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Understanding Helicobacter Pylori Pathogenesis: Prevalence And Molecular Mechanisms

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Abstract:
An estimated 4.4 billion individuals worldwide are affected by Helicobacter pylori infection, with its prevalence varying significantly across the globe, reaching its peak in Africa (70.1%) and lowest in Switzerland (18.9%). This persistence is particularly marked in developing nations and is primarily attributed to low socio-economic status and inadequate sanitation. Helicobacter pylori infection has been linked to various pathological conditions, posing a substantial challenge to the global health community.
This review offers an extensive overview of the prevalence of Helicobacter pylori infection and its correlation with disease outcomes, drawing upon numerous case studies and emphasizing the significance of detection through gastric biopsy. Additionally, this review presents recently uncovered molecular mechanisms that contribute to a deeper understanding of the underlying events that drive the epidemiological impacts. The data and mechanistic pathways presented herein contribute to more effective regulation of Helicobacter pylori infection and support the development of innovative pharmaceutical solutions for treating associated clinical conditions.

KEYWORDS: miRNA, Cancer, Helicobacter pylori, CagA.

Introduction

Helicobacter pylori, a Gram-negative bacterium primarily residing in the human stomach, is associated with a spectrum of gastrointestinal disorders, including peptic ulcer disease, chronic gastritis, gastric mucosa-associated lymphoid tissue lymphoma, vitamin B12 deficiency, anemia, gastric cancer, and even neurodegenerative diseases. Formerly regarded as a food contaminant, the groundbreaking work of Barry Marshall and Robin Warren, involving the successful isolation and cultivation of spiral bacteria, established H. pylori as a colonizer of the stomach, overturning the prior assumption of gastric sterility. This pivotal discovery led to the Nobel Prize in Physiology or Medicine in 2015, recognizing their "discovery of the bacterium Helicobacter pylori and its role in gastritis and peptic ulcer disease."

This newfound knowledge has not only aided in the development of preventive therapies but also in the formulation of drugs for treating a wide range of diseases arising from H. pylori infection, ultimately enhancing clinical management. Although the prevalence of Helicobacter pylori infection is declining in developed nations, it continues to spread rapidly in developing regions due to several factors. Diagnosis of H. pylori infection can be achieved through various tests and effectively treated with antibiotics. However, the escalating challenge lies in the rising antibiotic resistance. Compounding this issue is the current lack of preventive strategies, such as vaccination and early diagnosis, to counteract the onset of pathogenesis.

While numerous reviews on Helicobacter pylori pathogenesis have been previously published, this review prioritizes clinical study findings, providing a comprehensive perspective on the gravity of pathogenesis

resulting from H. pylori infection. It aims to introduce novel dimensions in the understanding of Helicobacter pylori pathogenesis, thereby contributing to the existing body of scientific knowledge.

Detection of CagA, VacA (S_1 and S_2), CagE,CagT, Hrg-A in Gastric biopsy tissue in patients with gastric diseases: metaplasia, dysplasia, gastric cancer, peptic ulcer, duode-nal ulcer, GERD and NERD

As per the findings of Habibullah, who conducted a screening of 84 gastric tissue samples using multiplex PCR to identify virulent strains of Helicobacter pylori along with virulent genes such as cagA, vacA (S1, S2), cagE, cagT, and HrgA. Their study revealed that in 81.7% of the samples, all five target genes were identified. Genotypes vacAs1 +ve, cagT +ve, hrgA +ve, cagE +ve, cagA +ve were more prevalent in 67.07% of the study population. The presence of cagA, cagE, cagT, and hrgA genes was observed in 81.7%, 85.4%, 92.7%, and 100% of the individuals, respectively. Notably, the vacAs1 subtype had a higher prevalence in patients with non-ulcer dyspepsia (50%) than in those with gastro-esophageal reflux disease, and overt gastrointestinal disease (78.57%).

