Proteins binding RNA transcripts from C9orf72 gene mutation

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Mutation in C9orf72 gene is the most common genetic cause of two fast progressing and incurable neurodegenerative disorders - amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The disorders are very close on genetic and neuropathological spectrum, while symptomatically different. In ALS motor neurons are affected, which leads to muscle atrophy, while FTD is presented by degeneration of frontal and temporal cortex. C9orf72 mutation is presented as increased number of hexanucleotide repeats of G4C2 sequence in gene non-coding region. Healthy individuals have up to 23 repeats, while in patients several hundred or several thousand repeats are present. Three mechanisms of action are proposed for the mutation. First, presence of extended repeats can cause haploinsufficiency of C9orf72 protein. Second, hexanucleotide repeats are transcribed to RNA in sense (G4C2) and antisense (C4G2) direction, both transcripts form mostly nuclear RNA foci in spinal cord and brain neurons of C9 ALS/FTD patients and are proposed to interact with RNA binding proteins important for normal cell functioning. Third, non-canonical translation of RNA repeats produces proteins with dipeptide repeats (DPRs), which are also to be toxic for the cell.
The aim of our research is to identify the proteins binding to sense and antisense RNA transcripts from the C9orf72 gene mutation and define their role in the development and progression of ALS and FTD. For identification of the proteins, we have set up RNA pull-down assay using long, biologically relevant RNA constructs in combination with mass spectrometry. For identification of interaction protein-RNA proximity ligation assay was set up. We have found proteins involved in various processes important for cell survival and normal function to be interacting with sense and antisense RNA repeats. We will present our latest findings on these interactions and their importance for disease development and progression.