The Protein Experts
Cytoskeleton, Inc.

Custom Services Newsletter

Q3 2016

This Issue Contains

Meetings

12th International Congress of Cell Biology
July 21-25
Prague, CZ

Gordon Research Conference - Plant and Microbial Cytoskeleton
August 14-19
Andover, NH

Society for Neuroscience 2016
November 12-16,
San Diego, CA
Booth # 2417

ASCB 2016
December 3-7,
San Francisco, CA
Booth # 928

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Introduction

Effective treatments for central nervous system (CNS) injuries, diseases, and disorders remain a serious challenge for preclinical research scientists and clinicians This newsletter discusses some compounds that are in clinical trials or proteins/pathways that warrant consideration as therapeutic targets (Fig. 1).

Rho family GTPase Modulation

Rho family GTPases control a myriad of neuronal processes, including neurite (axonal and dendritic) growth and differentiation, axonal guidance and pathfinding, and the development, growth, and maintenance of dendritic spines. In this context, the most studied and characterized Rho family GTPases are RhoA, Rac1, and Cdc42. Thus, these GTPases have also received the most attention in the arena of therapeutic intervention in various CNS injuries, diseases, and disorders[1,2].

Rho activity is involved in the neuronal response to CNS trauma such as spinal cord injury. The drug VX-210 (formerly known as Cethrin or BA-210; licensed to Vertex Pharmaceuticals), is a direct-acting, cell-permeable Rho inhibitor derived from C3 transferase, designed to offer both a regenerative and neuroprotective effect after acute spinal cord injury[3,4]. Blocking the Rho pathway allows growth cones to form and extend, processes critical to axon regeneration. As of February 2016, VX-210 is in Phase II/III clinical trials for treatment of acute spinal cord injuries[4-6]. Another Rho-directed drug is BA-1049 which inhibits Rho-associated protein kinase 2 (ROCK2). BA-1049 is being evaluated as a treatment for glaucoma and cerebral vascular disorders (e.g., cerebral cavernous malformations)[3].

Rac1 inhibitors have been evaluated for treatment of Alzheimer's disease (AD) and neurodevelopmental disorders (e.g., Fragile X syndrome [FXS])[2,7]. EHT1864, a Rac1 inhibitor, blocks production of Aβ peptides (Aβ 40 and Aβ 42) that form the plaques that characterize AD. EHT1864 indirectly inhibits γ-secretase activity which reduces the processing of the amyloid precursor protein[7]. The authors replicated these findings with the Rac1 inhibitor NSC23766[7]. NSC23766 also inhibits the exaggerated long-term depression activity in hippocampal neurons of an animal model of FXS (*Fmr 1* knockout mice)[2].

Cdc42 inhibition has been studied with ZCL278, a selective, cell permeable, small molecule inhibitor, discovered by high-throughput in silico screening that binds directly in the surface groove of Cdc42, a region essential for GEF binding. Because Cdc42 has a crucial role in establishing neuronal polarity, the effect of Cdc42 inhibition with ZCL278 on neuronal development was evaluated. ZCL278 inhibits Cdc42-mediated neuronal branching in primary neonatal cortical neurons. In addition, the extension of filopodia and microspikes from migrating growth cones were inhibited in the presence of ZCL278[8]. With an inhibitor such as ZCL278, it will be much easier to dissect the exact role that Cdc42 has in mediating pathways responsible for neuron growth and development.

Summary

The studies discussed here represent just the tip of the iceberg for potential treatments for a variety of CNS injuries, diseases, and disorders. Both targets and therapeutics can only be expected to grow exponentially in the coming years as more is learned about how Rho-family (and other members of the Ras superfamily) GTPases regulate CNS functions in normal and pathological conditions and rational drug design advances continue.

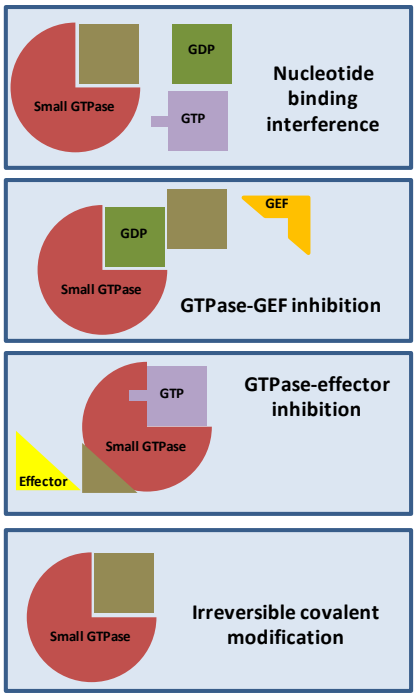

Figure 1. Modulation of GTPase activity by small molecules. Multiple mechanisms exist by which a small molecule (light brown square or triangle) can inhibit GTPase activity.

