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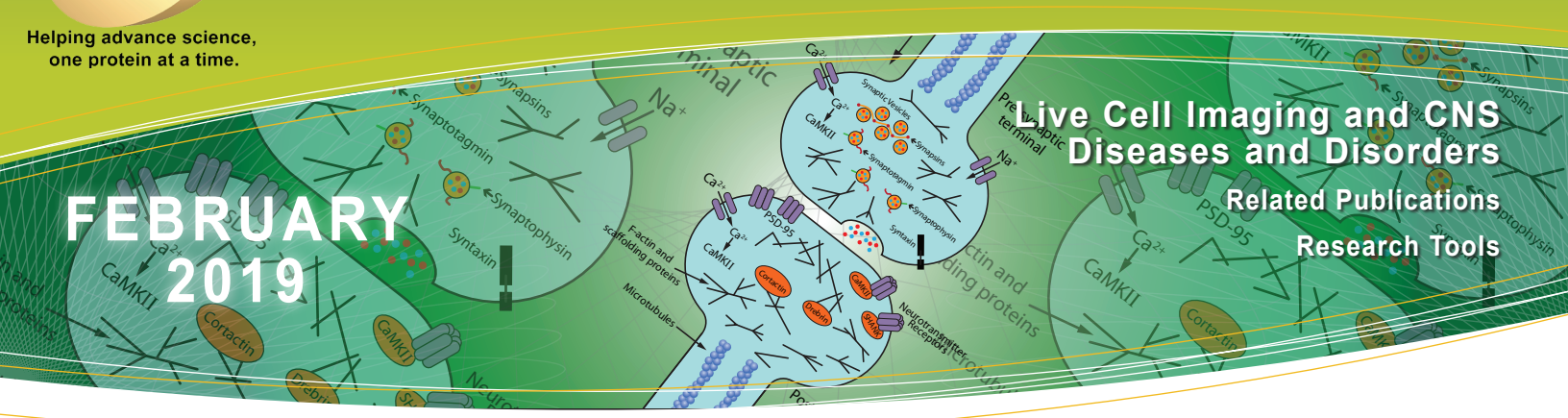
CYTOSKELETON NEWS

NEWS FROM CYTOSKELETON INC.

FEBRUARY 2019



Meetings

2019 Directed Cell Migration Gordon Research Conference
January 20-25
Galveston, TX

GRS: Cell Biology of Megakaryotes and Platelets
February 23-24
Galveston, TX

2019 Subgroup Mechanobiology
March 2
Baltimore, MD
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Live Cell Imaging and CNS Diseases and Disorders

Studying the causes of human central nervous system (CNS) diseases and disorders and developing impactful therapies requires in vitro and in vivo disease models that faithfully recapitulate the respective neuropathophysiology while also supporting the neurons with the necessary cellular machinery to respond to therapies in a manner that offers translational results[1-3]. Moreover, it is imperative to study the earliest neuropathophysiological changes as these provide the best opportunity for early diagnosis and intervention[4]. Changes in the structure and/or function of neuronal synapses are often the earliest pathological changes in many neurodegenerative diseases and CNS disorders[4-7]. To detect early synaptic dysfunction, scientists utilize live cell imaging which provides single cell resolution with real-time analysis of changing experimental conditions. This newsletter discusses the use of live cell imaging in in vitro and in vivo studies of CNS diseases and disorders.

Designing biologically-relevant in vitro and in vivo live cell imaging experiments requires healthy, mature neurons with proper synaptic structure and function (i.e., morphology and electrical activity, respectively) (Fig. 1). Primary imaging targets are pre- and post-synaptic structures and associated proteins (e.g., synaptic vesicle proteins, neurotransmitter receptors, ion channels, scaffolding proteins, and/or cytoskeletal proteins [F-actin, microtubules]) because they are among the earliest substrates to undergo pathological changes[4] (Fig. 1). Methods to assess changes in synaptic structure and/or function include measuring synapse density, neurite length, spine density, nodes (the location on the cell body from which neurites project), branching of dendrites, and/or electrical activity[4].

