Identification of cellular and molecular abnormalities of early brain development in Huntington's disease

Mgr inż. Karolina Świtońska-Kurkowska

ABSTRACT

Neurodegenerative diseases are the consequence of dysfunction of various populations of neurons and neural connections in the brain, leading to progressive motor and mental disorders. Long-term progression, changes in brain size and impaired learning in children at risk of the disease, suggest that molecular pathogenesis can be initiated even at the stage of embryonic development. A good model for studying the neurodevelopmental aspects of the pathogenesis of neurodegenerative diseases are polyglutamine diseases (polyQ). Their defined etiology is the expansion of CAG repeats in specific genes and the production of toxic proteins containing an excessively extended polyglutamine tract. One of these diseases is Huntington's disease (HD), caused by a mutation in the 1st exon of the *HTT* gene. A special form of HD is its juvenile form (JOHD), characterized by very long sequences of CAG repeats in the HTT gene. The number of 60 CAG repeats in the *HTT* gene may indicate the presence of neurodevelopmental changes in HD.

The primary goal of my research, as part of my doctoral thesis, was to identify molecular and cellular brain development disorders in JOHD. In order to achieve that, I formulated specific objectives. The first of them was the identification of disturbed neurodevelopmental processes in JOHD cells, even before the stage of differentiation and development, at the level of the iPSC lines. I pursued this goal with the use of high-throughput methods and bioinformatics analyses. In another important point of my work, I collected a number of available experimental data obtained in high-throughput studies of HD cells and other polyQ diseases. Using these data, I performed numerous *in silico* analyzes identifying potential (neuro)developmental molecular processes disrupted in HD, at multiple stages of development, and in the adult brain. In addition, *in silico* analyses aimed to identify the common neurodevelopmental components

of polyQ diseases. The next stage of my research involved the use of previously analyzed iPSC lines to generate fused brain organoids that reflected the early stage of brain development. The final goal of my PhD thesis, was to investigate whether selected disorders identified in fused brain organoids of HD can be observed in the biological material obtained from HD mouse model ($Hu^{128Q/21Q}$).

My analyzes of the RNA-seq results of the HD iPSC 71Q and 109Q cells showed that the altered genes and proteins in these cells potentially contribute to HD neuropathology. I identified genes related to the DNA damage response, involved in the p53 signaling pathway, regulating the acquisition of polarity by cells and regulating the TGFβ signaling cascade. Importantly, in the 71Q lines, I identified a number of genes encoding transcription factors and histones, the increased expression of which may lead to accelerated embryo development and earlier development of the nervous system. In the 109Q lines, I detected reduced expression of apoptosis-related genes that are direct interactors of TP53 in the cell. This may suggest that mHTT interacts with the TP53 protein and disrupts the expression and level of many other genes and apoptotic proteins. This leads to an excess of progenitors and potentially impairs differentiation into mature neurons. In the proteomics study, I confirmed the altered level of TP53 and ZFP30 proteins (Świtońska et al., 2019). Earlier studies of our group confirmed a decrease in the level of TP53 protein in 109Q iPSC cells (Szlachcic et al., 2015, 2017). The described deregulations indicate that the dysfunctions observed in adults with HD may be the result of early and cumulative abnormalities of embryonic development.

The results of subsequent comparative bioinformatics analyze highlighted the constant disruption of (neuro)developmental processes in JOHD cells, such as multi-stage embryo morphogenesis, neuronal growth and elongation, and synaptic transmission. My analyses have identified changes in genes involved in neuron formation, synaptogenesis and extracellular matrix formation in mouse models of some polyQ diseases. This indicates the neurodevelopmental similarities of this group of diseases (Świtońska-Kurkowska et al., 2021).

I examined the next embryonic stage of HD brain development at the level of early nerve cells. I created organoids of the cerebral cortex (*dorsal*) and striatum (*ventral*) and combined them into fused organoids containing the cell populations of the two brain regions most important in the neuropathology of HD. The fused organoids were significantly larger than the single *dorsal* and

ventral organoids, suggesting important interactions between the two brain regions, and allowing the study of cell populations previously unobserved in classical organoid systems. As in neurogenesis, the fused organoids contained markers for neural stem cells and mature neurons. I also detected changes in mRNA levels of markers of selected subpopulations of progenitors, inhibitory and excitatory neurons, and glial cells. The most interesting of the deregulated genes, with increased expression in all JOHD organoids, was *TTR*, a marker of the choroid plexus in the brain responsible for the production of cerebrospinal fluid. In the context of the neuropathogenesis of HD, this result was particularly important, because the ventricles of the brain are an area significantly enlarged in patients. Previously, the TTR protein had been identified in the cerebrospinal fluid of both symptomatic and presymptomatic HD patients. In my studies, increased levels of TTR protein were confirmed both in JOHD organoids and in the plasma of YAC^{128Q} mice. Therefore, my results indicate that both increased TTR gene mRNA expression in organoids and increased TTR protein levels may be biomarkers of JOHD. Moreover, I generated the so-called mosaic organoids, created by fusion of JOHD 71Q *dorsal* or *ventral* organoids with control 21Q *dorsal* or *ventral* organoids. I identified reduced levels of TTR protein in mosaic organoids compared to JOHD 71Q organoids. This indicates that this model can potentially be used to study the impact of increased amounts of non-mutated HTT protein in specific regions of the developing forebrain on the course of HD neuropathogenesis.

In summary, the changes I detected in the RNA-seq data point to specific (neuro)developmental processes already present in undifferentiated cells. I developed an organoid system to observe the early pathogenesis of HD. I identified molecular and cellular changes in the stages of pluripotency and HD brain development. I identified specific populations of nerve cells and their markers altered in HD. Among them, I defined the increased levels of TTR mRNA and protein as markers of the occurrence and severity of HD.