References

1. Cromm P.M. et al. 2015. Direct modulation of small GTPase activity and function. *Angew. Chem. Int. Ed*. **54**, 13516-13537.
2. Tejada-Simon M.V. 2015. Modulation of actin dynamics by Rac1 to target cognitive function. *J. Neurochem*. **133**, 767-779.
3. http://bioaxonebio.com/technology-overview/pipeline/
4. http://adisinsight.springer.com/drugs/800017953
5. Fehlings M.G. et al. 2011. A phase I/IIa clinical trial of a recombinant Rho protein antagonist in acute spinal cord injury. *J. Neurotrauma*. **28**, 787-796.
6. McKerracher L. and Anderson K.D. 2013. Analysis of recruitment and outcomes in the phase I/IIa Cethrin clinical trial for acute spinal cord injury. *J. Neurotrauma*. **30**, 1795-1804.
7. Desire L. et al. 2005. RAC1 inhibition targets amyloid precursor protein processing by γ-secretase and decreases Aβ production *in vitro* and *in vivo*. *J. Biol.Chem*. **280**, 37516-37525.
8. Friesland A. et al. 2013. Small molecule targeting Cdc42-intersectin interaction disrupts Golgi organization and suppresses cell motility. *Proc. Natl. Acad. Sci. USA*. **110**, 1261-1266.

Custom Modules

Our recently expanded Custom Services Department provides additional resources for your research projects.

Cytoskeleton is leading the way to develop novel motor- and GEF-based compound screens.

We are scientists dedicated to providing accurate data reported in a detailed and timely manner.

About Custom Services

Like our regular product offerings, the Custom Services Department emphasizes quality products and services. We understand that **accuracy** and **timeliness** are critical elements for a successful project. Choose from more than twenty defined modules (**for a full list, visit www.cytoskeleton.com/custom-services**), and then contact Technical Support (tservice@cytoskeleton.com) to guide you through the process.

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- Astra-Zeneca plc
- GlaxoSmithKline plc
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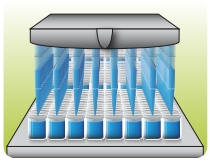
- Johnson & Johnson
- Bristol-Myers Squibb

Let's get started, it's as easy as 1,2,3 ...

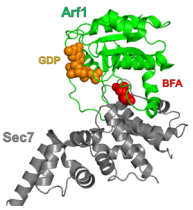
1. Choose a module and ask for a quote (24h turn around time)
2. Review quote, specifications, and deliverables
3. Place order and receive regular updates until project is finished

Compound Screening Modules



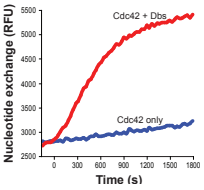
Type	Format	Deliverable	Module #	Timeline (wks)
GEF/GTPase exchange assay	GTP exchange factor plus Small G-protein (e.g. Rho or Ras) with mant-GTP reporter. Kinetic, fluorescence at 360nm/450nm	60 assays consisting of either 28 duplicate reactions plus 4 controls, or 5 x IC50s plus 1 x control IC50. PDF report with Executive Summary, Introduction, Methods, Results and Data Analysis.	CDS100	2
Eg5 Kinesin motor assay	Microtubule stimulated ATPase assay, kinetic, absorbance at 360nm	96 assays, consisting of 40 duplicate single concentrations (or 5 x IC50s), plus eight control wells. PDF Report with Executive Summary, Introduction, Methods, Results and Data Analysis.	CDS050 or CDS051	2
Cardiac Myosin motor assay	Ca^{2+}/Sarcomere (thin filament) stimulated ATPase assay, kinetic, absorbance at 360nm	Same as CDS052.	CDS056	2
Dynein motor assay	Microtubule stimulated ATPase assay, kinetic, absorbance at 360nm	Same as CDS052.	CDS065	2
Tubulin polymerization	Tubulin (>99% pure) Polymerization Assay, kinetic, fluorescence at 360nm/410nm	96 assays, with 40 duplicate single concentrations or 5 x IC50s, plus eight control wells (vinblastine, nocodazole or taxol). PDF Report with Executive Summary, Introduction, Methods, Results and Data Analysis.	CDS009 or CDS010	2

Gene Cloning and Protein Purification Modules



Type	Name	Deliverable	Module #	Timeline (wks)
Recombinant Small Protein	Small protein or protein domain (<30 kDa) with gene provided by client	Highly purified, His-tagged active protein lyophilized in 10 x 100 µg aliquots (or more depending on yield). Datasheet and assay method. Activity in line with published articles. *E. coli* expression.	REC012	3
Recombinant Small Protein plus cloning	Small protein or protein domain (<30 kDa) including gene synthesis	Same as above with gene synthesis.	REC022	6
Recombinant Kinesin Motor-Protein	Medium to large protein or protein domain (30-100 kDa)	Same as REC012.	REC032	3
Recombinant Kinesin Motor Protein plus gene cloning	edium to large protein or protein domain (30-100 kDa) with gene synthesis	Same as above with gene synthesis.	REC042	8
Native or eukaryotic protein expression & purification	Cited protein purification	Same as above plus using a published procedure.	REC052	4-20

Assay Development Modules



Type	Name	Deliverable	Module #	Timeline (wks)
GTP Exchange (fluor. kinetic, 360nm/460nm)	G-protein GTP exchange assay using Mant-GTP	Report with optimized protocol, based on data from titrating four variables ([ionic], [$MgCl_2$], [Mant-GTP] and temp.).	DEV026	4
GTPase assay (abs, endpoint, 650nm)	GTP hydrolysis assay, detecting phosphate	Same as above, except [Mant-GTP] is replaced by [G-protein] and if available [GAP protein].	DEV031	4
Motor ATPase (abs, kinetic, 360nm)	ATP hydrolysis assay, detecting phosphate	Report with optimized protocol, based on data from titrating five variables ([ionic], [$MgCl_2$], [Motor], [microtubules] and temp.).	DEV034	4