Live cell imaging of in vitro cultured mammalian neurons, including human-induced pluripotent stem cell (hiPSC)-derived neurons utilized due to their presumed greater biological relevance[8], has revealed unique mechanistic and functional findings relevant to human neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Apolipoprotein E (apoe-E) is a protein implicated in the pathogenesis of AD, but the role of apoe-E and its metabolites in neuron physiology is unappreciated. Differentiated SH-SY5Y neuroblastoma cells chronically treated with a 25 kDa fragment or full-length apoE display neuritogenesis (positive changes in neurite length and cell confluency as assessed by live cell imaging)[9]. Preliminary drug screening studies also benefit from this methodology. In AD, the two most common drug targets are amyloid beta (Aβ) and tau proteins as both of these proteins have early pathological effects on the synapse[5-7]. Recently, live cell imaging of hiPSC-derived neurons was performed in the screening of several candidate antibodies against pathological Aβ. Positive immunotherapeutic findings were assessed by quantifying changes in neurite length and dendritic branch points over a range of antibody incubation times and concentrations[10].

In another Aβ study, Hong et al.[11] imaged living hiPSC-derived neurons treated with Aβ oligomers isolated from post-mortem AD human brain extracts. The pathophysiology of the oligomers was assessed through quantification of changes in neurite length and synaptic plasticity with long-term potentiation recordings. Tau protein is the focus of a novel technology with the creation of the first genetically encoded Foster Resonance Energy Transfer (FRET) sensor sensitive to tau conformations. The sensor monitors the conformations of wild-type and pathological mutant tau proteins in the presence and absence of microtubules (MTs) in living HeLa cells and immortalized HT22 hippocampal neurons following pharmacological treatments[12]. This tau FRET sensor provides invaluable quantitative and qualitative data concerning modulation of tau binding to MTs, the ratios of soluble to insoluble (pathological) tau, and/or how to influence tau's conformation to favor non-pathological forms.

The complement to in vitro cell culture models is in vivo animal models. Live cell imaging of neurons in whole animals is often performed with two photon microscopy which allows observation of functional neurons with single cell resolution in their natural

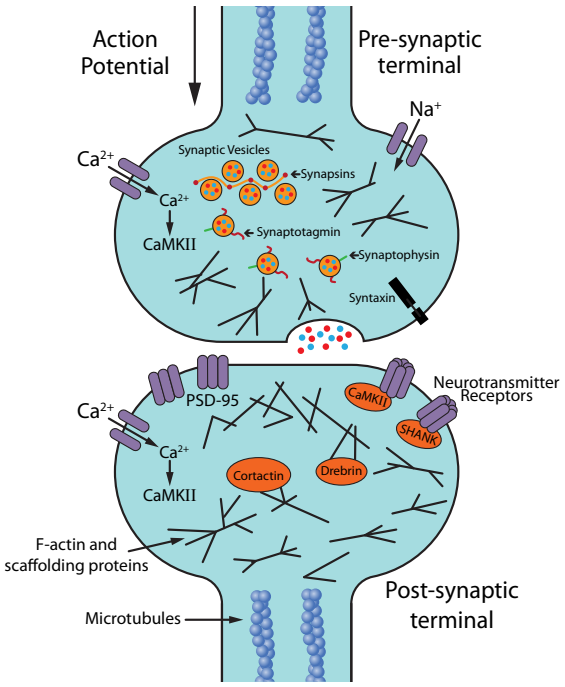

Figure 1: Molecular and cellular targets for live cell imaging of neurons: synapses, synaptic activity, and synaptic plasticity.

