‘Translation-related pathway and activated retrotransposons underlying the neurodegenerative disease ALS related to mutations in the *FUS* gene’

The goal of the project is to elucidate the molecular pathomechanism of amyotrophic lateral sclerosis (ALS) related to mutations in the *FUS* gene. ALS is a fatal neurodegenerative disease accompanied by progressive paralysis due to the degeneration of motor neurons in the motor cortex, brainstem, and spinal cord. Mutations in the *FUS* gene were detected in ~1% of ALS, leading to mislocalization of the FUS protein in cytoplasmic aggregates and abrogating its functions in the nucleus.

Previously, we have observed disturbances in human model cell lines with FUS impairment, with deletion of the *FUS* gene, or with the introduced point mutation related to ALS. The changes refer to two molecular pathways: i) increased levels of small nucleolar RNAs (snoRNAs) that corresponded to increased methylation and pseudouridylation of ribosomal RNAs at appropriate positions. Such changes can influence ribosome heterogeneity and may represent a translation (protein synthesis)-related mechanism that underlies disease progression (Gawade et al., 2023, Sci Rep). ii) Increased level of retrotransposons (mobile genetic elements) including LINE-1, and decreased level of PIWIL1 protein which in turn might result in abnormally processed class of small RNAs called piRNA (PIWI-interacting RNAs). Both PIWIL1 and piRNAs are involved in the regulation of retrotransposon gene expression. Retrotransposons are a source of DNA damage in neurodegeneration and can activate neuroinflammation.

In this project, we will use induced pluripotent stem cells (iPSCs) reprogrammed from fibroblasts derived from ALS patients with the FUS P525L mutation and their isogenic controls (cells in which the mutated gene was corrected to the wild type gene). Such cells can be further differentiated to various types of neural cells: neural progenitor cells (NPSs), motor neurons (MNs), astrocytes, and glial cells. Separated somas (cell bodies) and MN axons will be used as well. They will be an elegant model reflecting the ALS disease phenotype that is mostly observed in neural cells. Additionally, all types of cells will derive from the same patient with ALS and thus represent the same genetic background. Using these cells, we are going to confirm all results obtained in previously used model cell lines, HEK293T and SH-SY5Y.

In this project, experiments are planned to answer the following scientific questions: How do the methylation and pseudouridylation profiles of ribosomal RNA change in ALS-FUS neural cells compared to control cells? Does it influence on ribosome heterogeneity and affect translation efficiency or fidelity? Can ALS-FUS mutations activate retrotransposons that, in turn, stimulate neuroinflammation? Whether the regulation of PIWIL1 by FUS is affected in ALS, and what are the consequences of that? Is FUS involved in the processing of piRNAs along with PIWI proteins, and can ALS-related FUS mutations result in abnormal processing of piRNAs?

There is evidence on the importance of translation for the onset and progression of ALS disease. Furthermore, we suggest that there is a link between FUS, PIWIL1, retrotransposon activation, and neuroinflammation in ALS. I am confident that answering the questions presented in this project will contribute to understanding FUS-related neurodegeneration in ALS and can result in more precise and adequate treatment.