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CYTOSKELETON NEWS

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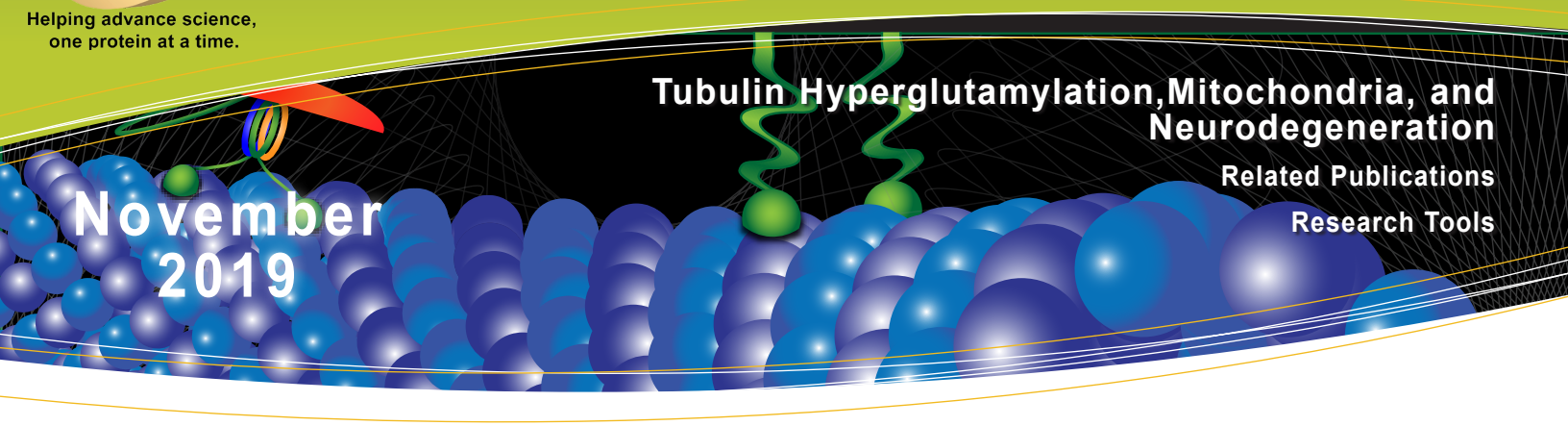

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Cold Spring Harbor Conference - Development and 3D Modeling of the Human Brain
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Tubulin Hyperglutamylation, Mitochondria, and Neurodegeneration

Microtubules (MTs) are composed of α/β-tubulin heterodimers and are one of three essential proteins that comprise the cytoskeleton of mammalian cells and have essential roles in cell development, growth, motility, mechanotransduction, and intracellular trafficking. Functional regulation of MTs is achieved through at least seven different post-translational modifications (PTMs) that usually occur post-polymerization and preferentially on the α/β-tubulin heterodimers of stable (vs dynamic) MTs. PTMs are highly dynamic and often reversible processes that regulate a protein's functions, binding partners, and/or subcellular localization by addition of a chemical group or a peptide to amino acid residue(s) within the target protein[1-4].

The polyglutamylation PTM, addition of variable length glutamate side-chains to primary sequence glutamate residues, was first described in the early 1990s. Glutamate residues in the C-terminal tails (CTTs) of α- and β-tubulins are the most common substrates[1-6] (Fig. 1). Glutamylation enzymes are members of the tubulin tyrosine ligase-like (TTLL) family of proteins[1-4,7,8]. Cytosolic carboxypeptidases (CCP; a.k.a. Nna) function as deglutamylases with CCP1, 4, 5, and 6 removing glutamate side-chain residues in mammalian cells. Three enzymes (CCP1, CCP4, and CCP6) catalyze the shortening of polyglutamate chains, while CCP5 specifically removes the branching point glutamate residue[1-4,9-11] (Fig. 1). Physiological polyglutamylation modifies MTs within neuronal cell bodies and processes (i.e., dendrites and axons) to regulate a variety of MT-based neuronal functions[1-4].

