N3 - Progress Report

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Modeling neurodegenerative diseases using stem cells

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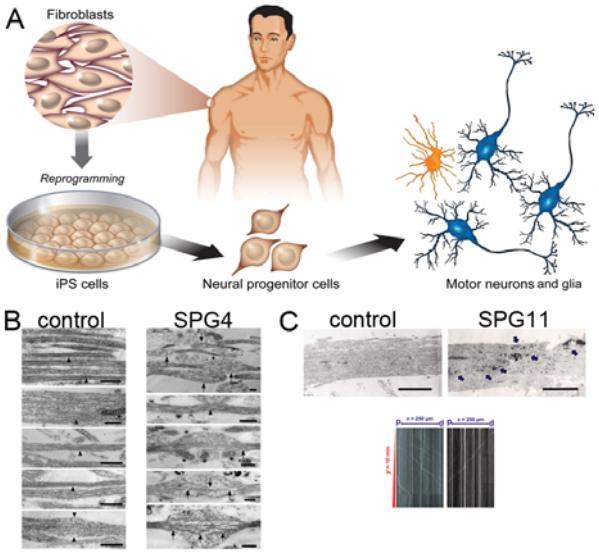
The overall goal in our laboratory is to investigate neurodegeneration using human stem cell derived models. During the last year, we investigated neuronal phenotypes in the most frequent autosomal dominant (SPG4) and recessive (SPG11) forms of hereditary spastic paraplegia (HSP). We were able to show a gene-dosage dependent rescue of impairments of the microtubule structure in patients' neurons with SPG4 mutations and axonal pathology in patients' neurons with SPG11.

The hereditary spastic paraplegias (HSP) are a heterogeneous group of motoneuron diseases characterized by progressive spasticity and paresis of the lower limbs. We generated neuronal cultures from induced pluripotent stem cells (iPSC) from patients' fibroblasts to model the two most frequent genetic forms (SPG4 and SPG11) that cause degeneration in the corticospinal tract.

Gene dosage dependent rescue of HSP neurite defects in SPG4 patients' neurons

Spastin, the protein encoding SPG4, is a member of the ATPase-associated (AAA) family of proteins with the main function to sever microtubuli. We investigated patients with an identical heterozygous nonsense mutation (p.R562X). The levels of Spastin expression and its isoforms were significantly decreased in SPG4 neurons. The neurite complexity of SPG4 glutamatergic projection neurons was severely impaired. Moreover these neurites displayed abundant neurite swellings, with loosely arranged, interrupted microtubules, and an imbalance of axonal transport, with an increase in retrograde transport for mitochondria. An important finding of this study is that elevation of Spastin levels by lentiviral expression of Spastin at low levels led to restoration of neurite complexity and reduction of neurite swellings in SPG4 neurons. Interestingly, there was also a decrease in Spastin expression in the fibroblasts of these patients, indicating a potential role for patient-derived fibroblasts as a pharmacological screening tool in the future.



A) Work flow for investigating disease phenotypes in human stem cell derived cells. B) Neurite swellings in SPG4 neurons. C) Axonal pathology and impaired axonal transport in SPG11 neurons.

In summary, we could show that the gene dosage of spastin (mutated in SPG4 linked HSP) determines the neuritic complexity. Moreover we provided the proof of principle that cellular phenotypes caused by the haploinsufficiency of spastin can be reverted by gene dosage dependent repair (Havlicek et al., HMG 2014).

Prof. Dr. Winner

Dysfunction of spatacsin leads to axonal pathology in SPG11 linked hereditary spastic paraplegia

Another study investigated the impact of spatacsin (mutated in SPG11) on neurons. An accumulation of vesicle-like structures and inclusions in human neurites from SPG11 patients points towards neurite pathologies in human neurons with SPG11 mutations. In mouse cortical neurons, spatacsin was located in a punctuated fashion in axons and dendrites. It colocalized with actin, tubulin and synaptic vesicle markers, and was present in synaptosomes. Knockdown of spatacsin evidenced that the loss of function of spatacsin leads to axonal instability by down-regulation of acetylated tubulin. Furthermore, time–lapse assays in spatacsin-silenced neurons highlighted an overall reduction in synaptic vesicles and anterograde vesicle trafficking indicative of impaired axonal transport.

