Abstract

“CHARACTERISTICS OF THE SCA3/MJD KI91 AND KI150 MOUSE MODELS: TISSUE PATHOGENESIS, MUTANT ATAXIN-3 INTERACTION AND BRAIN TRANSCRIPTOMIC ALTERATIONS”

Spinocerebellar ataxia type 3 (SCA3/MJD) is a neurodegenerative polyglutamine disease caused by the CAG triplet repeat expansion mutation in the ATXN3 gene. This results in forming of a long polyglutamine domain in the mutant ataxin-3 protein (ATXN3). The exact mechanism of SCA3 has not been determined, and therefore no targeted therapies currently exist.

Therefore, the main goal of my Ph.D. thesis was to determine the pathomechanism of SCA3 by generating a new mouse model of SCA3 with an exacerbated phenotype as an *in vivo* platform for studying pathogenesis, determining protein and tissue changes, protein-protein interactions, and rapid evaluation of potential SCA3 therapy. In the SCA3 mouse model with a mild phenotype, I did not observe early presymptomatic transcriptional changes but identified post-symptomatic mRNA changes. Such changes reflected a disturbance in brain cell populations associated with oligodendrocytes and microglia and energy metabolism. Despite the lack of transcriptional and behavioral changes at the very early stage of the disease in the mild SCA3 model, I identified altered localization and microaggregates of the mutant ATXN3 protein, useful as a marker in preclinical studies.

The next task of the research was to generate a new SCA3 Ki150 mouse model with an exacerbated phenotype, a preclinical-class model for testing effective therapies for the SCA3. Motor tests performed on the Ki150 indicate an exacerbated disease, such as reduced motor performance already at 1 month of age, and numerous and large aggregates of abnormal ATXN3 protein seen across the brain.

Next, protein interactions of normal and mutant ATXN3 were identified in Ki21 and Ki150 models. Parallel methods of brain lysate fractionation using orthogonal chromatography and co-immunoprecipitation were used, and then proteins and complexes were identified by proteomics for each method. The mutant and normal ATXN3 complexes were characterized by their size and content. Among others, large protein complexes with disturbed interaction with ATXN3 were detected, such as CCT5 and 6, Tcp1 (Chaperon Containing TCP1; CCT

complex; T complex), and Camk2a and Camk2b kinases responsible for calcium homeostasis and numerous proteasome proteins. I found that all these proteins have a characteristic circular structural form. Interestingly, one of the transient stages in the formation of polyQ fibrillar inclusions are the circular inclusions. Data analysis indicates that the mutated ATXN3 interacts abnormally with proteins of crucial cellular pathways involved in the pathogenesis of neurodegenerative diseases, including proteins involved in the translation, and transport of mitochondria in axons. Based on the identified interactors and their binding to ATXN3, a method of SCA3 targeted therapy with low molecular weight compounds was proposed.

In conclusion, the creation of a new model with an exacerbated phenotype and the study of protein interactions in this model allowed the identification of pathogenic biological processes in SCA3. The therapeutic strategy aimed at one of these processes resulted in a significant reduction in the level of the mutated ATXN3 protein in the fibroblast cells of patients with SCA3.