Neurodegeneration and Mitochondria

Hyperglutamylation of tubulin can cause neurodegeneration, first observed in the death of Purkinje cells in pcd (Purkinje cell degeneration) mice due to inactivating mutations in CCP1[9,12]. Purkinje neuron death was prevented and motor coordination improved following down-regulation of the tubulin-specific neuronal polyglutamylase (TTLL1) in the cerebellum of young pcd mice[9]. A recent study using Purkinje-cell-specific Ccp1-knockout mice confirmed not only that tubulin hyperglutamylation due to CCP1 inactivation can cause neurodegeneration, but that it does so by disrupting MT-based intraneuronal transport[13,14]. Mice lacking both Ccp1 and ttll1 did not display Purkinje cell death[13,14]. Even more intriguing is a mechanistic study which revealed how loss of CCP1 expression/activity causes neurodegeneration in pcd mice[14,15] (Fig. 1). Using primary cerebellar neurons cultured from pcd mice and CCP1-deficient retinal pigment epithelial cells (involved in retinal degeneration of pcd mice) produced the expected increase in tubulin polyglutamylation[14,15]. Furthermore, loss of Ccp1 reduced mitochondrial fusion and bi-directional (i.e., anterograde and retrograde) motility, as well as increased instances of mitochondrial fragmentation[14,15]. As with any preclinical models of neurodegeneration, the observation of genetic mutations in human subjects cements the importance of studying the disease not just from the perspective of learning more about neurodegenerative diseases, but to also devise therapeutic interventions. Recently, Shashi et al.[16] demonstrated that loss of CCP1 enzymatic activity (via either loss-of-function variants or missense variants with single amino acid changes) is responsible for a previously unexplained childhood-onset, progressive neurodegenerative condition.

Taken together, the above studies indicate that intraneuronal transport of mitochondria and maintenance of mitochondria functionality and morphology are compromised by tubulin hyperglutamylation. Importantly, mitochondria provide the energy needed for intracellular bi-directional transport and maintenance of ion gradients in neurons, two processes

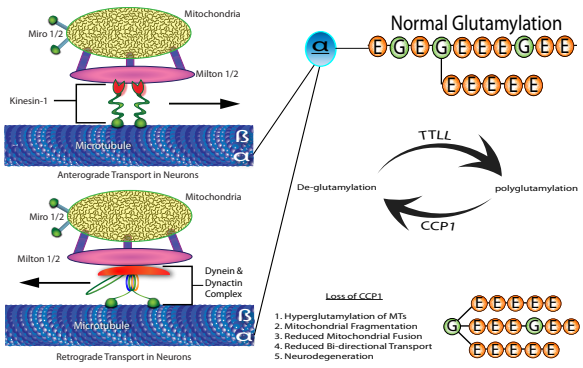

Fig. 1. Schematic representation of the bi-directional transport of mitochondria in neurons and effects of hyperglutamylation due to loss of CCP1 activity. The anterograde motor kinesin-1 and the retrograde motor dynein/dynactin complex directly interact with Miltons and Miros on mitochondria to drive their transport along MTs. Functional loss of CCP1, a deglutamylation enzyme, results in hyperglutamylation which negatively affects mitochondrial dynamics and results in neurodegeneration. The carboxyl-terminal amino acids of tubulin can be acted upon by TTLLs (glutamylation enzymes) and CCPs (deglutamylation enzymes), including CCP1. CCP1 cleaves glutamate residues from the polyE side chain down to the branch point. Inactivating mutations in CCP1 are responsible for Purkinje neuron degeneration in pcd mice. This figure is adapted from those in references 9 and 17.

Tubulin Kit PRODUCTS

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required for basic neuronal functions, including synaptic neurotransmission, synaptic vesicle recycling, and intracellular trafficking. Mitochondria are highly dynamic organelles that undergo continuous fusion, fission, and transport, processes which not only control mitochondrial morphology and number but also regulate mitochondrial function and location. Mitochondria themselves rely upon fast bi-directional intracellular transport along MTs to localize to specific cellular locations[17] (Fig. 1). The primary components of the motor-adaptor complex are the anterograde motor kinesin-1 (a.k.a. kinesin heavy chain [KHC] or KIF5), retrograde motor dynein (in complex with dynactin), Miro1 and 2, and Milton1 and 2[17]. Milton isoforms are mitochondrial adaptor proteins that recruit the molecular motors to mitochondria for MT-mediated transport and Miro isoforms are mitochondrial Rho GTPases. The anterograde motor kinesin-1 and the retrograde motor dynein/dynactin complex directly interact with Miltons and Miros on mitochondria to drive their bi-directional movement along the MTs[18,19].

