Zebrafish disease models for hnRNP A mutations in ALS

Acronym :
hnRNP A proteins in ALS

Principal Investigator:
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Summary of the research project

Amyotrophic Lateral Sclerosis (ALS) is a devastating disease, and currently the development of therapies is limited by the poor understanding of the disease-causing molecular mechanisms. A pathological hallmark in ALS is the depletion of heterogeneous nuclear ribonucleoproteins (hnRNP) such as TDP-43 or FUS from the nucleus and their aggregation in the cytoplasm. Just recently, the same protein mislocalisation was described in ALS and another ALS-type myopathy (IBMPFD/ALS) for mutant hnRNP A1 and hnRNP A2B1 (1).

The mislocalised hnRNP proteins imply two distinct pathomechanisms (Figure 1), which are currently under debate:

1) in disease state, the physiological function of hnRNP proteins could be lost or reduced, impairing various cellular processes essential for cell survival such as transcription, splicing, transport, and translational regulation.

2) the cytoplasmic aggregates of hnRNP proteins could convey toxicity, e.g. by sequestering essential RNAs and proteins to inclusions.

Each pathomechanism by itself or in combination could be responsible for the neurodegeneration seen in ALS patients.

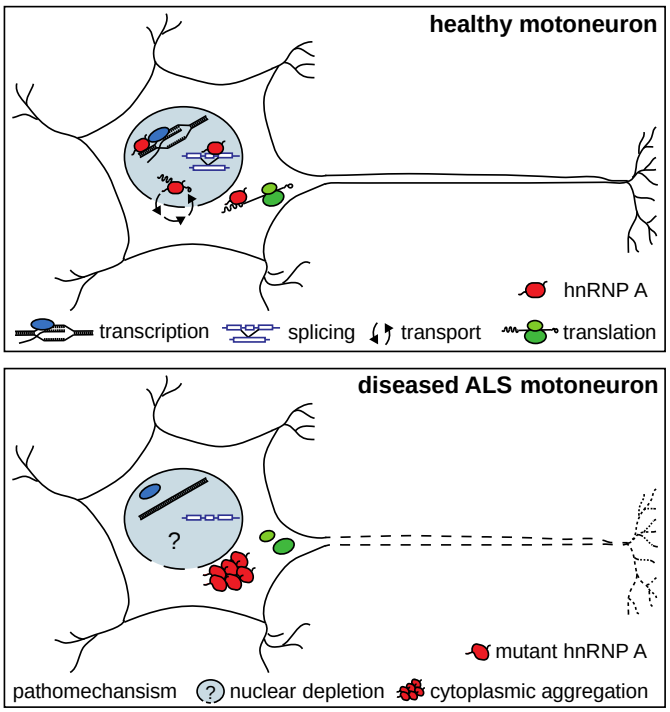

Figure 1 Pathomechanisms in ALS

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Patients carrying the recently identified mutations in hnRNP A1 and hnRNP A2B1 develop ALS with the typical pathological hallmarks of nuclear depletion and cytoplasmic aggregation (1). We therefore chose the two hnRNP A genes to model both pathomechanisms in zebrafish *in vivo*. We include hnRNP A3 for disease modelling in our study, since it binds to transcripts of the hexanucleotide repeat expansion of C9orf72, the most frequent cause of ALS (2). Moreover, it is detected in cytoplasmic aggregates while it is depleted from nuclei in patients with a C9orf72 hexanucleotide repeat expansion (2).

We will generate suitable disease models by genetically manipulating the hnRNP A gene family in zebrafish to identify the pathomechanism of the ALS-associated mutations in hnRNP A1 and hnRNP A2B1

