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Expression of sialyl Lex, sialyl Lea, Lex and Ley glycotopes in secreted human ovarian cyst glycoproteins

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Human blood group A, B, H, Ii, Le^a and Le^b antigens and their determinants expressed on ovarian cyst glycoproteins have been studied for over five decades. However, little is known about sialyl Le^x and sialyl Le^a glycotopes, which play essential roles in normal immunity, inflammation and cancer cell metastasis. Furthermore, Le^x and Le^y were classified as glycotopes of unknown genes. Identification of these Lewis epitopes was hampered by the lack of specific antibodies. In this study, the occurrence of sialyl Le^x, sialyl Le^a, Le^x and Le^y reactivities in cyst glycoproteins was characterized by enzyme-linked immunosorbent assays. The results indicated that most human ovarian cyst glycoproteins carried Le^x (8/25) and or Le^y (17/25) glycotopes. The expression (epitopes) of the new genes described in previous reports are Lex and Ley glycotopes; the reactivities of sialyl Le^x and sialyl Le^a glycotopes in secreted cyst glycoproteins may be affected by the conditions of purification; the relationship between Ley and human blood group ABH was confirmed; recognition profiles of sialyl Le^x, sialyl Le^a, Le^x and Le^y present in the carbohydrate chains of water-soluble cyst glycoproteins were illustrated; possible attachments of glycotopes to the internal carbohydrate complex of cyst glycoproteins have been reconstructed; proposed biosynthetic pathways for the formation of sialyl Le^a, sialyl Le^x, Le^x, Le^y, ALe^y and BLe^y determinant structures on Type-I and Type-II core structures of human ovarian cyst glycoproteins are also included in this study.

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Analytical glycomics: Biopharmaceutical applications

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Full characterization of the N-glycosylation moieties of biopharmaceuticals is of high importance, especially when glycovariants may impact the biological effect. Well over half of the new generation protein therapeutics are monoclonal antibodies, in which the attached oligosaccharides not only affect their physicochemical properties and stability, but also their receptor binding activity, circulating half-life and last but not least, their immunogenicity. Therefore, high performance glycoanalytical techniques are of great demand for N-glycosylation analysis of therapeutic antibodies, especially during clone selection, process development and lot release. Analysis of complex carbohydrates is a very challenging task due to the lack of their chromophore/fluorophore activity and, in many instances, easily ionizable groups, necessitating derivatization before electric field mediated analysis. Full N-glycosylation characterization may also require sequencing with consecutive exoglycosidase digestion steps, followed by capillary electrophoresis analysis. In this presentation, the state of art liquid phase separation methods will be conferred for comprehensive structural elucidation of protein N-glycosylation, mostly using capillary electrophoresis and its combination with mass spectrometry (CESI-MS). Assisted by the emerging field of glycoinformatics, assignment of the identity of the separated glycan structures will be demonstrated by using the recently introduced GUCal software.

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