N3 - Progress Report

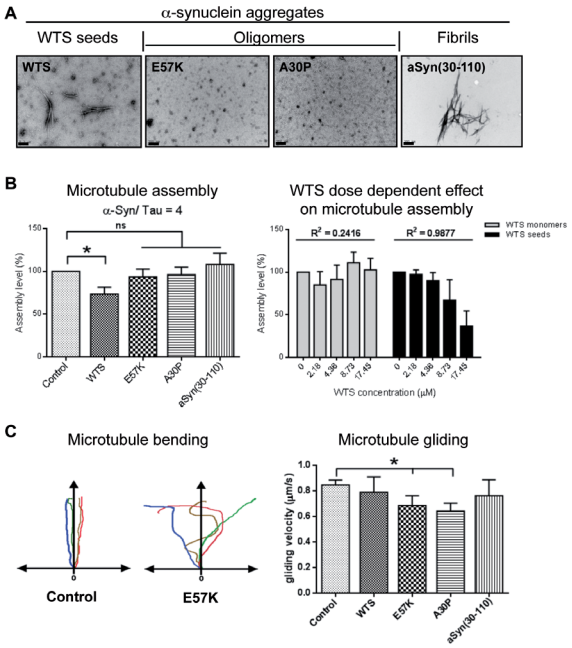
01.10.2010 - 30.09.2016

Modeling neurodegenerative diseases using stem cells

Prof. Dr. Beate Winner, IZKF - Junior Research Group 3

The overall goal of research in our laboratory is to model neurodegenerative diseases using stem cells and derivatives. Specifically, we investigate neurodegeneration and regeneration in synucleinopathies including Parkinson's disease (PD). Protein aggregation of mis-folded proteins is associated with several synucleinopathies. We are interested in studying the mechanism and functional consequences of oligomerization for neurite degeneration, axonal transport, and cellular membranes.

Deposits and aggregates of α-synuclein within neurites are a patholgical hallmark of multiple neurodegenerative diseases including dementia with Lewy bodies (DLB) and Parkinson's disease (PD). Pharmacological treatment cannot halt the progressive neuronal degeneration. While the destructive character of α-synuclein aggregation is evident and includes synaptic dysfunction and associated neurodegeneration, the underlying mechanisms and the cascade of events leading to α-synuclein-mediated toxicity and the relevance to human disease are still unclear. Furthermore, although α-synuclein oligomers are one of the toxic species of α-synuclein, their interaction with neuronal physiology is still not fully understood. Our recent data describe a structure-functional interaction of different α-synuclein species with the microtubule-based axonal transport machinery in a cell-free system and its consequences on human dopaminergic neurons.



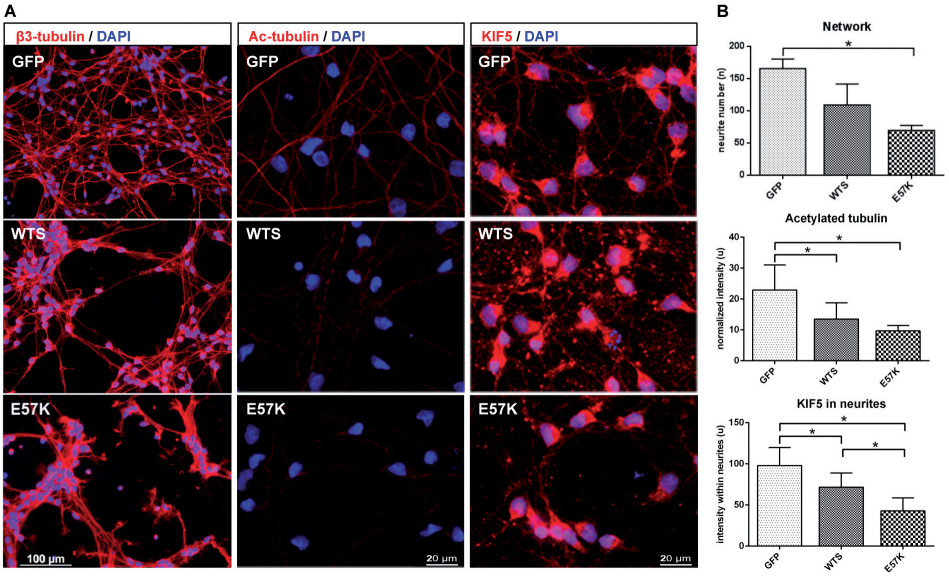
Interaction of a-synuclein aggregates with microtubule-kinesin system in vitro. (A) α-synuclein aggregates. (B) Tau-dependent microtubule assembly is inhibited by α-synuclein seeds. (C) α-synuclein oligomers inhibit kinesin-dependent microtubule gliding.