In another study, 174 patients with clinical isolates suffering from various gastric conditions, including gastric cancer, gastric ulcer, duodenal ulcer, and non-ulcer dyspepsia, were examined for Helicobacter pylori. Among the patients, duodenal ulcer (6.9%), non-ulcer dyspepsia (7.1%), gastric cancer (85.7%), and nearly 97.8% of subjects with strains encompassing complete cag-PAI were associated with gastric ulcers.

The influence of food prepared in hygienic and unhygienic conditions on the transmission of Helicobacter pylori. Their study involved 1000 individuals, and three gastric biopsies were collected to identify the presence of Helicobacter pylori. The prevalence of Helicobacter pylori was reported at 70.8% among individuals who frequently consumed food prepared under less hygienic conditions, while it was 60% less prevalent in individuals who consumed food prepared under hygienic conditions. The study provided compelling evidence that food prepared in unhygienic conditions and a lack of personal hygiene play a pivotal role in the transmission of Helicobacter pylori bacteria.

Role Of Micro Rnas (Mirnas) In Helicobacter Pylori Pathogenesis And Gastric Cancer

Gastric carcinogenesis is a complex condition driven by a blend of genetic predisposition, epigenetic alterations in oncogenes and tumor suppressor genes, environmental factors like tobacco smoking, high-salt intake, and the presence of chronic Helicobacter pylori infection. Precisely pinpointing a single factor is often challenging, as the disease outcome results from the intricate interplay of multiple risk factors. The diagnosis of this disease at advanced stages presents a significant challenge to the healthcare system, as only palliative treatments are viable at that point, with limited curative options and a high mortality rate further exacerbating the situation.

Symptoms associated with gastric cancer include weight loss, anemia, nausea, vomiting, reduced appetite, and notably, around 40% of patients do not report dyspeptic symptoms in their medical history. The survival rates for gastric cancer are distressingly low, with 1-year and 5-year rates at a mere 42% and 24%, respectively. Helicobacter pylori-induced gastritis is the primary risk factor underlying gastric carcinogenesis, and eradicating H. pylori presents a prospective avenue for preventing the disease before it progresses to malignancy in the gastric mucosa. Accumulating evidence from animal studies, clinical observations, and research in humans supports the notion that Helicobacter pylori is a potent carcinogen in gastric adenocarcinoma.

Helicobacter pylori expresses various virulence factors that disrupt host cell biological processes, upsetting cellular homeostasis. These virulence factors detrimentally alter the immune response, creating a conducive environment for carcinogenesis. Multiple molecular mechanisms and cellular pathways are believed to be involved in gastric carcinogenesis, and the role of microRNAs (miRNAs) has gained substantial attention in recent years. miRNAs are small, non-coding RNAs that participate in RNA silencing and the post-transcriptional regulation of gene expression. Consequently, they play a significant role in cell cycle progression, proliferation, apoptosis, autophagy, necro-apoptosis, and invasion. Their role in metastasis and carcinogenesis has been thoroughly investigated, as the expression of miRNAs is frequently altered in various cancers.

While numerous reviews have been published linking virulence factors secreted by Helicobacter pylori with gastric carcinoma, limited publications address the role of miRNAs in Helicobacter pylori-mediated pathogenesis and gastric carcinogenesis. This review seeks to fill this knowledge gap.

