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Grant Title: Untangling lumenal UDP-sugar transport in humans

Abstract

1. Objectives


The SLC35 family of transporters from humans comprise over thirty members with many implicated in the delivery of nucleotide sugars into the Golgi apparatus and endoplasmic reticulum (ER). While a range of substrate specificities have been allocated to the human SLC35 family only two SLC35 transporters have been linked to congenital disorders in glycosylation. We have been applying a proteo-liposome transporter assay coupled to tandem mass spectrometry to characterize substrate specificities of human NSTs. Preliminary findings indicated that the SLC35D1 transporter was a general UD-sugar transporter while the uncharacterized SLC35E1 member of the family appeared to be a specific UDP-Xyl transporter.

2. Methods

We employed commercial human CRIPSR/Cas9 cell lines in an attempt to confirm the *in vitro* activities of these two human transporters. Two independent loss-of-function CRISPR/Cas9 cell lines targeting SLC35D1 were profiled using antibodies directed at human proteoglycans, lectin arrays, nucleotide sugar compositions and analysis of glycans cleaved from proteoglycans. Employing the proteo-liposome transport assay we conducted a complete biochemical analysis of both SLC35D1 and SLC35E3 to determine K_M and other substrate specific parameters. By employing phylogenetic analysis, we were also able to identify and characterize a UDP-GlcNAc transporter in the reference plant Arabidopsis related to SLC35D1 as well as two UDP-xylose transporters in *Cryptococcus neoformans* homologous to SLC35E3.

3. Results

The profiling of proteoglycans in human cell lines proved difficult and was likely the result of enriched growth conditions impacting the demand for the generation of proteoglycans. While the loss of the general UDP-sugar transporter (SLC35D1) from the cell lines resulted in changes to UDP-GlcA levels, no effect on proteoglycans could be observed. In contrast, the *in vivo* characterization of loss-of-function mutations in the plant UDP-GlcNAc transporter resulted in the absence of lumenal GlcNAc on substrates within the Golgi apparatus. This included *N*-linked glycans and resulted in only the formation of mannose-rich *N*-glycans as well as sphingolipids lacking GlcNAc. While these changes resulted in a significant biochemical phenotype, plant growth and development was unaffected. The elimination of the two UDP-xylose transporters in *C. neoformans* demonstrated that, unsurprisingly UDP-xylose transport is required for xylose incorporation into capsule and proteins. Interestingly these transporters were necessary for *C. neoformans* to cause disease in mice, although not for fungal viability in the context of infection. While this work highlights the difficulties in working in cell lines, the project reiterated the notion of functional conservation and the sequence level in the SLC35 transporter family, which is also highly conserved across kingdoms.