Abstract

Mucopolysaccharidoses are a group of diseases that belong to lysosomal storage disorders. A common sign of these monogenic multisystem diseases is a gene mutation leading to a deficiency of the lysosomal enzyme participating in glycosaminoglycan degradation. It results to their accumulation in the tissues and organs, where they cause a progressive damage.

There is no efficient treatment available for most mucopolysaccharidoses. Moreover, the research is complicated because of the low prevalence and type of affected tissues. Animal models of these human diseases are used for an evaluation of newly developed therapeutic approaches. However, they also have many limitations due to the different pathogenesis and catabolic pathways of the accumulated substrates between humans and animals. Therefore, animal models are replaced by human cell models.

In this thesis, the development of four mucopolysaccharidoses human cell models is reported (MPS IIID, MPS IVA, MPS IVB, MPS VI). Corresponding genes (*GNS*, *GALNS*, *GLB1*, *ARSB*) were inactivated using CRISPR/Cas9 technology, where plasmids containing specific inserts are delivered to the target human induced pluripotent stem cells (iPSC), using electroporation.

Isolated clones, which represent iPSC disease models, were characterized by Sanger sequencing, enzyme assays and staining of some pluripotent markers.

Chondrogenic differentiation of characterized iPSC allows phenotype comparison of the mucopolysaccharidoses and detection of glycosaminoglycan accumulation in one of the cell types most affected by mucopolysaccharidoses.

Thus, prepared human cell models of mucopolysaccharidoses represent a promising tool for understanding the disease pathogenesis of specific tissue damage and the study of new therapeutic approaches.