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Human α-1,4-galactosyltransferase as an enzyme regulating Shiga toxin binding

Abstract

The human α-1,4-galactosyltransferase, encoded by the *A4GALT* gene, is an unusually promiscuous glycosyltransferase. It synthesizes the terminal Galα1→4Gal structure of the P^k and P1 antigens. These glycosphingolipid antigens belong to the P1PK histo-blood group system. A single amino acid substitution (p.Q211E) enhances the promiscuity of the enzyme, rendering it able to attach Gal both to another Gal residue but also to GalNAc, giving rise to NOR1 and NOR2 glycosphingolipids. The P^k antigen (Gb3) is present on erythrocytes of most individuals, whereas presence of P1 antigen underlies two main phenotypes: P_1, if the P1 antigen is present, and P_2, if P1 is absent. The genetic background of P_1/P_2 polymorphism has eluded clarification for years, and the results of this study show that rs5751348 offers the best predictive value for the P_1/P_2 phenotypic polymorphism. Significantly, the P^k antigen is the major receptor for Shiga toxins produced by enterohemorrhagic *Escherichia coli*, but the role of P1 antigen in Shiga toxin binding has never been examined. Results of this study contributed to the elucidation of P_1/P_2 polymorphism background. The human α-1,4-galactosyltransferase has been long believed to transfer Gal only to glycosphingolipid acceptors, but the data presented in this thesis demonstrate that the enzyme is able to add galactose also to glycoprotein acceptors. The consensus α-1,4-galactosyltransferase, as well as p.Q211E mutein, can synthesize the P1 glycotope (terminal Galα1→4Galβ1→4GlcNAc) on complex type N-glycans, with the mutein exhibiting an elevated level of activity. To date, glycosphingolipids were considered the only receptors for Shiga toxins. However, results of this study revealed that N-glycoproteins carrying P1 glycotopes are recognized by Shiga toxin 1 but not Shiga toxin 2 B subunits. Furthermore, such glycoproteins may act as functional receptors for Shiga toxin 1, but not for Shiga toxin 2. Thus, Shiga toxin 1 can recognize and use P1-terminated N-glycoproteins in addition to its canonical glycosphingolipid receptors to enter and kill the cells, while Shiga toxin 2 can use glycosphingolipids only. Since the interaction with Shiga toxins is a consequence of human α-1,4-galactosyltransferase activity, the enzyme may be considered a regulator of Shiga toxin binding.