Understanding the role of integrin glycosylation in 3D migration of healthy and CDG patient-derived fibroblasts

Internship proposal at the Department of Cell Biology, Radboudumc
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Background:

Glycosylation is the most common form of modification and involves the process of covalent attachment of sugar moieties (glycans) to proteins and lipids. These glycan chains, which are often neglected, actually play important roles in for example protein folding and trafficking as well as the modulation of ligand binding properties and receptor clustering. However, the exact molecular mechanisms by which glycosylation affects protein function are poorly understood due to the high diversity and complexity of the glycosylation process.

An important cell function, involved in development, wound healing and infection is **cell migration**, which is critically controlled by cell-cell and cell-extracellular matrix (ECM) interactions. Cell-ECM interactions are facilitated by a family of transmembrane receptors called integrins. **Integrins** are heavily glycosylated and several studies have shown that aberrant glycosylation disrupts integrin function by affecting membrane expression, ligand binding and receptor dimerization[1]. For some integrins the role of the individual glycan chains has been studied showing that glycans modulate integrin conformation and consequently promote or inhibit ligand binding. For the β1 subunit, which is an important subunit in collagen binding, it has been shown that removal of one glycosylation site localized at the headpiece of the integrin molecule significantly increases ligand binding[2].

Mutations in genes involved in the glycosylation machinery result in **congenital disorders of glycosylation (CDG)**, a group of diseases characterized by defects in glycan assembly in the endoplasmic reticulum (CDG type I) or glycan processing in the Golgi apparatus (CDG type II). CDG is a rare and usually severe disease with patients displaying a variety of symptoms affecting multiple tissues such as brain, muscle and intestine. For this internship project we have access to CDG patient fibroblasts, providing the unique opportunity to use these patient-derived cells to study migration in different glycosylation defects.

The **aim of this project** is primarily to investigate whether integrin β1 glycosylation modulates fibroblast migration and in addition, we hope to provide molecular mechanisms for some of the symptoms displayed by CDG patients.

Research plan:

To look at integrin expression and activation status in the different CDG patient fibroblasts we will immunostain the cells with antibodies specific for the active form or all forms of the β1 subunit and measure the fluorescent intensity by **flow cytometry**. Integrin glycosylation can be determined with a **Western blot**, by comparing the protein sizes in CDG patient to control cells as well as before and after treatment with specific glycan cleaving enzymes. In addition a **lectin blot** can be performed, making use of proteins recognizing specific glycan motifs (lectins).

To look at the effect of impaired glycosylation on integrin clustering immunostaining of integrins will be visualized by **Airyscan**, a super-resolution microscopy technique. A **3D collagen matrix model** will be used to look at 3D migration of fibroblasts using live imaging with **wide-field time-lapse microscopy** followed by manual tracking and quantification of migration speed and directionality. To look at integrin clustering in 3D migrating cells, the samples will be fixed, immunostained and imaged by **confocal laser scanning microscopy**. Finally, the force exerted by the fibroblasts by pulling on the collagen fibres, which is needed for migration, can be quantified by a **collagen gel contraction assay.**

Literature

1. Gu *et al.* (2009) Importance of N-Glycosylation on α5β1 Integrin for Its Biological Functions. Biol. Pharm. Bull. 32(5):780-5.
2. Cai et al. (2017) The importance of N-glycosylation on β3 integrin ligand binding and conformational regulation. Sci. Rep. 7(1):4656