Activation of retrotransposons underlying neurodegenerative disease ALS related to mutations in FUS protein

The aim of the project is to better understand the neurodegeneration resulting from the activation of retrotransposons due to mutations in *FUS* gene related to amyotrophic lateral sclerosis (ALS). FUS is primarily a nuclear DNA and RNA binding protein, involved in various cellular processes related to the regulation and metabolism of nucleic acids. Mutations in FUS have been correlated with the development of ALS and cause mislocalization of FUS into the cytoplasm, creating toxic aggregates in motor neurons and glial cells of patients. ALS is a degenerative disease of the nervous system that gradually causes the loss of motor neurons, resulting in difficulty in muscle movement.

The activity of transposable elements, which are a type of mobile genomic elements, has been linked to neurological diseases and neuroinflammation, which is something this project would also like to address. One group of transposable elements, called LINE-1 retrotransposons, are able to actively insert themselves into various locations of the genome, posing a threat to its integrity. However, molecules called piRNAs (PIWI-interacting RNAs) in association with PIWI proteins suppress retrotransposons activity which help maintain genomic integrity.

Interestingly, our preliminary results revealed that the expression of PIWIL1, a member of the PIWI protein family, is reduced in cells where the *FUS* gene has been knocked out or there is an ALS-linked mutation, compared to wild type cells. Moreover, I observed increase in the expression of LINE-1 retrotransposon transcripts in these cells as well. This increase of LINE-1 has been observed in studies related to neurodegeneration. Moreover, research revealed variations in the expression of piRNAs in samples from deceased Parkinson's disease and Alzheimer's disease patients. Previous research in fruit flies (Drosophila) has suggested a connection between abnormal expression of the PIWI homologue and FUS-related ALS pathogenesis. Moreover, the FUS protein homologue in Drosophila was shown to be involved in piRNA biogenesis. However, there is currently no evidence linking FUS, PIWIL1, and retrotransposon activation in human cells.

Collected information prompted us to ask the following questions: Can ALS-related FUS mutations lead to the activation of LINE-1 retrotransposons? Whether PIWIL1 regulation by FUS is affected in ALS, and what are the consequences of that? Whether FUS is involved in the processing of piRNAs along with PIWI proteins and whether ALS-related FUS mutations lead to abnormal processing of piRNAs? Does neuroinflammation occur through stimulation of innate immune receptors by increased expression of retrotransposons? Experiments in this project will be performed on induced-pluripotent stem cells (iPSCs) with ALS-linked FUS mutation reprogrammed from fibroblasts derived from ALS patient. iPSCs will be ultimately differentiated into motor neurons (MNs) and astrocyte cells, which are the main cell types affected by ALS neurodegeneration.

My plan is to address these questions through microscope immunofluorescence analyses, in which I want to check whether FUS, PIWIL1, and proteins encoded in the LINE-1 retrotransposon colocalize in cytoplasmic aggregates in FUS-ALS MNs and astrocytes. Moreover, through high-throughput sequencing of RNAs isolated from MNs and astrocytes, I want to discover unusual changes in the processing and expression of piRNAs, inflammatory genes, and transposable elements. I am also interested in checking the level of PIWIL1 and LINE-1 and testing whether PIWIL1 directly affect LINE-1 transposon expression. I will do it by approaches with overexpression and silencing of FUS and PIWIL1. I will also check the effect of silencing of LINE-1 on inflammation and innate immunity-related gene expression.

The proposed study could unravel the mechanism of neurodegeneration involving FUS, PIWIL1, piRNAs, and retrotransposons. Answering the questions put forward in this project will contribute to understanding the role of FUS in the regulation of retrotransposon activity through PIWIL1 and will elucidate the influence of transposon activation on ALS-related neurodegeneration. Linking retrotransposon activation and neuroinflammation will significantly contribute to this field and help create potential therapies in the future. Activated retrotransposons may be involved in stimulating inflammation-associated genes through their sensing by innate immune receptors. To summarize, this project has the potential to find a possible link between activated transposable elements and neurodegeneration in FUS-related ALS.