Congenital disorders of glycosylation: Generation and functional analysis of CRISPR-Cas9-driven PMM2 point mutations in human antigen-presenting cell models

Fulfilled until summer 2018

Internship proposal at the Department of Cell Biology, Radboudumc
Supervisors: Paola de Haas, Wiljan Hendriks, Alessandra Cambi
Contact: Paola.dehaas@radboudumc.nl

Background:

PMM2-CDG is the most prevalent type of congenital glycosylation disorders, affecting the assembly of sugars in the process of protein glycosylation. It is caused by mutations in phosphomannomutase 2, an enzyme that converts mannose 6-phophate to mannose 1-phosphate, which in turn is converted to GDP-mannose that serves as building block for assembly of glycan chains. PMM2 insufficiency results in loss of complete N-glycans in almost every cell of the body and patients therefore present a large spectrum of symptoms. Approximately 20% of the patients does not survive the first year of life, which is mainly due to microbial infections.

Several studies indicate that glycosylation is important for proper immune functioning (Monticelli *et al*, 2016). For example, the glycan-binding selectins and galectins determine leukocyte trafficking and cell-extracellular matrix (ECM) interactions, respectively. Furthermore, glycosylation plays a role in immunological synapse formation and it modulates the threshold of T cell receptor and B cell receptor activation. However, the molecular mechanisms and the impact of PMM2 defects on the immune system, especially in key immune cell types such as T cells or antigen-presenting cells, have not been thoroughly studied due to the lack of appropriate model systems.

Research plan:

Our aim is to develop and study *in vitro* models of PMM2-CDG using a novel strategy to select for **CRISPR-driven genome editing via homology-directed repair (HDR)**. To this end we will introduce two prevalent PMM2-CDG mutations (R114H and F119L) into the genome of the human monocyte-like cell line THP1, which is able to differentiate into macrophage and dendritic cell types. We tested several guide-RNAs targeting the PMM2 locus and obtained results illustrated the need to increase efficacy of CRISPR-Cas9 gene editing events via the homologous recombination DNA repair pathway.

Very recently a **coselection strategy** to enrich for cells that underwent nuclease-driven HDR was described. It exploits the multi-target capability of CRISPR-Cas9 by selecting for ouabain resistant alleles of the ubiquitous sodium potassium ATPase to enrich for custom genetic modifications at the locus of interest (Agudelo *et al*, 2017). By adding our PMM2-directed guide-RNAs and corresponding HDR oligonucleotide template to the Cas9 and ATPase-directed gRNA expression plasmid and ouabain resistance HDR template, we envision effective recovery of properly engineered THP1 cells. Obtained genotypes will be checked with **T7 nuclease assays and PCR melt curve analyses**, followed by **Sanger sequencing** of individual THP1-derived clones.

Eventually the THP1-derived models will be used to investigate the effect of PMM2-CDG mutations on immune cell functioning. Membrane protein levels for integrins, pathogen recognition receptors and the major histocompatibility complex will be immunodetected using **flow cytometry**. Also, their **phagocytic capacity will be microscopically probed** with labeled antigen-coated beads or zymosan. To study effects on cell migration, the **rolling and adhesion on ICAM1 coated coverslips** will be monitored while applying a medium flow that resembles the blood circulation. Collectively, these experiments will shed light on the immune component in PMM2-CDG.

Literature:

Monticelli *et al.* (2016) Immunological aspects of congenital disorders of glycosylation (CDG): a review. J Inherit Metab Dis 39: 765‑780.

Agudelo *et al.* (2017) Marker-free coselection for CRISPR-driven genome editing in human cells. Nat Methods 14: 615-620.