Internship Proposal

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Project Title:
Neurodegenerative Disease Spinocerebellar Ataxia Type 37: Focus on Pathogenic Mechanisms and Treatment

Level:

Master Student

Project Summary:

Spinocerebellar ataxias (SCAs) are hereditary neurodegenerative diseases, characterized by cerebellar neurons loss leading to motor incoordination (speech difficulties, limb and gait ataxia). Even though several mutations causing SCAs have been reported so far, there are many SCAs-affected families for which no mutation has been discovered. In 2017, my group discovered an ATTTC repeat in the gene DAB1 as the cause of SCA37 (Seixas et al., 2017). This mutation is located in 5' UTR introns of DAB1 gene, which regulates neuronal migration and positioning during brain development. After our discovery, similar ATTTC repeat insertions have been reported in seven types of familial adult myoclonic epilepsy (FAME). As human brain tissue is very scarce, to determine why cerebellar neurons dye in SCA37, we have developed both cellular and animal models. In human transfected cells, the ATTTC repeat is transcribed and the AUUUC repeat RNA forms nuclear RNA aggregates. In other diseases, expanded repeat RNAs can interact and sequester other proteins in the cell, inhibiting the activity of these proteins, which increases the cellular toxicity. In zebrafish, the microinjection of the pathogenic AUUUC repeat RNA increases the lethality and the number of developmental malformations in the embryos. Considering these malformations and the developmental function of DAB1, we have investigated how early in life this pathogenic RNA causes neuronal damage by generating two zebrafish models.

Work to be developed by the student:

The overexpression of the pathogenic AUUUC repeat RNA interferes with neuronal axon outgrowth, migration and synaptic connections. In ATTTC repeat transgenic zebrafish animals, we will compare the number of Purkinje neuronal cells between ATTTC repeat expressing transgenic animals and controls. To address this, we will dissect adult animal

brains and perform brain sections; immunofluorescence with anti-zebrin II cerebellar marker and confocal microscopy will be carried out in these brain slices. In SCA37 post-mortem brains from affected individuals, cerebellar loss of Purkinje cells was accompanied by abundant astrogliosis. Therefore, we will also carry out immunofluorescence with anti-GFAP astrocyte marker and confocal microscopy to compare whether an increase of astrocytes is detected in the cerebellum of ATTTC repeat transgenic animals compared with controls. Regarding the SCA37 disease mechanism, we hypothesize that cerebellar Purkinje cell loss is triggered by the formation of abnormal RNA aggregates and sequestration of RNA-binding proteins by the AUUUC repeat RNA. To investigate this, we will perform RNA fluorescent in situ hybridization (FISH) with a probe against the AUUUC repeat RNA in brain slices of ATTTC repeat transgenic animals and controls. In summary, this project focuses on investigating neuronal loss in a SCA37 transgenic zebrafish model, identifying the abnormal RNA aggregates as drivers of neuronal death in the brain.

References:

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