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Recombinant mucin-type proteins as tools for studies on the interactions between *Helicobacter pylori* and its carbohydrate receptors

AKADEMISK AVHANDLING

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Hörsal Arvid Carlsson, Medicinaregatan 3,
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av

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Avhandlingen baseras på följande delarbeten:

I. Mthembu YH , Jin C , Padra M, Liu J, Olofsson –Edlund J, Ma H, Padra J, Oscarson S, Borén T, Karlsson NG, Lindén SK and Holgersson J. Recombinant mucin-type proteins carrying LacdiNAc on different O-glycan core chains fail to support *H. pylori* binding. Molecular Omics (doi: 10.1039/C9MO00175A)

II. Mthembu YH, Olofsson-Edlund J, Jin C, Cherian R, Liu J, Karlsson NG, Borén T, and Holgersson J. Identification of the O-glycomes of mucin-type receptors for BabA- and SabA-mediated *Helicobacter pylori* adhesion. (*Submitted*)

III. Mthembu YH, Jin C, Padra M, Liu J, Karlsson NG, Linden SK and Holgersson J. O-glycan core chain specificity of A4GNT and the effect of GlcNAcα4Gal determinants on *Helicobacter pylori growth. (In-Manuscript)*

IV. Flowers SA, Thomsson KA, Ali L, Huang S, Mthembu YH, Gallini R, Holgersson J, Schmidt TA, Rolfson O, Björkman LI, Sundqvist M, Karlsson-Bengtsson A, Jay G, Eisler T, Krawetz R and Karlsson NG. Core-2 O-glycans are required for galectin-3 interaction with the osteoarthritis related protein lubricin. (*Submitted*)

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ABSTRACT

Glycan-protein interactions are important in pathogen adhesion and infections. *H. pylori* has adhesins which enables it to bind to glycans on the gastric mucosa and, in the long run, cause gastric cancer. The reported current antibiotic regimen used in the treatment to eradicate *H. pylori* fails in 20% of the patients. A multivalent glycan inhibitor could offer a suitable alternative to antibiotics by acting as a competitive inhibitor for the cell receptors, leading to the binding and elimination of the microbe. This thesis is focused around the use of genetically engineered CHO-K1 cells producing a recombinant mucin-type fusion protein, P-selectin glycoprotein ligand-1/mouse IgG2b (PSGL-1/mIgG2b), which is used as a scaffold for multivalent presentation of engineered bioactive *O*-linked glycans. Through the engineering of carbohydrate determinants mediating attachment or affecting the growth of *H. pylori*, potential inhibitors of *H. pylori* infection were created (paper I, II and III).

In paper I, we show that B4GALNT3 added a β1,4-linked GalNAc to GlcNAc (LDN) irrespective of whether the latter was carried by a core 2, core 3 or extended core 1 chain. There was no correlation between *H. pylori* binding and the expression of LDN determinants on gastric mucins or a mucin-type fusion protein carrying core 2, 3 and extended core 1 *O*-glycans.

In paper II, The *H. pylori* experiments demonstrated that only PSGL-1/mIgG2b proteins with Le^b on core 3 inhibited BabA-mediated binding. On the other hand, the series of sialylated PSGL-1/mIgG2b proteins all demonstrated various degrees of inhibition of SabA-mediated binding, suggesting that SabA accepts various substitution of sLe^x for binding.

In paper III, we show by Western blot and LC-MS/MS that core 1, core 2, core 3 and extended core 1 chains could all carry the GlcNAcα4Gal determinant following transient transfection of CHO-K1 cells. Preliminary results showed that PSGL-1/mIgG2b carrying the GlcNAcα4Gal-terminal on core 1 and core 2 *O*-glycans did not inhibit the growth of *H. pylori*.

In paper IV, we show that the interaction of galectin-3 with the lubricating protein, lubricin, derived from osteoarthritis as opposed to healthy joints is dependent on core 2 *O*-glycans.

In conclusion, we have shown that glyco-engineering of a mucin-type fusion protein in CHO-K1 cells generates a powerful tool for investigations on *O*-glycan biosynthesis and microbial, in this case *H. pylori*, adhesion. The use of a mucin-type fusion protein as a carrier of frequent *O*-glycan substitution not only may increase the avidity of the reporter protein for its binding partner under study, but in addition mimics the structural context in which bioactive carbohydrate determinants are presented and used as microbial attachment sites at our mucosal surfaces.

Keywords: *O*-glycans, mucins, glycosyltransferases, *Helicobacter pylori*, microbial adhesion

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