Abstract

Human autopsy or biopsy tissue samples, mouse models and cell cultures of various types represent the most common materials in the investigation of cell pathogenesis of inherited diseases. This dissertation is devoted to all these approaches in the study of two X-linked lysosomal storage diseases, Fabry disease (FD,α-galactosidase A (AGAL) deficiency) and mucopolysaccharidosis type II (MPSII, idunorate-2-sulfatase (IDS) deficiency).

The primary goal of the work was analysis of lipid blood group B antigens with terminal α-galactose (B-GSL) in the pancreas of FD patients with blood group B (FD-B).,In addition to the main glycosphingolipid (GSL) substrate, globotriaosylceramide (Gb3Cer), B-GSLs represent another minor substrate of AGAL. The deposition of undegraded B-GSL has been demonstrated in FD-B pancreas where it was significantly higher than in other organs such as the kidneys and lungs which accumulate mainly Gb3Cer. High concentration of lipid and non-lipid B-antigens was primarily confirmed in exocrine acinar epithelial cells of FD-B, accompanied by massive accumulation of ceroid (secondary sign of lysosomal storage). Unlike acini, the endocrine portion of the pancreas remained unaffected by accumulation of AGAL substrates. This interesting phenomenon of cell biology shows how a specific blood group can influence organ manifestations in patients. Since the kidneys represent the most affected organ in FD, distribution of selected GSLs and their molecular types (isoforms) was examined in the tissue sections of FD mouse model in the next part of this work. Five increased isoforms of Gb3Cer was identified in renal cortex by mass spectrometry imaging with declining trend towards medulla. Increased positivity of Gb3Cer was mainly due to storage in cortical tubules. These results were consistent with both histochemical findings and tandem mass spectrometry but the MSI showed extra details of molecular composition of undegraded Gb3Cer (or other lipids).

The use of cell cultures derived from patients is the classic pathway for the study of disease phenotype. Human primary cultures, however, have limitations not only in material availability but also in difficulty to obtain cell types relevant to disease. Induced pluripotent stem cells (iPSC) remove a large part of these limitations. They represent a new direction for exploring cellular pathology and pathobiochemistry of inherited diseases during early development and a platform to test therapeutic interventions. Therefore, our important goal was to generate iPSC lines for selected lysosomal diseases that will serve as a basis for differentiation into selected cell types affected by storage. In this work, the iPSC lines of FD (FD-iPSC) and MPSII (MPSII-iPSC) were established.

Two states of iPSCs have been identified so far, varying mainly by X chromosome inactivation (XCI) in female cells, referred to as primed (cells after reprogramming with inactivation of one of the X chromosomes) and naïve (derived from the primed cells with both X-chromosomes active). Change of culture media of FD-iPSC and MPSII-iPSC leading to the transition of primed iPSC to their naïve state, led to the change in the morphology of the iPSC colonies in both diseases, but the change in the XCI ratio was recorded in the FD-iPSC clone, only. Since MPS II also affects the CNS, the generated MPSII-iPSC lines were subsequently differentiated into a mixture of neural cells. Increased expression of lysosomal markers and presence of abnormal structures without limiting membrane were largely proven in glial cells. Glycosaminoglycan (GAG) accumulation was confirmed in a mixture of terminally differentiated patient's neural cells. Addition of recombinant IDS to the culture medium had only a mild preventative effect. The persistence of GAG accumulation is probably due to their increased incidence on the plasma membrane, where they cannot be degraded by recombinant IDS fully activated only in acidic environment of lysosomes.

FD-iPSC lines were differentiated into spontaneously beating clusters of cardiomyocytes (CM). The reason of their preparation are frequent cardiomyopathies in FD patients. Increased concentration of Gb3Cer, less numerous and disorganized fibers were detected in FD-CM. Effective internalization of commercially available recombinant AGAL was confirmed by co-localization with lysosomal marker Cathepsin D. Functional CM will be further used for testing of therapeutic approaches, e.g., by pharmacological chaperones. We have further addressed the question of whether a structurally related enzyme α-N-acetylgalactosaminidase (NAGA) can participate on the degradation of AGAL substrates as suggested by earlier work on dermal fibroblasts in the FD. Our metabolic experiments with radiolabeled AGAL substrates in iPSC based lines with knocked-out genes for AGAL, NAGA or both enzymes, rather indicate the presence of another α-galactosidase involved in B-GSL degradation.