

Cellular processes require strict control of gene expression. One of the mechanisms taking part in this is epigenetic regulation. In our area of interest is regulation of neurogenesis processes by epigenetic mechanisms. Particularly interesting for us is poorly recognized Prdm3 protein (PR-Domain Zinc Finger Protein3). It is known that Prdm3 can play a functions as a methyltransferase. However, there are still many questions to be solved: how does this protein recognize DNA sequences, what type of sequences, what genes are affected during neurogenesis, does it work alone or in protein complex?

We plan to induce neuronal differentiation in P19 line cells (mouse embryonic stem cells) with retinoic acid as a model of neurogenesis *in vitro*. Our preliminary results indicate that Prdm3 can have a significant impact on the neural differentiation. In order to prove this, we will use the CRISPR-Cas9 method, which will allow us to knockout the Prdm3 gene expression. For this reason, we are also planning to evaluate the expression of a number of genes involved in neurogenesis. Results of other groups suggest that Prdm3 can influence on the expression of other genes by the formation of protein complexes. We will use liquid chromatography coupled to mass spectrometry (LC-MS/MS) to indicate the composition of protein complexes. The obtained results will be confirmed by co-immunoprecipitation (co-IP) and immunoblotting (Western Blot). To determine the significance of protein composition in the complex during neurogenesis we will use the RNA interference method (siRNA). It is known that Prdm3 protein has got high impact on chromatin condensation state. These mechanisms have a colossal importance in controlling the transcription factor access to DNA sequences. For this reason, we will examine the effect of these factors on chromatin structure state during neurogenesis using the NoME Seq method (Nucleosome Occupancy and Methylome Sequencing). This method will be used to evaluate the GpC and CpG methylation levels in the sequences of the selected genes, but also for the simultaneous nucleosomes positioning. Analysis will be carried out by sequencing. We are planning to evaluate sequences of previously recognized targets by Prdm3 proteins.

Our research touches the epigenetic mechanisms involved in controlling neurogenetic processes. We believe that acquired knowledge about these phenomena might be used to design novel tools for regenerative medicine.