Subsequent studies attempted to analyze miRNA expression profiles in Helicobacter pylori infection. Several studies have been conducted to assess miRNA expression profiles in gastric cancer patients infected with Helicobacter pylori. These studies have revealed that approximately 31 miRNAs were deregulated in Helicobacter pylori-infected gastric cancer patients compared to uninfected gastric cancer patients. The link between Helicobacter pylori-induced inflammation and miRNA levels can be established through factors such as neutrophil infiltration and chronic inflammation. MiRNA expression levels have been associated with the degree of inflammation, although no association was found with intestinal metaplasia or glandular atrophy. Subsequent studies suggest that Helicobacter pylori infection may influence miRNA levels in gastric mucosa, thereby impacting the host's immune response. The miRNAs that are aberrantly expressed in response to infection differ from those expressed in various gastric diseases. Some key miRNAs that were found to be dysregulated in both Helicobacter pylori infection and gastric malignancies include downregulated miRNAs such as miRNA-31, miRNA-203, miRNA-141, miRNA-375, miRNA-449, miRNA-210, and overexpressed miRNAs like miRNA-155, miRNA-17, miRNA-21, and miRNA-146a. These miRNAs play a crucial role in gastric neoplasms and have become significant molecular markers in Helicobacter pylori pathogenesis.

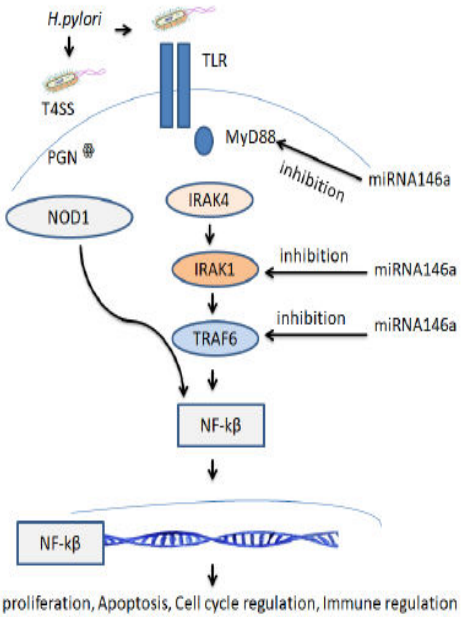

Figure1 TLR, NF-κB and NOD signaling pathways

Role Of Mirnas In Dysregulation Modulating Host Inflammatory Responses During Helicobac-Ter Pylori Infection

During Helicobacter pylori infection, host cells engage the innate immune system through Toll-like receptors (TLRs) and NfkB signaling. Notably, NfkB signaling is activated when Peptidoglycan (PNG) released by Helicobacter pylori is injected, subsequently leading to the activation of IL-8. This activation of IL-8 has a profound impact on cell cycle, cell proliferation, and cell survival, ultimately contributing to carcinogenesis.

Previously, several studies have elucidated the role of miRNAs in regulating both the innate and adaptive immune systems. Helicobacter pylori infection manipulates miRNA expression to circumvent the host immune response and sustain its pathogenicity. In the context of Helicobacter pylori infection, miR-155 and miRNA-146a have been identified as key players in the negative regulation of the inflammatory response. Aberrant expression of miRNA-146a has been associated with the development of gastric cancer and the downregulation of the innate immune system. The upregulation of miRNA-146a suppresses levels of TNF-α, IL-1β, and IL-8, which are pivotal in both innate and adaptive immune responses, specifically in promoting inflammation.

3.1 Role of miRNAs in Cell Invasion and Metastasis in the Helicobacter Pylori Infection

Helicobacter pylori infection leads to the downregulation of miRNA-449, which typically targets the Met proto-oncogene. The Met proto-oncogene encodes hepatocyte growth factor receptors. When Met proto-oncogene is deregulated, it transforms into an oncogene, setting the stage for malignancy, angiogenesis, increased cell proliferation, and metastasis.

Additionally, miRNA-21, which is found at elevated levels in gastric cancer, plays a role in enhancing gastric cell invasiveness. Furthermore, miRNA-21 is implicated in angiogenesis and metastasis by influencing the function of matrix metalloproteinases (MMPs). In the context of Helicobacter pylori infection, the upregulation of miRNA-21 leads to increased MMP levels (specifically MMP-1, MMP-2, MMP-3), thereby contributing to pathogenesis and carcinogenesis.

