ABSTRACT

Title of the Thesis: Therapeutic targeting of autophagy in gastric cancer.

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Helicobacter pylori is a well-established inhabitant of the human stomach, and a major risk factor for the progression of gastritis, peptic ulcers, and even gastric cancer. *H. pylori* can be transmitted through contaminated food and water. In response to infection, host cells often stimulate autophagy as a protective mechanism to maintain cellular homeostasis. However, *H. pylori* has evolved strategies to subvert the host's autophagic machinery to persist within the epithelial cells of the stomach. Mechanisms by which *H. pylori* accomplishes its survival are still being investigated, but it often involves a complex interplay between bacterial effector and host cell proteins. High Mobility Group Box 1 (HMGB1) a nuclear host protein serves as a key regulator of autophagy and exhibits increased expression levels in several diseases and various types of cancer including gastric cancer. However, the role of HMGB1 in autophagy during *H. pylori* infection has not been explored to date. Hence, HMGB1 is targeted as a therapeutic candidate for the treatment of gastric disorders.

Human gastric cancer cell line (AGS) were infected with *H. pylori* strains SS1 and 26695. Western blot was used to examine autophagy protein expression. Autophagy and lysosomal activities were observed by fluorescence assays. Glycyrrhizin was used to inhibit HMGB1, and an *in vivo* mouse model of *H. pylori* infection was established to study the effect of glycyrrhizin treatment. Co-immunoprecipitation assays were performed in AGS and RAW 264.7 cells to study autophagy modulation by *H. pylori.*

We demonstrated that *H. pylori* infection modulates autophagy pathway and concomitantly increases the expression of HMGB1 in gastric cancer cells and macrophage cells. Autophagy modulation by *H. pylori* leads to its intracellular growth within the defective autophagosomes. To unravel HMGB1's role during autophagic dysregulation, we inhibited HMGB1 expression using glycyrrhizin, a known inhibitor of HMGB1. Inhibition of HMGB1 results in increase of autophagic flux which in turn, effectively reduces intracellular *H. pylori* burden, even in the case of antibiotic-resistant strains. Elevated autophagic response by glycyrrhizin was validated by using lysosomal inhibitors, chloroquine, and bafilomycin.

To gain deeper insights into the mechanisms underlying bacterial clearance, for the first time we reported that inhibition of HMGB1 by glycyrrhizin rescued the gastric cancer cells from lysosomal membrane permeabilization induced by *H. pylori* infection. Following that, glycyrrhizin treatment restored the lysosomal membrane integrity. The restored lysosomal function increases autolysosome formation and inhibits intracellular *H. pylori* growth. Additionally, glycyrrhizin treatment inhibited inflammation and repaired gastric tissue damages in mice. Interestingly we also found that increased HMGB1 during *H. pylori* infection interacts with multiple autophagy proteins, (including Beclin1, UVRAG, and WIPI2) that might modulate the autophagy mechanism.

Therefore, our study provides clear evidence that inhibiting HMGB1 restored lysosomal activity, thus mitigating the impact of *H. pylori* infection. It also demonstrated the potential of glycyrrhizin as an antibacterial agent to address the problem of antimicrobial resistance.

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