Biochemical studies of carbohydrate blood group antigens

Carbohydrate phenotype in relation to cellular glycosyltranferases

AKADEMISK AVHANDLING

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av

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Avhandlingen baseras på följande delarbeten:

I **Diswall M**, Ångström J, Schuurman H-J, Dor FJ, Rydberg L, Breimer ME. (2007) Studies on glycolipid antigens in small intestine and pancreas of alpha1,3galactosyltransferase knockout miniature swine. *Transplantation 84(10): 1348-56*

II **Diswall M**, Ångström J, Schuurman H-J, Dor FJ, Rydberg L, Breimer ME. (2008) Glycolipid studies in small intestine and pancreas of alpha1,3galactosyltransferase knockout miniature swine: alpha1,3GALT-KO animals lack alpha-GAL antigens and contain novel bloodgroup H compounds. *Transplantation Proceedings 40(2): 543-6*

III **Diswall M**, Ångström J, Karlsson H, Phelps C, Ayares D, Teneberg S, Breimer ME. Studies of alpha1,3-galactosyltransferase knock-out pig glycolipids and their reactivity with human and baboon antibodies. *Manuscript*

IV Löfling J, **Diswall M**, Eriksson S, Borén T, Breimer ME, Holgersson J. (2008) Studies of Lewis antigens and *H. pylori* adhesion in CHO cell lines engineered to express Lewis b determinants. *Glycobiology 18(7): 494-501*

GÖTEBORGS UNIVERSITET

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The possibility to alter the cell surface carbohydrate expression by insertion or deletion of glycosyltransferase genes is a powerful technique to study the biological function of selected carbohydrate antigens. However, shifting the equilibrium between competing glycosyltransferases might lead to unexpected phenotypic effects, such as accumulation of other carbohydrate antigens and exposure of antigen structures that normally are cryptic or present in minute amounts on the cell surface.

The present work explores the relationship of glycosyltransferase repertoire (genotype) and the resulting cell membrane glycolandscape (phenotype) from a glycosphingolipid perspective using two model systems where glycosyltransferase genes have been deleted or introduced. In addition to contributing to basal glycobiology, the experimental models have the potential to serve clinical purposes in the xenotransplantation and microbial adhesion fields. Glycosylation is not template driven, explaining why the cell membrane carbohydrate expression cannot be predicted from genotyping, but rather requires phenotyping. For phenotyping in this sense, antibody/lectin recognition of cell membrane antigens is insufficient necessitating structural determination.

Neutral and acidic glycolipids from tissues and cells were isolated by means of organic solvent extraction and repeated chromatography steps. Individual glycolipid components were purified by high performance liquid chromatography. The antigenic properties of the glycolipids were examined for reactivity with mono- and polyclonal antibodies, lectins and sera on thin layer chromatography plates. Structural elucidation was conducted by the combined use of mass spectrometry and proton nuclear magnetic resonance spectroscopy.

Our studies report an indisputable correlation between glycosyltransferase gene setup and the resulting glycolandscape phenotype. The study is also indicative of the renowned complexity of glycosylation, *e.g.* the glycosyltransferase dependence on the underlying protein/lipid backbone and the species-, individual- and organ-specific glycosyltransferase activity. In addition, we have identified several novel glycosphingolipids in pig tissues for which function and importance remain to be elucidated.

Key words: glycosphingolipid, glycosyltransferase, genetic modification, carbohydrate, mass spectrometry, proton NMR spectroscopy, xenotransplantation, GalT-KO pig

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