Conversely, miRNA-106a levels are heightened in cancer cells and are associated with a metastatic phenotype. However, in contrast to these findings, it has been reported that miRNA-218 expression levels are down regulated in gastric cancer, and this downregulation is linked to invasion and metastasis. This effect may be attributed to the activation of ROBO1 signaling by its receptors, which results in enhanced cell migration.

Role Of Mirnas In Apoptosis And Cell Survival

The overexpression of oncomiRs plays a crucial role in promoting gastric cancer cell survival by regulating pro-apoptotic proteins, notably Bcl-2. Several miRNAs, including miRNA-93, miRNA-25, and miRNA-106b, not only govern the cell cycle but also mediate apoptosis by inhibiting the expression of the pro-apoptotic Bim protein. Furthermore, miRNA-130b has been observed to target RUNX3, resulting in the inactivation of the Bim protein, a significant apoptotic marker in gastric cancers.

miRNA-150 plays a role in regulating EGR2 function, which is associated with apoptosis, by activating pro-apoptotic factors like BNIP3L and Bak. In contrast, miRNA-200bc/429 levels are decreased in gastric cancer cells and are found to target BCL2 and XIAP, leading to reduced expression and increased apoptosis.

Additional miRNAs, such as miRNA-101 and miRNA-512-5p, influence the BCl-2 family. In gastric cells, the levels of these miRNAs are decreased, resulting in elevated levels of Mcl-1. Furthermore, miRNA-449 levels are decreased in Helicobacter pylori-infected gastric cells, and their levels are absent in gastric tumors. Research by Xiao et al. (2009) indicated that miR-155 levels increase during Helicobacter pylori infection and carcinogenesis, leading to the deregulation of apoptosis by down regulating FADD. MiRNA-21, a well-known oncomiR involved in the development of various cancers, including gastric cancer, exhibits elevated expression in Helicobacter pylori-infected gastric cells. This disruption in the balance between apoptosis and proliferation promotes cell proliferation while inhibiting apoptosis.

4.1 Modulating Cytoskeleton Proteins Role in Heli-cobacter Pylori Infection

The reorganization and turnover of the actin cytoskeleton in the cytosol is a highly dynamic process that responds to various stimuli and plays a significant role in critical physiological processes, including cell adhesion to neighboring cells, cell invasion, extracellular matrix interactions, migration, maintenance of cell shape, and phagocytosis. Actin typically exists in two forms: filamentous actin and monomeric globular actin, with the latter formed by the polymerization of G-actin monomers in a specific direction. A wide range of upstream signaling molecules, such as E-cadherin, integrins, components of the extracellular matrix (ECM), lysophosphatidic acid, and tumor necrosis factor-alpha, mediate the transmission of extracellular information to the actin cytoskeleton. This allows for rapid responses to changes in the cellular environment, especially in processes like cell migration where coordinated reorientation of the actin lattice is vital.

One of the notable characteristics of Helicobacter pylori infection in gastric epithelial cells is the dynamic rearrangement of the actin cytoskeleton, which promotes cell migration and invasive growth. Helicobacter pylori employs multiple mechanisms to reshape the cytoskeleton, with the most extensively studied ones being mediated by the effector proteins CagA and T4SS. These mechanisms trigger signaling transduction pathways within host cells, targeting various proteins such as GTPases, adaptor proteins, kinases, actin-binding proteins, and others involved in actin lattice regulation. Although multiple mechanisms contribute to cell mobility, one that has been examined in detail is discussed here.

Helicobacter pylori expresses CagL at the tip of the T4SS, and CagL can directly bind to β1 integrins found on gastric epithelial cells. This interaction activates β1 integrin, leading to increased FAK and Src activity during the early stages of Helicobacter pylori infection. Subsequently, FAK phosphorylates paxillin, contributing to the activation of c-Abl-phosphorylated Crk signaling. The regulation of the actin cytoskeleton is orchestrated by SFKs, FAK, and Abl kinase-mediated activation of Crk proteins via the DOCK180/Rac1/WAVE/Arp2/3 pathway, facilitating epithelial cell migration.

