinoma (HNSCC) cell lines[15]. In a follow-up study, the authors studied expression of microRNA-138 in the same cell and tumor lines and concluded that microRNA-138 negatively regulates RhoC expression and activation[16]. In both papers[15,16], RhoC activity was measured using the RhoA G-LISA, substituting the RhoA antibody with a RhoC antibody.