Personalized phenotype characterization of glycogen storage disorder type III patients primary skin fibroblasts to evaluate novel therapeutic agents.

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Abstract

Glycogen is a branched polysaccharide of glucose units stored as the main cell energy resource which its synthesis, gluconeogenesis and its degradation, glycogenolysis is catalyzed by an enzymatic system. Failure of any of these enzymes can lead to a group of diseases collectively known as glycogen storage diseases (GSDs). GSDs are normally associated with hereditary deficiencies in enzymes of glycogen and glucose metabolism causing glycogen accumulation. My Ph.D. Thesis focuses on GSD type III (GSDIII, Cori disease), which is caused by glycogen debranching enzyme (GDE) deficiency leading to accumulation of glycogen due to its reduced degradation. GSDIII usually starts as a liver disorder characterized by hepatomegaly, hypoglycemia, hyperlipidemia and hyperketonemia. These result from the limited ability to breakdown glycogen to glucose, leading to excessive use of lipid oxidation as an alternative energy source with ensuing attenuation of lipid uptake by adipocytes and increase in ketone bodies as byproducts of fatty acid oxidation (FAO).

The aim of my Ph.D. is to characterize the disease phenotype of primary fibroblasts from patient with GSDIII using image based high content analysis (HCA) and evaluate the effects of novel glycogen reducing compounds for GSDs on the GSDIII patients’ cells. Using our conceptually novel and established HCA approach, we plan to identify disease biomarkers and cellular pathways that are relevant to the patient’s cells and disease severity that will be investigated by integrating different “omics” approaches. This will enable more accurate assessment of the novel GSD compounds treatment effects on the different patient cell samples according to their respective cellular phenotype and pathological GSDIII subtype condition.

Preliminary experiments shown support the feasibility of this Ph.D. Thesis proposal and its research aims. First phenotypic image based high content analysis of 6 GSD III patient fibroblast samples grown at 3 different culture conditions showed significant differences at organelar level (mitochondria, and lysosome) which has been well documented previously to be affected by the disease in liver and muscle of different established GSD III models. We plan to look deeper into both of these organelle GSD III phenotypes by studying the differences in the mitochondria and lysosomal activities. While performing these experiments we observed that some GSDIII fibroblasts samples take longer to detach under trypsin treatment which might be due to extracellular matrix excessive production by spontaneously differentiated myofibroblasts within the GSDIII fibroblasts population due to the disease status. To test this hypothesis, we performed a time lapse detachment assay to measure trypsin treatment effect on the cells that corroborated our previous observation with GSD III cells and performed immunofluorescence staining for myofibroblast specific marker actin alpha smooth muscle (alpha-SMA) showing to be positive in some GSDIII samples (~ 20% of the cells express this marker) but negative in all healthy controls tested. Based on these observations we plan to investigate the disease mechanism that drives this abnormal differentiation pathway in some GSDIII primary fibroblasts probably related to the patients’ GDE deficiency. In parallel with the phenotype
characterization of the GSDIII fibroblasts samples we plan to test the treatment effects and describe the mode of action of our novel GSD compounds.