The binding of CagL to integrins results in the translocation of CagA, a pathogenic factor of Helicobacter pylori, into the host cell cytoplasm via a type IV secretion process. In the presence of Src kinase family members, CagA undergoes rapid phosphorylation on specific EPIYA sequence repeats, forming tyrosine-phosphorylated CagA (CagAP-Tyr). CagAP-Tyr then interacts with various host cell factors (X) in its phosphorylated and non-phosphorylated forms. The phosphorylation of CagA sets off several downstream effects, including the dephosphorylation of unidentified cellular proteins, rearrangements of the host cell actin cytoskeleton, and cell scattering. This experiment provided critical evidence linking Helicobacter pylori infection to cytoskeletal rearrangement. Additionally, tyrosine-phosphorylated CagA interacts with Shp-2 and Csk to inactivate FAK and Src in the later stages of Helicobacter pylori infection. The inactivated Src is then replaced by activated Abl kinases to maintain CagA phosphorylation, but the inactive Src leads to the tyrosine dephosphorylation of Src target molecules such as ezrin, vinculin, and cortactin. Cortactin is subsequently serine-phosphorylated by Helicobacter pylori-activated ERK1/2 kinases, playing a pivotal role in cell elongation.

In addition to CagA, the VacA cytotoxin is also implicated in cell adhesion and cytoskeleton rearrangement. It has been demonstrated that VacA binds to fibronectin in vitro in a dose-dependent manner, indicating that VacA may interact with fibronectin, influencing integrin receptor-induced cell signaling and cytoskeleton-dependent cell functions.

Nix, Pink, Parkin, Lamp, And Other Autopha-Gy And Mitophagy Proteins In Helicobacter Py-Lori Infection

NIX, also known as BNIP3-like, plays a crucial role in both cell death and autophagy, two essential cellular processes responsible for disposing of waste and preventing pro-carcinogenic activities. A study has revealed that in the context of Helicobacter pylori infection, miR-30d, which is upregulated, contributes to increased intracellular survival of Helicobacter pylori by inhibiting several core proteins in the autophagy pathway. These targeted proteins include ATG2B, ATG12, ATG5, BNIP3L (NIX), and BECN1, collectively forming the core of the autophagy process. Additionally, miR-30b, which is also upregulated during chronic Helicobacter pylori infection, targets ATG1 and Beclin-1 (BECN1), leading to the inhibition of autophagosome formation. This, in turn, provides a favorable environment for the survival and growth of bacteria.

Mitophagy, the selective sequestration of mitochondria into autophagosomes, is initiated by the loss of mitochondrial membrane potential resulting from membrane disruption and ion leakage. Three key proteins involved in this process are NIX, PINK1, and Parkin. Under normal conditions, PINK1 (Phosphatase and Tensin homologue-induced kinase 1) is targeted to mitochondria but rapidly undergoes proteolysis. However, when mitochondria are damaged and their membrane potential is lost, PINK1 accumulates on the mitochondrial surface due to inhibited proteolysis. PINK1 on the outer mitochondrial membrane is a crucial component for recruiting the E3 ubiquitin ligase Parkin, ultimately leading to mitophagy. Subsequently, the BCL2-related protein NIX (Nip3-like protein X; BNIP3L), located on the mitochondrial outer membrane, attracts other components of the autophagic machinery.

During Helicobacter pylori infection, the bacterium injects the virulent factor VacA into the cytosol of gastric epithelial cells. VacA permeates the mitochondrial membrane, disrupting its membrane potential, which, in turn, attracts the PINK1-Parkin system, inciting mitophagy and autophagy processes.

Conclusion

Helicobacter pylori infection is significantly associated with various pathologies, and its widespread prevalence and detrimental consequences underscore the urgency to address this illness promptly. While comprehensive research is essential to uncover the numerous molecular mechanisms involved.

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