Cytoskeleton, Inc.

Custom Services Newsletter

Q3 2015

This Issue Contains

Meetings

Society for Neuroscience 2015
October 17-21,
Chicago, IL
Booth #1019

ASCB 2015
December 12-16,
San Diego, CA
Booth #1134

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Introduction

Microtubules (MTs) are comprised of α/β tubulin heterodimers which have polymerized into cylinderical structures. MTs serve as an essential component of a cell's cytoskeleton as they regulate and participate in a variety of cellular functions that include motility, morphology, intracellular transport, signal transduction, and cell division (Fig. 1). The cell cycle consists of the sequential G1, S, G2, and M phases with MT polymerization and depolymerization (i.e., MT dynamics) playing a key role in the normal progression of this cycle to insure proper cell division (Fig. 1). The disruption of MT dynamics, and thereby the cell cycle, leads to cell death. As such, MTs are a well-recognized and often-studied target for cancer drug discovery efforts[1-4].

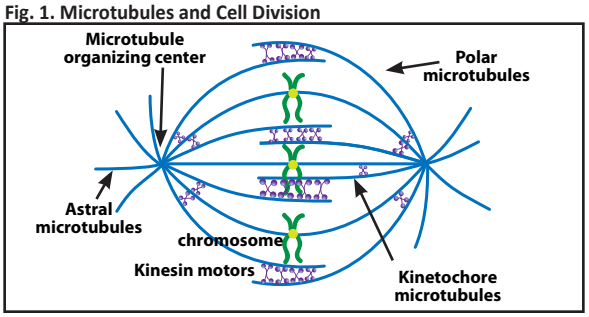
Tubulin Polymerization Inhibitor Screenings

Tubulin polymerization inhibitors function in one of two ways: 1. stabilization of MTs which leads to inhibition of MT dynamics and 2. depolymerization of MTs[1-4]. While the overwhelming target of anti-cancer compound screenings is MTs (motor kinesins are another target), different tubulin polymerization inhibitor screening methods are utilized[5-8]. An early step in cancer drug discovery studies is usually an initial high throughput screen of a large number of compounds (hundreds of thousands) against a line (or lines) of cancer cells. Further screening is performed on a small number of compounds, selected based on the potency of their anti-cancer effect. One such screen is *in vitro* tubulin polymerization under cell-free conditions which not only confirms that the compound(s) inhibit tubulin polymerization, but also enables calculation of the compounds' V_{max} and IC_{50}/EC_{50} values[7]. These screens are often performed by either researchers in the laboratory or contract research organizations (CROs) as part of a custom screening service. CROs are cost and time-effective due to potential complications related to low yields and purity of tubulin along with any needed assay optimization. In either case, the screening uses tubulin protein and/or polymerization assay kits that are commerically available.

Cytoskeleton, Inc. offers custom tubulin screening services that use tubulin and polymerization assay kits produced and validated by Cytoskeleton scientists. The tubulin proteins/kits have been used in multiple cancer drug discovery studies, specifically for follow-up screening efforts once a potent compound has been identified with the larger primary screens[5-8]. Below we briefly discuss these papers, focusing on the key role that *in vitro* tubulin polymerization cell-free assays play in cancer drug discovery.

When screening novel compounds for their effect on tubulin polymerization, comparisons are made against well-characterized tubulin inhibitors, some of which are current anti-cancer treatments. In Cheng et al.[5], the most promising drug was chosen based on large scale screenings of a variety of drugs for anti-proliferative effects against multiple cancer cell lines. Cytoskeleton's tubulin polymerization assay/tubulin protein was used to demonstrate that the inhibitory effect of the compound had a mechanism of action similar to vincristine[5]. Similarly, Mu et al.[6] used Cytoskeleton's tubulin products to screen a novel synthetic compound that selectively targeted cancer cells, finding that the compound inhibited tubulin polymerization in a much more moderate manner than vincristine[6]. Besides vincristine, inhibitors of tubulin polymerization also can act at or near the colchicine binding site[7,8]. Nagarajan et al.[7] characterized a compound that inhibited tubulin polymerization via the colchicine binding site. Likewise, Bernard et al.[8] described novel compounds that reversibly inhibit MT formation through binding near the colchicine site.

Fig. 1. Microtubules and Cell Division



Summary

Cancer drug discovery studies utilize a variety of screening tools, including cell free *in vitro* tubulin polymerization screens. Cytoskeleton Inc. offers custom tubulin screening services supported by over 20 years of experience. For those scientists who are comfortable with performing the screens themselves, Cytoskeleton offers tubulin in various purities as a stand-alone protein and as part of a polymerization kit that includes all necessary screening reagents For more information or a quote, please contact us at tservice@cytoskeleton.com.

References

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2. Jordan A. et al. 1998. Tubulin as a target for anti-cancer drugs: Agents which interact with the mitotic spindle. *Med. Res. Rev*. **18**, 259-296.
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6. Mu Y. et al. 2015. The novel tubulin polymerization inhibitor MHPT exhibits selective anti-tumor activity against Rhabdomyosarcoma *in vitro* and *in vivo*. *PLoS ONE*. **10**, e0121806.
7. Nagarajan S. et al. 2015. Tubulin inhibitor identification by bioactive conformation alignment pharmacophore (BCAP)-guided virtual screening. *Chem. Biol. Drug. Des*. doi: 10.1111/cbdd.12568.
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Custom Modules

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

Cytoskeleton is leading the way to develop novel kinesin, dynein, and myosin 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.

Clients Include:

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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
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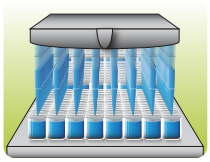
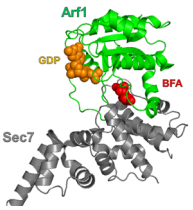
Compound Screening Modules



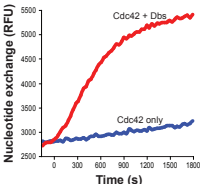
Type	Format	Deliverable	Module #	Timeline (wks)
Eg5 Kinesin motor assay	Microtubule stimulated ATPase assay, kinetic, absorbance at 360nm	96 assays, consisting of 40 duplicate single concentrations (or 5 x IC50s), plus eight control wells. PDF Report with Executive Summary, Introduction, Methods, Results 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
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

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