OP-0109 Protein profiling reveal *SLC35b4* responds to glucose stimulation and acts through modulating molecular Hsp60 among others

Brigitte Wex, Rémi M. Safi, Gregory Antonios, Perla Z. Zgheib, Dania B. Awad, Firas H. Kobeissy, Rami A. Mahfouz, Marwan M. El-Sabban and **Soha N. Yazbek***

**Medical Laboratory Sciences Program, Faculty of Health Sciences, American University of Beirut, Beirut, Lebanon*

Introduction

Cumulative contribution of the identified diabetes genetic variants accounts for only 10% of total heritability. Our screen for diabetes genes in a unique mouse model (B6-A/J CSS) revealed:

- Over 143 genes may control diabetes predisposition
- Genes are context dependent with large non-additive effect size

Functional studies on the identified genes uncovered a novel role of *SLC35b4*

- In vivo* alteration of hepatic gene expression of *SLC35b4* correlated with insulin resistance and gluconeogenesis
- In vitro* alteration of *SLC35b4* mRNA altered gluconeogenesis

SLC35b4:

- Solute receptor gene on chromosome 7q3 expressed in most tissues
- Transports UDP-N-acetylglucosamine (UDP-GlcNAc) and UDP-xylose (UDP-xyl)
- Function thought to mainly take place in the Golgi
- Literature linked it to several metabolic disorders in humans involved in obesity-induced diabetes
- SNP in the human *SLC35b4* gene was associated with variation in body mass index (BMI), metabolic syndrome, fasting glucose, pro-insulin levels, fat stores and obesity

Aims

We aimed to investigate *SLC35b4*'s role in the control of hepatic glucose production, an important mechanism for the response to insulin and for the alteration of glucose levels. This study investigated the localization of *SLC35b4* and its role in the regulation of protein expression, and compared the differential expression between a knockdown of *SLC35b4* and controls in HepG2.

Methods

SLC35b4's responsiveness to glucose, expression and localization were assayed using western blot and immunostaining. Localization was confirmed using DUO-Link PLA. 2D gel electrophoresis and MALDI-TOF were used to identify differentially expressed proteins between a knockdown of *SLC35b4* and controls in HepG2 cells, and pathway analysis was performed using Elsevier's Pathway Studio version 10.0.

Results

Immunostaining for *SLC35b4* in HepG2 cells showed that *SLC35b4* is up-regulated in response to glucose stimulation.

Western blots of *SLC35b4* protein in response to glucose stimulation showed that *SLC35b4* is up-regulated by 60% in glucose treated HepG2 cells

Results cnt'd

Subcellular localization of *SLC35b4* transporter in HepG2 cells by indirect immunofluorescence and DUO-Link PLA showed that *SLC35b4* is widely spread in the Golgi apparatus and the ER.

Immunostaining for *SLC35b4* in normal liver tissue showed that *SLC35b4* protein is expressed similar to HepG2 cells.

2D gel electrophoresis and MALDI-TOF showed that *SLC35b4* knockdown in HepG2 cells altered the expression of multiple downstream proteins; four of which passed our stringent inclusion criteria and were successfully identified by MALDI (Hsp60, HSPA8, TUBA1A, Eno1). Panther pathway analysis revealed their connection to *SLC35b4* and the insulin-glucose homeostasis pathway.

Discussion and Conclusion

- Literature has clearly linked Hsp60 to diabetes in humans, mice and beta cell cultures; mainly the increase in the levels of Hsp60 in both serum and saliva of diabetic patients.
- SLC35b4* Knockdown downregulated Hsp60 by 22 folds.
- Hsp60 being evidently modified by O-GlcNAcylation and the association of this modification with diabetic phenotype provides strong implication of the mechanism through which *SLC35b4* could influence the downstream effect on Hsp60.
- Our report gives the first evidence of the causal effect that Hsp60 can play in hyperglycemia. Knockdown of *SLC35b4* decreased the protein expression of Hsp60 which, as has been previously reported by our investigator, could have led to the increase in endogenous glucose production caused by *SLC35b4* knockdown.
- We also found a significant down-regulation in HspA8 and ENO1 supporting the functional link of both to hyperglycemia.
- The 4th gene that was significantly down regulated was TUBA1, a finding that implicates *SLC35b4* in modulating O-GlcNAcylation.

We hypothesize that the uptake of UDP-GlcNAc by *SLC35b4* carrier may have a negative feedback on the gluconeogenesis, leading to a decrease in glucose production. The finding that some of the downstream proteins are strongly modified by O-GlcNAcylation presents evidence to support this hypothesis