Master Internship Project

General project title: PGM1 deficiency – A heterogeneous myopathy with opportunities for treatment

Group leader: Dr. D.J. Lefeber

Daily supervisor: PhD candidate F. Conte - Federica.Conte@radboudumc.nl

Period: 6 months

Starting date: October/March 2019

1. PGM1 deficiency: a recently characterized CDG

This internship will be developed in the field of glycosylation disorders, specifically congenital disorder of glycosylation (CDGs). This heterogeneous group of metabolic and multiorgan disorders include over fifty different genetic diseases caused by defects in the synthesis or in the attachment of glycan moiety of glycoproteins and glycolipids (Jeaken, 2013; Jaeken, 2011; Lefeber et al., 2011).

In 2009 a new muscular disorder was reported by Stojkovic et al. in one adult patient with muscle glycogenosis (exercise-induced muscle cramps and rhabdomyolysis). This disorder was initially defined as a pure/isolated metabolic myopathy (*muscle glycogenosis type XIV*) caused by a genetic defect affecting phosphoglucomutase type 1 (PGM1) gene. Since PGM1 enzyme was known to play an essential role in the homeostasis/metabolism of carbohydrates (interconversion of glucose-1-phosphate to glucose-6-phosphate), the muscular features were initially correlated with the defects in the energy production. However, in the following years (Tegtmeyer et al. 2014; Timal et al., 2012) more patients with mutations on PGM1 have been reported and more light started to be shed on PGM1 deficiency: it turned out to be a multiorgan disorder affecting a wide number of organs and tissues, such as heart, skeletal muscles, glands, liver, kidneys and skeleton (Küçükçongar et al., 2015; Tegtmeyer et al. 2014; Pèrez et al., 2013). In addition, Tegtmeyer et al. (2014) confirmed via glycomics approaches (LC/MS) that in all these patients an abnormal serum transferring glycoprofile was present, suggesting that PGM1 mutation lead not just to abnormal glycolysis regulation and glycogen accumulation (defective energy production), but also to abnormal protein glycosylation. Since then, PGM1 deficiency was definied as a 'new' *congenital disorder of glycosylation* (CDG).

The available *in vivo* data suggest that inherited PGM1-CDG fits a *loss-of-function* phenotype that may include protein misfolding, in addition to conventional catalytic defects, caused by a set of more than 35 genetic PGM1 mutations identified as potentially pathogenic (Beamer, 2015; Lee et al., 2014; Conte et al., in preparation). However no clear correlation between gene defect location and clinical severity has been found. In addition, it has been extimated that over 50% of all human proteins need to undergo glycosylation to become fully functional (including hormons, enzymes, kinases, etc…): the eveidence that in PGM1 patients glycosilation is defective, may be the key point to interpret and maybe solve some (or all) the clinical phenotypes (e.g. growth delay in such patients may be due to misglycosilated hormonal proteins).
However, the molecular mechanisms underlying the development of this CDG and its clinical features (especially on muscle and cardiac tissues) are by as yet unsolved.

2. Internship project summary

This Master Internship (under the supervision of PhD candidate F. Conte) will focus on the optimization and application of ^{13}C-based tracer metabolomics on PGM1 cell models, specifically primary dermal fibroblasts and hiPS-derived cardiomyocytes. The study of these models, in combination with previous data generated by F. Conte in other cell models, will help shedding light on how bioenergetic metabolism and protein glycosylation are affected by PGM1 deficiency in different tissues, offering precious information for therapy design.

Specifically, the trainee will be involved in:

(A) Primary fibroblast culture;
(B) Enzyme activity assay and westerblot on fibroblasts samples;
(C) hiPSCs culture and hiPS-derived cardiomyocytes characterization via IF and qPCR;
(D) Mass-spec based target metabolomics (Tandem Quadrupole UPLC/MS);
(E) Other biochemical techniques (e.g. enzyme activity assay);

3. Project timeline

Field	Task	Month 1	2	3	4	5	6
hiPSCs and cardiomyocytes	hiPSCs: culture						
	Cardiomyocyte characterization						
	Metabolomics experiments						
	TBA-based metabolomics analysis				Data analysis	Data analysis	
Primary fibroblasts	Fibroblasts: culture						
	^{13}C-based labelled experiments						
	TBA-based and TEAA-based metabolomics analysis						Data analysis

Note I: the cell culture work will be shared with F. Conte.
Note II: this timeline is only a general indication of the project lalyout and can be subjected to changes.
Note III: the Tandem Quadrupole UPLC/MS is used by multiple members of Prof. Lefeber's group, hence the scheduling of the analysis is decided based on full agreement of all members. The timeline showed above is only an indication of the period of the analysis.
Note IV: the project will be paused for ~2 weeks during Christmas holidays. These two weeks will be recuperate (added) at the end (if needed).

4. Requirements

This project cover several technological fields, from stem cells to metabolomics, representing a great possibility for CV building. However, it requires high commitment and full-time schedule, hence priority will be given to the candidates who have already accomplished all their courses.

The project will cover a period of at least 6 months.

The final presentation to the research group has to be finalized within 4 weeks after the ending date of the lab work (unless technical problems emerged).

The first draft of the final report has to be submitted to the supervisor within max 5 weeks from the ending date of the lab work.

Fluent English speaking/writing required.

5. References

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