## Functional analysis of potential UDP-*N*-acetylglucosamine transporters

UDP-*N*-acetylglucosamine (UDP-Glc*N*Ac) is one of the most important substrates used in the glycosylation. Until now, the SLC35A3 (A3) protein was considered to be the main transporter of this nucleotide sugar. The studies focused on characterizing A3 protein were mainly based on heterologous yeast systems, which produce significantly different types of glycans than mammalian cells. Additionally, studies on the A3 protein have shown that almost complete silencing of the *SLC35A3* gene does not significantly affect the level of *N*-acetylglucosamine (Glc*N*Ac) in *N*-glycan structures in mammalian cells. Due to the inaccuracies related to the transport of UDP-Glc*N*Ac, as well as literature reports on other proteins from the SLC35A family acting as a potential transporter of this nucleotide-sugar, the aim of this study was to characterize selected transporters which could be involved in delivery of UDP-GlcNAc for the synthesis of N- and O-glycans. The first stage of this work was inactivation of the *SLC35A3* gene (A3KO, KO-knock-out) in the CHO line, using the CRISPR-Cas9 system. Since the A3 protein interacts with the UDP-galactose transporter (SLC35A2, A2), and these proteins are in close proximity to the same Mgat glycosyltransferases, the *SLC35A3* gene was disabled in cells lacking the A2 protein (A2KO, Lec8), obtaining double knockouts (A2/A3KO) in three cell lines: HepG2, HEK293T and CHO. As part of this doctoral thesis, *N*-glycans from cell lysates, and from SEAP protein (*Secreted Alkaline Phosphatase)* and *O*-glycans in SLC35A3 knockouts, SLC35A2 knockouts, and double knockouts in three cell lines: HepG2, HEK293T and CHO were characterized. The obtained results showed that SLC35A3 inactivation did not inhibit Glc*N*Ac incorporation into *N*- and *O*-glycans. Cell-specific changes in branched *N*-linked glycans formation have been observed. Almost complete inhibition on *N*-glycans branching in the HEK293T A2/A3KO and Lec8 A3KO lines has been demonstrated, which supports the idea of this two proteins being functionally related. Due to the observed changes in *N*-glycan branches, it was investigated what effect the inactivation of investigated transporters had on the amount and secretion of Mgat1, Mgat2 and Mgat5. These glycosyltransferases are responsible for–catalyzing the addition of Glc*N*Ac to *N*-glycans, initiating branching. They are also able to form complexes with A2 and A3 proteins. It has been shown that lack of functional A2 and A3 proteins has an impact both on the quantity and the secretion of the tested glycosyltransferases, but it is also a cell-specific effect. Differences in the secretion of transferases may be caused by changes in the composition of multi-protein complexes, which lack studied transporters. As part of the doctoral thesis, cell lines lacking the functional proteins SLC35B4 (B4), (HepG2 B4KO) and SLC35D1 (D1), (HepG2 D1KO,

HEK293T D1KO) were also generated. Both proteins are considerate as UDP-GlcNAc transporters as well. In generated mutants no significant changes in synthesized *N*- and *O*-glycans were observed. These findings suggest that SLC35A3 may not to be the primary UDP-Glc*N*Ac transporter. This work demonstrates the necessity to search for the main/additional UDP-Glc*N*Ac transporter or for the possibility that different mechanisms of UDP-Glc*N*Ac transport into the Golgi apparatus may exist.