An In-Vivo Study Controlling Gene Expression in CagA Positive *Helicobacter pylori* to Determine the Effects of the CagA Gene on the Development of Gastric Cancer

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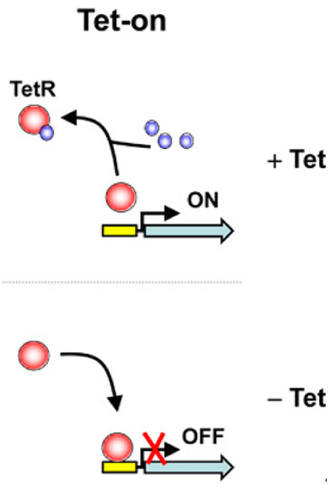
Introduction

Helicobacter pylori (H. pylori) is a carcinogenic stomach bacterium that is present in more than 50% of the world's population. The bacteria infect the stomach's epithelial lining and, as a result, most people develop gastritis which causes burning abdominal pain and can lead to stomach ulcers. However, those infected are also at a greater risk of developing gastric cancer, the third leading cause of cancer deaths worldwide and the fifth most diagnosed cancer as of 2018. H. pylori is responsible for about 3% of those cases, making up about 30,000 gastric cancer diagnoses annually. As a result, H. pylori, a class I carcinogen, has been identified as one of the most common causes of gastric cancer.

Helicobacter pylori bacteria is a spiral-shaped, gram-negative rod that has motility due to its flagella. As it resides in the a host's stomach, usually too acidic for most bacteria, it is able to thrive due to the presence of a urease enzyme. This enzyme creates a neutral environment for the bacteria by producing ammonia. Its multiple adhesion molecules allow H. pylori to interact with the stomach's epithelial lining and promote an inflammatory response. It is this increase in inflammation which has been linked to the formation of gastric adenocarcinomas and mucosa associated lymphoid tissue (MALT) lymphoma. However, because of the many different strains of H. pylori, not everyone infected with H. pylori is equally at risk of developing gastric cancer from the bacteria. Someone who has H. pylori that is positive for the cytotoxin-associated gene A (CagA) protein has a greater risk of developing gastric cancer than someone who is negative. CagA is a virulence factor that is translocated into epithelial cells using the type 4 secretion system (T4SS). T4SS, which acts as a needle appendage, injects CagA into the epithelial cells where it undergoes tyrosine phosphorylation. This induces an oncogenic reaction as it causes proinflammatory cytokines to be produced. Thus, the introduction of CagA into the epithelial cells causes the cells to grow and transform leading to the development of gastric cancer.

Possible Future Treatment Methods

To control the negative effects of CagA positive H. pylori, the use of a Tet inducible system was explored. The Tet-On system uses a tetracycline repressor (TetR) to regulate gene expression. Whether TetR affects gene expression is dependant on the presence or absence of an inducer (tetracycline). When the inducer is present, the transcription of a certain gene is allowed as the tetracycline blocks the TetR from binding to specific tet operator (tetO) sequences located in the promoter region of the target gene. When this inducer is absent, transcription of a certain gene is repressed due to the binding of TetR to tetO.



This can stop the expression of a certain gene and thus, inhibits the translation of the protein encoded by that gene. The purpose of this study would be to test whether blocking the expression of the CagA protein decreases the risk of developing gastric cancer. The Mongolian gerbil model would be the most suitable animal model to use due to its ability to develop gastric cancer, unlike the mouse model.

This study would help verify the etiology of gastric cancer in patients with CagA positive H. pylori. It also provides proof of concept for a new treatment for this widespread bacteria which could possibly reduce the chances of people getting gastric cancer before serious symptoms present themselves. The study of gene regulation through the use of Tet inducible systems in relation with the knockdown expression of the CagA protein can provide valuable information for possible treatments in the future. The results of this study may be influential for the creation of future drugs to treat CagA positive H. pylori, as well as other gene regulatory treatments for varying diseases.

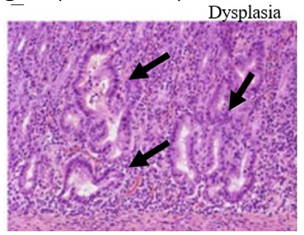
Hypothesis

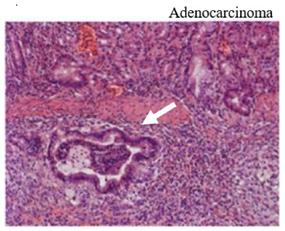
The inactivation of the CagA protein decreases the risk of developing gastric cancer caused by CagA positive H. pylori.

Aims

To establish a causal relationship through an in-vivo study

To establish a causal relationship, the CagA negative and CagA positive H. pylori bacteria will be used to infect Mongolian gerbils. Since this animal model is strictly outbred, I will need to use more gerbils to conduct my test and get statistically significant results. 72 gerbils will be split into 3 groups: 24 infected with CagA positive H. pylori bacteria, 24 infected with CagA negative H. pylori bacteria and 24 in a control group. The first two groups listed will be infected with the H. pylori via gavage. After being infected, the gerbils will be tagged and released into an animal reserve resembling their natural habitat. The tags will indicate which group the gerbils belong to. Four gerbils from each group would be sectioned every month for 6 months and gastric inflammation will be measured as it can detect the beginning stages of gastric cancer development. An haematoxylin and eosin (H&E) stain will be used to measure the inflammation of the gerbil's stomachs. With the help of a histopathologist, the inflammation will be measured on an inflammation score and Student's t-test will be used to compare the inflammation scores of the gerbil's infected with CagA positive H. pylori and those infected with CagA negative H. pylori as well as the control group which is predicted to have no inflammation.





To determine whether the inactivation of the CagA protein through the use of a Tet-On system decreases the risk of developing gastric cancer through an in-vivo study

The CagA gene, which encodes the CagA protein, will be regulated by a tetracycline repressor (TetR). TetRs block the expression of the CagA gene by binding to the tetOs located in the promoter region of the CagA gene sequence. To determine whether the inactivation of the CagA protein decreases the risk of developing gastric cancer, another in-vivo study will be carried out with the Mongolian gerbil. The expression of the CagA gene will be controlled with the use of a Tet-On system. To control whether the TetR binds to tetO, the inducer, tetracycline will be used. When tetracycline is present, the CagA gene will be transcribed. When tetracycline is absent, CagA gene expression will be blocked due to the binding of TetR to tetO. 120 gerbils will be split into 5 groups each containing 24 gerbils: a control group, a group of gerbils infected with CagA positive H. pylori not administered tetracycline, a group of gerbils infected with CagA positive H. pylori constantly administered tetracycline, a group of gerbils infected with CagA positive H. pylori administered tetracycline for only 4 of the 6 months and a group of gerbils not infected with the bacteria but constantly administered tetracycline regardless. The gerbils will be tagged to indicate which group each gerbil belongs to and released into the reserve. The tetracycline will be administered to the appropriate gerbils by incorporating this drug into their water. Four gerbils from each group will be sectioned every month for 6 months and gastric inflammation will be measured.

Outcomes and Future Directions

It is predicted that the gerbils constantly administered tetracycline will have a higher inflammation score than those administered tetracycline for only a limited period of time. This would indicate that the inactivation of the CagA protein decreases the risk of developing gastric cancer. With this test, I will be able to provide a rationale indicating that if a future drug was created to stop the activity of the CagA protein, then that drug would prevent gastric cancer if given to patients with gastric inflammation caused by CagA positive H. pylori.