α-synuclein oligomers impair neuronal microtubule-kinesin interplay

We used recombinant α-synuclein proteins (wild type, single point mutants, and the fragment 30-110) to obtain different aggregate species in vitro. We evaluated the direct effect of these species on microtubule-based cytoskeleton in a cell-free system and in a human dopaminergic neuronal cell line. Our data show that wild type α-synuclein (WTS) monomers and aggregates bind to proteins required for microtubule-based anterograde axonal transport, such as kinesin, tubulin, microtubules, microtubule associated protein 2 (MAP2), and Tau. Interestingly, WTS aggregates composed of oligomers and short fibrils (WTS seeds) impaired microtubule polymerization in vitro promoted by axon-specific Tau protein in a dose-dependent manner. On the other side, α-synuclein oligomers decreased kinesin-microtubule motility in a model system of kinesin-driven transport in vitro. In a human dopaminergic neuronal cell line, the neurite network morphology was severely disrupted by mild overexpression of α-synuclein oligomers and less potently by a-synuclein seeds. Neurite morphology disruption in these cells correlated with a significant reduction of microtubule stability and impaired amounts of kinesin and kinesin-

Prof. Dr. Winner

dependent cargo in neurites. Thus, our study describes complex interactions of α-synuclein species with proteins involved in axonal transport and microtubule network. We propose a sequence of pathologic events in neurites induced by α-synuclein aggregation that involve impairment of microtubule-kinesin functionality microtubule network disruption by toxic soluble α-synuclein aggregates - oligomers and seeds. These alterations act together with other pathologic effects of α-synuclein oligomers, such as membrane thinning and leakage, and together represent critical early mechanisms of synucleinopathies. These results provide new intriguing insights into the pathomechanisms of very early stages of synucleinopathi*es.* (Prots et al., JBC 2013)



α-synuclein oligomers (E57K) and seeds (WTS) cause disruption of microtubule-kinesin cytoskeleton in neuronal cell line. (A) Staining and (B) quantification of neuritic network, acetylated tubulin and kinesin amount.

Contact:
Prof. Dr. Beate Winner
Phone: +49 9131 85 39301
e-mail: beate.winner@med.uni-erlangen.de

Invited lectures

Seminar BioSysNet, Munich, 07.03.2013, I. Prots "Contribution of a-synuclein oligomers to axonal dysfunction"

12th Tom Wahlig Symposium, Dresden, Germany, 22.03.13, F. Perez-Branguli, „SPG11: A new member of synaptic family?"

Young Investigators Symposium of the Stem Cell Society Singapore. Biopolis, Singapore, 31.05.2013, S. Havlicek, „Modeling Autosomal Dominant Hereditary Spastic Paraplegia (SPG4) Using Human Induced Pluripotent Stem Cells"

2nd Eurogenesis meeting, Adult neurogenesis in physiology and disease, Bordeaux, Frankreich, 26.06.13, B. Winner, „Modeling synucleinopathies using stem cells"

Gage lab symposium, La Jolla, USA, 09.11.13, B. Winner, „Modeling hereditary spastic paraplegia using stem cells"

Awards

Steven Havlicek: IZKF Erlangen poster prize for the poster „Gene-dosage dependent neurite defects in a human induced pluripotent stem cell model of SPG4 related hereditary spastic paraplegia". 13th IZKF PhD workshop, 7.10.2013, Erlangen.

Publications during funding period

Havlicek S, Kohl Z, Mishra HK, Prots I, Eberhardt E, Denguir N, Wend H, Plötz S, Boyer L, Marchetto MC, Aigner S, Sticht H, Groemer TW, Hehr U, Lampert A, Schlötzer-Schrehardt U, Winkler J, Gage FH, Winner B (2013) Gene dosage dependent rescue of HSP neurite defects in SPG4 patients's neurons. Hum Mol Genet. 2013 Dec 30. [Epub ahead of print] PMID: 24381312

Marxreiter F, Ettle B, May VE, Esmer H, Patrick C, Kragh CL, Klucken J, Winner B, Riess O, Winkler J, Masliah E, Nuber S. (2013) Glial A30P alpha-synuclein pathology segregates neurogenesis from anxiety-related behavior in conditional transgenic mice. Neurobiol Dis. 2013;59: 38-51

Prots I, Veber V, Brey S, Campioni S, Buder K, Riek R, Böhm KJ, and Winner B (2013) Alpha-synuclein oligomers impair neuronal microtubule-kinesin interplay. J. Biol. Chem. 288: 21742-21754