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Novel Modifiers for Inherited Neurodegenerative Disorders - Spinal Muscular Atrophy and Ataxia –

Neurodegnerative disorders, including autosomal recessive spinal muscular atrophy (SMA), amyotrophic lateral sclerosis, Parkinson disease, hereditary spastic paraplegias and ataxias, have been shown to share common pathomechanisms, such as endocytic defects and Ca^{2+} dysregulation. This fact highlights the potential of cross-disease modifiers, which specifically modulate these common pathways; an example of this phenomena is plastin 3 (PLS3) overexpression, which modulates endocytosis. PLS3 overexpression has been identified as a genetic modifier in humans who remained unaffected, despite carrying the genetic predisposition for SMA, a detrimental neurodegenerative disease and the leading genetic cause of infant lethality. SMA is caused by homozygous deletion or mutation of the *survival motor neuron 1* (*SMN1*) gene, whereas SMA disease severity is mainly determined by the number of *SMN2* copies, a nearly identical copy gene of *SMN1*, which mainly produces aberrantly spliced transcripts. Recently, the first SMA therapy has been approved that is based on antisense oligonucleotides (ASO) correcting *SMN2* splicing, namely Spinraza. However, for severely affected SMA1 individuals - representing 60% of SMA patients - the elevated SMN levels may still be insufficient. Thereby, SMN-independent protective modifiers, such as PLS3 overexpression and neurocalcin delta (NCALD) reduction, represent an attractive therapeutic target for a combinatorial approach. However, the exact underlying mechanism of this protection is largely unknown. In order to approach this question and to identify novel modifiers, we focused on PLS3 interacting partners. In previous work, we identified calcineurin-like EF-hand protein 1 (CHP1) as a novel direct PLS3 interacting partner. Remarkably, loss-of function of CHP1 causes autosomal recessive cerebellar ataxia. In this context, the aims of this work are the following: 1) analysis of CHP1 reduction as a novel modifier of SMA, 2) the identification of common interacting partners of PLS3 and SMN, and 3) analysis of PLS3 overexpression as a cross-disease modifier for ataxia caused by *Chp1* mutation in mice.

In the first part of this work, we show that the interaction between PLS3 and CHP1 is Ca^{2+} independent. Although CHP1 is ubiquitously present, it is particularly abundant in the central nervous system and at SMA relevant sites, including motor neuron (MN) growth cones and neuromuscular junctions (NMJs). Hereby, CHP1 is dynamically increasing during neuronal development. Strikingly, we found elevated CHP1 levels in spinal cord and brain but not in *transversus abdominis* muscle of SMA mice. Remarkably, CHP1 downregulation restored impaired axonal growth in *Smn*-depleted NSC34 MN-like cells. Congruently, CHP1 overexpression inhibited neurite outgrowth, implying that CHP1 is a negative regulator of neurite growth. However, CHP1 reduction, by genetically mutant *Chp1* allele, the *vacillator* mutation, did not improve the SMA phenotype of severely-affected SMA mice. Therefore, we aimed to test the effect of CHP1 reduction on an intermediate SMA mouse model. Importantly, subcutaneous injection of low-dose SMN-ASO in presymptomatic neonatal mice doubled the mean survival span of severely-affected SMA mice, while additional CHP1 reduction by heterozygous *Chp1* mutation, further prolonged the mean survival by 1.6-fold. Strikingly, CHP1 reduction ameliorated well-described hallmarks of SMA pathology, including electrophysiological defects, reduced motor axon length, reduced number of proprioceptive synapses per MN soma, smaller NMJ size and impaired maturity, as well as smaller muscle fibre size, compared to low-dose SMN-ASO alone. Mechanistically, we found that *Chp1* knockdown in MN-like cells tripled macropinocytosis, whereas clathrin-mediated endocytosis (CME) remained unaffected. Thereby, *Chp1* knockdown rescued the impaired macropinocytosis in *Smn*-depleted cells. Furthermore, upon *Chp1* knockdown we found a significant

increase of calcineurin activity. Calcineurin is a Ca^{2+}-calmodulin-dependent phosphatase which collectively dephosphorylates proteins involved in endocytosis, whereas calcineurin activity was reduced in *Smn*-depleted cells. In line with this, we showed that dynamin 1 is hyperphosphorylated in SMA MNs, which was restored to control level by the heterozygous *Chp1* mutant allele. Taken together, in this work we conclusively show that CHP1 reduction is a novel SMA modifier, which ameliorates hallmarks of SMA pathology by elevating calcineurin phosphatase activity, thereby improving impaired endocytosis and eventually neurotransmission. Most importantly, we demonstrate that CHP1 reduction is an attractive SMN-independent therapeutic target for a combinatorial therapy.

In the second part of this work, we aimed to investigate in a collaborative work with A. Hart (Brown University, Boston, USA) the functional connection between SMN and PLS3. For this purpose, we investigated putative common interacting partners of SMN and PLS3. Thereby, we identified heterogeneous nuclear ribonuclear protein F (hnRNP F) and H as shared interacting partners by co-immunoprecipitations and co-localisation analysis. Interestingly, in this context, the Hart laboratory showed that the knockdown of *sym-2*, the ortholog to hnRNP F/H, suppresses endocytic defects in a *C. elegans* SMA model and rescues its neuromuscular defects. These findings contribute to a better understanding of the SMN-PLS3 complex and indicate that SMN, PLS3 and hnRNP F/H functionally share the endocytic pathway.

In the last part of this thesis we investigated whether transgenic *PLS3* overexpression is a cross-disease modifier for autosomal recessive ataxia, caused by loss of CHP1 in the *vacillator* (*vac*) mouse model. Remarkably, we observed that PLS3 overexpression delays the ataxic gait progression at early disease stages but does not rescue it. In line with this, although Purkinje cell axons were still absent, PLS3 overexpression significantly increased the Purkinje cell number in the cerebellum of $Chp1^{vac/vac}$; $PLS3^{tg/tg}$ mice compared to $Chp1^{vac/vac}$ mice, indicating that PLS3 overexpression delays Purkinje cell death in *vacillator* mice and thereby eventually ameliorates the motor performance at early disease stages. First results indicate that PLS3 overexpression most likely improves the previously reported impaired NHE1 membrane targeting in the *vacillator* mouse model, which may ameliorate the pH homeostasis, and eventually delays Purkinje cell degeneration. However, further studies are crucial to fully understand the underlying mechanism.