

UNCOVERING ST3GAL3 FUNCTION: FROM PATHOGENIC VARIANTS TO GLYCOSPHINGOLIPIDS REMODELING

Penati S.¹, Dei Cas M.¹, Montavoci L.¹, Casati S.², Caretti A.¹ and Trinchera M.³

¹ *Department of Health Sciences, San Paolo Hospital, Università degli Studi di Milano, Milan, Italy*

² *Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy*

³ *Department of Medicine and Surgery (DMC), University of Insubria, Varese, Italy*

Sialyltransferase ST3GAL3 catalyzes the transfer of a sialic acid residue to the C-3 position of a galactose, completing the oligosaccharide chains of glycoproteins and gangliosides. Initially considered mainly responsible for the biosynthesis of glycotopes such as sialyl-Lewis a, loss-of-function variants of ST3GAL3 were later associated with a rare disorder characterized by epileptic encephalopathy in patients presenting normal levels of circulating CA19.9. In vitro studies revealed a substrate preference for glycosphingolipids containing Gal β 1,3GlcNAc (lacto-series) and Gal β 1,3GalNAc (ganglio-series) backbones. Altogether, these observations suggest that ST3GAL3 plays a key role in generating a specific subset of minor gangliosides. We optimized an HPLC-MS/MS method to quantitatively profile glycosphingolipids in plasma and fibroblast samples from patients carrying ST3GAL3 pathogenic variants. The enzyme activity of wild-type and variant ST3GAL3s was assessed upon transfection into HEK 293-T cells using both artificial (LNB-pNP) and natural substrates, and the formation of sialylated products was quantitated via LC-MS/MS. The rescue capacity of sialyltransferases ST3GAL4 and ST3GAL6 was also evaluated in-vitro. Additionally, a panel of HEK 293-T knockout clones targeting glycosphingolipid-related genes was generated using CRISPR/Cas9 genome editing to dissect substrate specificity and ST3GAL redundancy in a cell model. All ST3GAL3 variants showed complete loss of enzymatic activity, except p.A13D, which retained minimal function. In patient-derived fibroblasts, a significant decrease in sialyl-lactotetraosylceramide (sLc4) and a concomitant accumulation of its precursor Lc4 were observed. Similar alterations were not consistently detectable in plasma. Knockout models confirmed that ST3GAL3 preferentially modifies Lc4 although some ambiguity remains regarding the exclusive role in sLc4 (Gal β 1,3GlcNAc backbone) and GM1b (Gal β 1-3GalNAc backbone) biosynthesis. These results highlight the loss of ST3GAL3 enzyme function as the pathogenic mechanism in the disease and suggest Lc4 as key substrate. Further work is ongoing to clarify the role in sLc4 and GM1 biosynthesis.