An important question is if these findings extend to other neurodegenerative diseases? It is already well known that pathogenic tubulin PTMs, including those that affect binding of MAPs (microtubule-associated proteins), play a key role in Parkinson's disease (PD) and various tauopathies[12,20]. Roles for MT polyglutamylation in other neurodegenerative diseases remains an open question, but mitochondrial dysfunction is observed in PD, Alzheimer's disease (AD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS)[17]. The importance of understanding the roles of mitochondrial dynamics in the pathogenesis of neurodegenerative diseases has intensified after the identification of several key regulators (e.g., Drp1, OPA1, mitofusins) of mitochondrial fusion and fission. Furthermore, there is a clear need to examine the possible interplay between tubulin PTMs, mitochondrial dysfunctions, and neurodegenerative diseases[17].

Summary

Despite recent gains in understanding tubulin polymodifications, much remains to be discovered, including identification of non-enzymatic regulators of glutamylation/deglutamylation (e.g., CSAP [cilia and spindle-associated protein])[21] and all tubulin deglutamylases, as well as a complete understanding of how glutamylation (and other PTMs) affect binding of MAPs (e.g., tau, molecular motors)[9-11,21] and the activity of MT-severing enzymes[21]. Identification of the complete population of glutamylation and deglutamylation enzymes and related regulators will reveal new therapeutic targets for neurodegenerative diseases that have dysfunctional intracellular transport as a pathophysiological hallmark. To assist researchers, Cytoskeleton offers a variety of Signal-Seeker Detection Kits which allow the measurement of endogenous levels of PTMs, tubulin activity and binding assay kits, live cell imaging probes for microtubules, and purified tubulin proteins.

Signal Seeker™ Kits

Product	Assays	Cat. #
Signal-Seeker™ Phosphotyrosine Detection Kit	30	BK160
Signal-Seeker™ Ubiquitination Detection Kit	30	BK161
Signal-Seeker™ SUMOylation 2/3 Detection Kit	30	BK162
Signal-Seeker™ Acetyl-Lysine Detection Kit	30	BK163
Signal-Seeker™ SUMOylation 1 Detection Kit	30	BK165

Live Cell Imaging Products

Product	Ex / Em	Amount	Cat #
SiR-Tubulin™ Kit Includes SiR-Tubulin, and Verapamil	630 / 680 nm	50 nmol	CY-SC002
SiR700-Tubulin Kit 35 nmol SiR700-Tubulin and 1 µmol verapamil	680 / 720 nm	50 nmol	CY-SC014

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Dynein motor protein	1 x 50 µg	CS-DN01-A
Kinesin heavy chain motor domain protein GST tagged: Homo sapiens recombinant	2 x 25 µg 1 x 1 mg	KR01-A KR01-XL
KIF3C kinesin motor domain protein GST tagged: Homo sapiens recombinant	2 x 25µg	KF01-A
KIFC3 kinesin motor domain protein GST tagged: Homo sapiens recombinant	2 x 25 µg	KC01-A
Custom purification of kinesin motor	Custom	Inquire for Price

Tubulin Kits

Product	Assays	Cat #
Tubulin polymerization HTS assay using >97% pure tubulin, OD based - Porcine	24	BK004P
Tubulin polymerization assay using >99% pure tubulin, OD based - Porcine	24-30	BK006P
Tubulin polymerization assay using >99% pure tubulin, fluorescence based	96	BK011P
Microtubule Binding Protein Spin-Down Assay Biochem Kit	50-100	BK029
Microtubule/Tubulin In Vivo Assay Biochem Kit	30-100	BK038