Analysis of Glycoprotein Production in Antibiotic Resistant Strains of *Helicobacter pylori*

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Helicobacter pylori (*Hp*) is a pathogenic bacterium that can cause peptic ulcer disease and gastric cancer, which is the second most fatal cancer worldwide (1). The most common treatment of *Hp* infection involves "triple therapy" with a combination of antibiotics (2, 3). However, current therapies are not sufficiently effective in eliminating the bacteria because of a growing number of antibiotic resistant strains (2, 3). Furthermore, even when these methods are successful, they affect beneficial bacteria found in the patient gut, which can lead to obesity, autoimmune disorders, malnutrition, and allergies (4, 5). Therefore, research on new, alternative therapies for curing *Hp* infection is critical.

One potential target of new treatments are the sugar-coated proteins, called glycoproteins, found on the surface of *Hp*. The structures of *Hp*'s sugars (glycans) are different from those of humans. *Hp*'s ability to modify its proteins with sugars is linked to its pathogenesis. To study these glycoproteins and reveal new drug targets, the Dube lab has utilized metabolic oligosaccharide engineering (MOE), a technique in which unnatural sugars are incorporated into *Hp*'s glycoproteins (6). The unnatural sugars are then detected in a subsequent chemical step, allowing for the visualization of glycoproteins. Through this technique the Dube lab has identified 125 previously unknown glycoproteins, 20 percent of which are linked to pathogenicity (7).

Recent work in the Dube lab has focused on understanding the genes involved in glycoprotein synthesis within the bacteria and the functional defects associated with compromised glycoprotein biosynthesis. Out of twenty mutant strains that were constructed by deleting putative glycosylation genes, thirteen of these mutants were defective in glycoprotein production (as revealed by dramatically decreased incorporation of the unnatural sugar) (8). Select mutant strains had reduced motility, biofilm formation, and ability to adhere to host cells when compared the to the wild type (8). Reintroducing the wild type version of these mutated glycosylation genes into the bacteria restored glycoprotein synthesis, biofilm formation, and adherence to host cells (8). These results suggest that *Hp*'s glycosylation machinery are potential drug targets.

While screening antibiotic resistant strains of *Hp*, the Dube lab serendipitously found two strains that had the same defects in glycosylation defects as the strains with intentionally targeted mutations. This summer I sought to study these antibiotic resistant strains of *Hp*, one that is resistant to clarithromycin ($Clar^R$) and one that is resistant to levofloxacin ($Levo^R$) in order to further understand the genes involved in glycoprotein synthesis. I performed MOE on the antibiotic resistant strains and confirmed that both $Levo^R$ and $Clar^R$ contained glycoprotein biosynthesis defects. I then worked to confirm this result through glycan release and analysis, an approach which provides a better look at the structural differences in mutant and wild type glycans (9). Because this assay is relatively new to the lab, I began by validating the method through studying a series of controls. I examined the fluorophore used to label the glycans (anthranilic acid), a simple sugar (glucose), a well-characterized glycoprotein (k-casein), and wild type *Hp*.

I then looked at how two features of *Hp* that are directly linked to pathogenesis are effected by the glycosylation deficiencies in the two antibiotic resistant test strains. I compared the ability of $Levo^R$, $Clar^R$, and wild type *Hp* to form a biofilm, which *Hp* form in order to protect themselves from the host's defenses and antibiotics. I found that in comparison to the robust biofilm formed by the wild type *Hp* and visualized by staining with crystal violet, $Levo^R$ and $Clar^R$ did not form appreciable biofilm. These data likely indicate that the glycoprotein defects in these two strains are linked to decrease in abilities related to *Hp*'s ability to cause disease. Secondly, I examined how motility – which is absolutely required for *Hp* to move through and colonize the host's stomach – is affected by measuring how colony diameter changes on soft agar plates over the course of eight days. I found that my antibiotic resistant strains did not demonstrate significant motility, indicating that the glycoprotein defects in these strains reduce the ability of the bacteria to perform functions linked to pathogenicity. Together these two assays both suggest that glycoproteins are critical for *Hp*'s fitness.

Moving forward, I will further confirm the reduced glycoprotein production of $Levo^R$ and $Clar^R$ *Hp* strains through my now validated glycan release and analysis approach. I intend to study the ability of these two antibiotic strains to adhere to host cells, a function that is linked to pathogenicity. Finally, I will determine whether the functionality of these bacteria can be restored through introducing wild type copies of glycosylation genes. Ultimately, this project will identify novel drug targets that could be harnessed to treat antibiotic resistant bacteria.

References

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