Examination of Putative *Helicobacter pylori* Glycoproteins

Onyi Esonu, 2012

*Helicobacter pylori* is a motile, gram-negative bacteria that colonizes the gastrointestinal tract of more than half the world’s population. Although most infected individuals are asymptomatic, studies have identified *H. pylori* as the causative agent of certain gastric cancers, the development of duodenal ulcers, and gastritis. As current methods of treatment are becoming increasingly ineffective due to poor patient compliance and antibiotic resistance, novel methods of treatment are needed in response.

Exploiting the differences between human and *H. pylori* cells allows an alternative pathway to antibiotics. One striking difference between human cells and *H. pylori* is their sugar coating. For example, *H. pylori*’s flagellin proteins are modified by pseudaminic acid (PseNAc), a carbohydrate that is unique to a small number of bacterial pathogens. Disruption of the biosynthetic pathway that creates this sugar leads to the formation of non-functional flagella that leave *H. pylori* immotile and unable to infect potential hosts. As pseudaminic acid is not made or used by eukaryotic cells, it presents a method of selectively targeting *H. pylori* cells in infected individuals. Although pseudaminic acid has been linked to virulence, little is known about the identity, structure and function of other glycan-modified proteins in *H. pylori*.

The Dube lab has used metabolic oligosaccharide engineering (MOE) to incorporate the unnatural sugar peracetlyated N-azidoacetylglucosamine (Ac₄GlcNAz) into *H. pylori*’s glycoproteins through its natural metabolic pathways. Biochemical probes can be attached to the sugar’s azide residue, permitting the detection and/or destruction of *H. pylori* cells containing it. Previous work by Kanokwan Champasa ’11, using MOE, has proven the existence of over 50 glycosylated proteins that are biosynthesized by *H. pylori*, including the virulence factor Cag14.

The goal of my research project is to verify the identities of the putative glycoproteins found by Champasa through molecular cloning, MOE and mass spectrometry. Using the *H. pylori* – *E. coli* shuttle vector pHel2, which has been engineered to allow for the controlled expression of desired proteins, I will express, purify and isolate select putative glycoproteins from *H. pylori* for analysis of their glycosylation state.

Recent results indicate that the pHel2-Cag14 plasmid was successfully created and cloned in *E. coli*. In order to generate sufficient quantities of Cag14 for analysis of its glycosylation state, conditions for the replication of pHel2-Cag14 must be optimized in *H. pylori*. Once the transformation protocol has been optimized, pHel2-Cag14 will be transformed into *H. pylori* cells grown in the presence of Ac₄GlcNAz. The expressed glycoproteins will be purified using a Ni²⁺ column, and their glycans detected by a glycostain kit and visualized via molecular probes that can be attached to the azide residue of Ac₄GlcNAz and analyzed using mass spectrometry. These experiments will confirm whether the proteins identified in *H. pylori* using MOE are modified by glycans.

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**Funded by: Student Faculty Research Grant**