News
Publications
Research Tools

LIVE CELL IMAGING PRODUCTS

Continued from Page 1

state and within the larger setting of an interconnected support network[13]. In this way, live cell imaging in animals is transformative with the opportunity to visualize and analyze dynamic cellular processes in real-time. Additionally, single cell in vivo imaging allows for quantification of neuronal functional responses concomitant with sensory input, behavior, and/or pharmacological treatments[13]. In vivo two photon live cell imaging was utilized in the hunt for the mechanism of action of an anti-PD drug that targets α-synuclein[14] and for evaluating the feasibility and optimal timing, host, and donor conditions for transplanting embryonic cells into damaged adult brain regions to replace dead or dying neurons[15-17]. Moreover, in vivo imaging can reveal new therapeutic approaches. In the MPTP mouse model of PD, live cell imaging showed that the structural and functional synaptic plasticity of motor cortical neurons was affected by the selective loss of dopaminergic neurons in MPTP-treated mice, raising the possibility that modulating neuronal activity in particular regions of motor cortex is a viable option for treating the motor impairments associated with PD[18].

Summary

Live cell imaging is an invaluable tool in the study of CNS diseases and disorders as it offers insight into not only the basic disease/disorder biology, but also the functional consequences of pharmacological, genetic, and/or behavioral therapy interventions in real time. When coupled with advances in super resolution microscopy and automated live cell imaging[19], there are unparalleled opportunities to focus on the first pathological synaptic changes that can be halted or reversed with early therapeutic intervention. At Cytoskeleton Inc., valuable research tools such as live cell imaging probes for F-actin, microtubules, DNA, and lysosomes are available, as well as purified cytoskeletal proteins and activation assays and antibodies, to advance these research goals.

Live Cell Imaging Products

Description	Ex / Em	Amount	Cat. #
SiR-Actin Kit Includes SiR-Actin and Verapamil	630 / 680 nm	50 nmol	CY-SC001
SiR-Tubulin Kit Includes SiR-Tubulin and Verapamil	630 / 680 nm	50 nmol	CY-SC002
Cytoskeleton Kit Includes SiR-Actin, SiR-Tubulin, and Verapamil	630 / 680 nm	50 nmol each	CY-SC006
SiR-DNA Kit Includes SiR-DNA and Verapamil	630 / 680 nm	50 nmol	CY-SC007
SiR-Lysosome Kit Includes SiR-Lysosome and Verapamil	630 / 680 nm	50 nmol	CY-SC012
SiR700-Actin Kit Includes SiR-Actin and Verapamil	690 / 720 nm	35 nmol	CY-SC013
SiR700-Tubulin Kit Includes SiR700-Tubulin and Verapamil	690 / 720 nm	35 nmol	CY-SC014
SiR700-DNA Kit Includes SiR700-DNA and Verapamil	690 / 720 nm	35 nmol	CY-SC015
SiR700-Lysosome Kit Includes SiR700-Lysosome and Verapamil	690 / 720 nm	35 nmol	CY-SC016

G-LISA Activation Assay Kits

Product	Reactions	Cat. #
G-LISA RhoA Activation Assay Biochem Kit (colorimetric format)	96	BK124
G-LISA RhoA Activation Assay Biochem Kit (luminescence format)	96	BK121

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Actin Biochem Kits

Actin Biochem Kit	Reactions	Cat. #
Actin Binding Protein Spin-Down Assay Biochem Kit rabbit skeletal muscle actin	30-100 assays	BK001
Actin Binding Protein Spin-Down Assay Biochem Kit human platelet actin	30-100 assays	BK013
Actin Polymerization Biochem Kit (fluorescence format) rabbit skeletal muscle actin	30-100 assays	BK003
G-Actin/F-actin In Vivo Assay Biochem Kit	30-100 assays	BK037

Tubulin Biochem Kits

Product	Reactions	Cat. #
Tubulin Polymerization Assay Biochem Kit (absorbance format), porcine tubulin	24-30	BK006P
Tubulin Polymerization Assay Biochem Kit (fluorescence format): 99% pure porcine tubulin	96	BK011P
Microtubule Binding Protein Spin-Down Assay Biochem Kit	50-100	BK029
Microtubule/Tubulin In Vivo Assay Biochem Kit	30-100	BK038