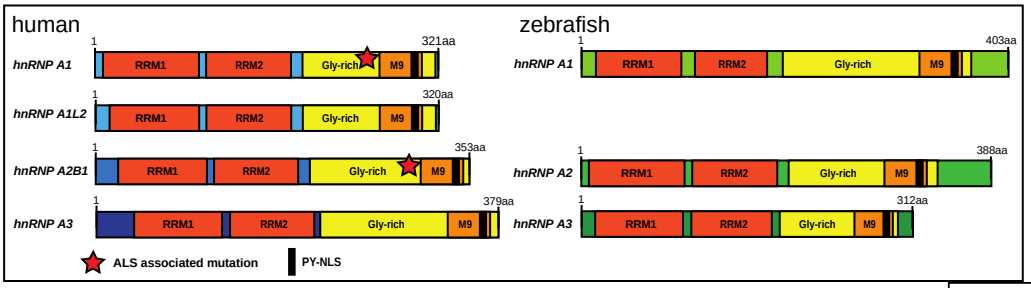

Figure 2 ***hnRNP A1*, *hnRNP A2B1* and *hnRNP A3* genes are conserved**

HnRNP A proteins are highly conserved across species (Figures 2 and 3) allowing a relevant analysis for clinical research

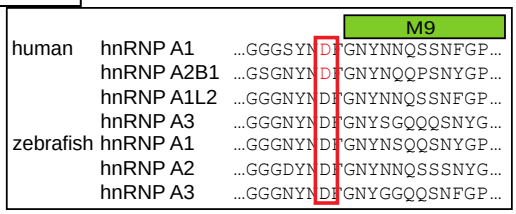

Figure 3 **Conserved Aspartate (D) adjacent M9**

Our work will describe the consequences of the ALS-causing mutations in hnRNP A1 and hnRNP A2B1 to unravel the pathomechanism on a molecular level. Once we understand the pathogenesis of ALS, we can design better potential therapeutic strategies. Furthermore, having a genetic zebrafish disease model for ALS opens the possibility to perform large unbiased chemical screens to identify therapeutic compounds. Compounds that rescue or improve the mutant phenotype hold the potential to serve as an ALS therapy.

Relevant research articles for this project are :

1. Kim HJ, Kim NC, Wang YD, Scarborough EA, Moore J, Diaz Z, et al. Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. Nature. 2013;495(7442):467-73.
2. Mori K, Lammich S, Mackenzie IR, Forne I, Zilow S, Kretzschmar H, et al. hnRNP A3 binds to GGGGCC repeats and is a constituent of p62-positive/TDP43-negative inclusions in the hippocampus of patients with C9orf72 mutations. Acta neuropathologica. 2013;125(3):413-23.
3. Hruscha A, Krawitz P, Rechenberg A, Heinrich V, Hecht J, Haass C, et al. Efficient CRISPR/Cas9 genome editing with low off-target effects in zebrafish. Development. 2013;140(24):4982-7.
4. Schmid B, Hruscha A, Hogl S, Banzhaf-Strathmann J, Strecker K, van der Zee J, et al. Loss of ALS-associated TDP-43 in zebrafish causes muscle degeneration, vascular dysfunction, and reduced motor neuron axon outgrowth. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(13):4986-91.

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Her 5 most relevant publications:

Hruscha A, Krawitz P, Rechenberg A, Heinrich V, Hecht J, Haass C, and Schmid B. Efficient CRISPR/Cas9 genome editing with low off target effects in zebrafish, Development. (2013) 140, 4982-4987

Schmid B, et al. Loss of ALS-associated TDP-43 in zebrafish causes muscle degeneration, vascular dysfunction, and reduced motor neuron axon outgrowth. PNAS, 2013 Mar 26;110(13):4986-91.

van Bebber F, Hruscha A, Willem M, Schmid B*, and Haass C*. Loss of Bace2 in zebrafish affects melanocyte migration and is distinct from Bace1 knock out phenotypes. J Neurochem. 2013 Feb 13;127(4):471-81. *equal senior authors

Plucińska G, Paquet D, Hruscha A, Godinho L, Haass C, Schmid B*, Misgeld T*. *In vivo* imaging of disease-related mitochondrial dynamics in a vertebrate model system. J Neurosci. 2012 Nov 14;32(46):16203-12. *equal senior authors

Paquet D, Bhat R, Sydow A, Mandelkow EM, Berg S, Hellberg S, Fälting J, Distel M, Köster RW, Schmid B, Haass C. A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation. J Clin Invest. 2009 May 11;9(5):1382-95.