ABSTRACT

Neurodegenerative diseases such as Alzheimer, Parkinson, and polyglutamine diseases are a serious and growing burden for modern societies. The diseases are characterized by severe motor and/or cognitive impairment resulting from progressive damage to specific populations of neurons and nerve connections in the brain, ultimately resulting in disability and loss of patients' independence. Moreover, neurodegenerative diseases are becoming more common since life expectancy is increasing. Although neurodegenerative diseases share some common features, their exact pathomechanism is still unknown, and therefore no effective therapy exists. Unlike Alzheimer disease and other neurodegenerative diseases with an insufficiently defined etiology, polyglutamine diseases (polyQ) result from a dominant mutation - the expansion of CAG repeats in single genes. The group of polyQ diseases also include spinocerebellar ataxia type 3 (SCA3), caused by the expansion of CAG repeats in exon 10 of the *ATXN3* gene encoding the ataxin-3 protein.

The main goal of the research conducted as a part of my Ph.D. thesis was discovering the pathomechanism, that leads to the development of spinocerebellar ataxia type 3. Next to the primary goal of my project, I also defined specific aims. The first specific aim was to characterize the phenotype of the SCA3 Ki91 homozygous knock-in model and distinguish the pre-symptomatic and symptomatic stages of SCA3 using behavioral and cellular tests. The second important aim of my research was to identify the molecular processes that give rise to neurodegenerative disease (pre-symptomatic phase), and then the key processes for SCA3 pathogenesis during disease progression in the Ki91 model. I have achieved this goal by performing large-scale analysis of changes in the protein levels in 2 brain regions: the cerebral cortex and the cerebellum of the Ki91 model in distinct SCA3 stages. A further aim was selecting proteins that could serve as SCA3 biomarkers and a detailed analysis of molecular processes that could be altered in the SCA3 Ki91 model using functional tests and microscopy. In my Ph.D. research, I have used the Ki91 mouse knock-in model with a human sequence of exons 7-11, containing 91 CAG repeats in the mouse *Atxn3* gene. This model was designed at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences (Switonski et al., 2015). Behavioral tests were performed on mice from 2-month-old till 18- months of age in the cohort generated by me (n = 18 per genotype). I have analyzed the differences in the protein levels with the high-throughput LC-MS / MS mass spectrometry in the age of mice, which I have selected as the most crucial time points for SCA3 development based on behavioral results. Next, in order to reveal the cellular processes and molecular pathways, which might contribute to the SCA3 pathogenesis, I have performed an analysis of dysregulated proteins using bioinformatic tools.

After analyzing the results, I have distinguished 4 phases of the disease, including the pre-symptomatic phase, in which I did not observe any motor or other symptoms that were described in the publication (Wiatr et al., 2019). Definition of the pre-symptomatic stage in the disease model is essential because it enables the precise distinction between processes that cause neurodegeneration from those that are a result of disease progression or natural aging. I have found that altered proteins in the pre-symptomatic phase of SCA3 were involved in translation, DNA damage repair, neurogenesis, and neural structure formation, as well as energy metabolism and cellular transport. In addition, I have also analyzed the level of protein phosphorylation and discovered a global reduction in phosphorylation in the cerebral cortex and cerebellum of Ki91, and altered levels and phosphorylation of several kinases, such as Pak1. A large group of proteins with reduced phosphorylation were important regulators of cytoskeleton function and axonal transport. The second publication gathered the results that were a continuation of my research in the subsequent stages of the disease, ranging from weight loss in young animals to severe motor impairment in 18-month-old mice (Wiatr et al., 2021). Proteins with altered levels in the older animals are involved in processes such as energy metabolism, cytoskeleton formation, transport along the axon, synaptic transmission, degradation in the proteasome, and apoptosis. In the later phases, the number of proteins that localized in lysosomes, synaptic vesicles, and synapses, and which are involved

in metabolism, increases. Based on the results obtained in the analysis of altered proteins and molecular processes, I have developed a model of SCA3 pathogenesis consisting of disturbances in the transport of synaptic vesicles, mitochondria, and autophagosomes along the SCA3 axon. Therefore, in the next stage, I have performed experiments to demonstrate that early pathogenic SCA3 processes occur in axons. I have shown a deficit in the energy metabolism of Ki91 cerebellar neurons in a functional test in which the rate of cell acidification (glycolysis rate) and oxygen consumption were measured. I have demonstrated the accumulation of neurofilaments and vesicles (labeled with Rab7) in the bodies and axons of the Ki91 cerebellar neurons. Then, I obtained an *in vitro* culture of cortical and cerebellar neurons, from which I have isolated the axons and the somatodendritic parts separately. For this purpose, I have used modified Boyden chambers, which separate axons from the bodies of neurons through a porous filter. Next, I have analyzed which proteins localize in altered amounts in axons in comparison to the somatodendritic part of neurons derived from the SCA3 Ki91 model and compared to the control (C57). The analysis of axonal proteins that demonstrated altered levels allowed for initial assessment of whether the disturbance of axonal transport occurs in the SCA3 Ki91 model and which organelles or proteins it concerns. Proteins enriched in SCA3 axons are primarily proteins of the translational machinery, while proteins depleted in SCA3 axons include cytoskeletal proteins that regulate the transport and build vesicles.

Overall, I have identified many changes at the protein level in the SCA3 model that indicate disruption of molecular processes and pathways such as energy metabolism and axonal transport. The results of my Ph.D. thesis set new directions of research that will bring us closer to understanding the pathomechanism of SCA3 underlying neurodegeneration.