The present study provides the first evidence that human SPG11 mutations and loss of function of spatacsin share neurite pathologies and show that SPG11 is implicated in axonal maintenance and cargo trafficking. Understanding the cellular functions of spatacsin will allow deciphering mechanisms of motor cortex dysfunction in autosomal recessive hereditary spastic paraplegia. (Perez-Branguli, Mishra et al., HMG 2014).

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Invited lectures

Seminar Series, Institute of Physiology, 21.01.2014, Uniklinik RHTW Aachen, Modeling motor neuron disease using human iPSC derived neurons (Beate Winner)

Keystone symposia, Adult Neurogenesis, 15.05.2014, Stockholm, Adult Neurogenesis in Parkinson's Disease (Beate Winner)

Awards

Neurowind e.V. Travelgrant for German Stem Cell Network Conference, Nov. 4th 2014, Bonn, Annika Sommer, PhD student

Travel grant for Early Career Researchers in Overseas for 37th Annual Meeting of the Molecular Biology Society of Japan, November 25 - 27, 2014, RIKEN Center for Integrative Medical Sciences, Yokohama City (Japan) to Himanshu Mishra, PhD student

Award "Best project proposal: The nature of stem cells in adult neurogenesis" at the 8th Route 28 Summit in Neurobiology, September 5-11, 2014 Frauenchiemsee, Germany, to Martin Regensburger, MD

Selected publications during funding period

Pérez-Brangulí F, Mishra HK, Prots I, Havlicek S, Kohl Z, Saul D, Rummel C, Dorca-Arevalo J, Regensburger M, Graef D, Sock E, Blasi J, Groemer TW, Schlötzer-Schrehardt U, Winkler J, Winner B. Dysfunction of spatacsin leads to axonal pathology in SPG11 linked hereditary spastic paraplegia. HMG, 2014 23(18):4859-74

Havlicek S, Kohl Z, Mishra HK, Prots I, Eberhardt E, Denguir N, Wend H, Plötz S, Boyer S, Marchetto MCN, Aigner S, Sticht H, Groemer TW, Hehr U, Lampert A, Schlötzer-Schrehardt U, Winkler J, Gage FH, Winner B. Gene dosage dependent rescue of HSP neurite defects in SPG4 patients' neurons. HMG, 2014; 23(10):2527-41

Purohit P*, Perez-Branguli F*, Prots I*, Borger E, Gunn-Moore F, Welzel O, Loy K, Wenzel EM, Grömer TW, Brachs S, Holzer M, Buslei R, Fritsch K, Regensburger M, Böhm KJ, Winner B, Mielenz D. The Ca2+ sensor protein Swiprosin-1/EFhd2 is present in neurites and involved in kinesin-mediated transport in neurons. Plos One, 2014; 9(8):e103976. *contributed equally

Ettle B, Reiprich S, Deusser J, Schlachetzki JC, Xiang W, Prots I, Masliah E; Winner B, Wegner M, Winkler J. Intracellular alpha-synuclein affects early maturation of primary oligodendrocyte progenitor cells. Molecular and Cellular Neuroscience. 2014;62:68-78

May VE, Ettle B, Poehler AM, Nuber S, Ubhi K, Rockenstein E, Winner B, Wegner M, Masliah E, Winkler J. Alpha-synuclein impairs oligodendrocyte progenitor maturation in multiple system atrophy. Neurobiology of Aging, 2014;35(10):2357-68

Rockenstein E, Nuber S, Overk CR, Ubhi K, Mante M, Patrick C, Adame A, Trejo-Morales M, Riek R, Winklder J, Gage FH, Winner B, Masliah E. Synaptic accumulation of oligomer prone a-synuclein exacerbates synatpci degeneration and neuronal loss in a transgenic mouse model. Brain, 2014;137(5):1496-513

Winner B, Marchetto MC, Winkler J, Gage FH. Human-induced pluripotent stem cells pave the road for a better understanding of motor neuron disease. HMG, 2014; 23(R1):R27-34