**Name of Journal:** *World Journal of Clinical Cases*

**Manuscript NO:** 78629

**Manuscript Type:** MINIREVIEWS

**Role of nickel-regulated small RNA in modulation of *Helicobacter pylori* virulence factors**

Freire de Melo F *et al*. Small regulatory RNA NikS and *Helicobacter pylori*

Fabrício Freire de Melo, Hanna Santos Marques, Fabian Fellipe Bueno Lemos, Marcel Silva Luz, Samuel Luca Rocha Pinheiro, Lorena Sousa de Carvalho, Cláudio Lima Souza, Márcio Vasconcelos Oliveira

**Fabrício Freire de Melo,** **Fabian Fellipe Bueno Lemos, Marcel Silva Luz, Samuel Luca Rocha Pinheiro, Lorena Sousa de Carvalho, Cláudio Lima Souza,** **Márcio Vasconcelos Oliveira,** Institution Multidisciplinar em Saúde, Universidade Federal da Bahia, Vitória da Conquista 45029-094, Brazil

**Hanna Santos Marques,** Campus Vitória da Conquista, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista 45083-900, Brazil

**Author contributions:** All authors equally contributed to this paper with conception and design of the study, literature review and analysis, manuscript drafting, critical revision, and editing, and approval of the final version; all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Supported by** CNPq Brazil (National Council for Scientific and Technological Development) - FFM, No. 317005/2021-09.

**Corresponding author: Fabrício Freire de Melo, PhD, Professor,** Institution Multidisciplinar em Saúde, Universidade Federal da Bahia, Rua Horminio Barros 58, Vitória da Conquista 45029-094, Brazil. freiremeloufba@gmail.com

**Received:** July 7, 2022

**Revised:** August 14, 2022

**Accepted:** September 6, 2022

**Published online:**

**Abstract**

*Helicobacter pylori (H. pylori)* is a Gram-negative bacterium that infects about half of the world's population. *H. pylori* infection prevails by several mechanisms of adaptation of the bacteria and by its virulence factors including the cytotoxin associated antigen A (CagA). CagA is an oncoprotein that is the protagonist of gastric carcinogenesis associated with prolonged *H. pylori* infection. In this sense, small regulatory RNAs (sRNAs) are important macromolecules capable of inhibiting and activating gene expression. This function allows sRNAs to act in adjusting to unstable environmental conditions and in responding to cellular stresses in bacterial infections. Recent discoveries have shown that nickel-regulated small RNA (NikS) is a post-transcriptional regulator of virulence properties of *H. pylori*, including the oncoprotein CagA. Notably, high concentrations of nickel cause the reduction of NikS expression and consequently this increases the levels of CagA. In addition, NikS expression appears to be lower in clinical isolates from patients with gastric cancer when compared to patients without. With that in mind, this minireview approaches, in an accessible way, the most important and current aspects about the role of NikS in the control of virulence factors of *H. pylori* and the potential clinical repercussions of this modulation.

**Key Words:** *Helicobacter pylori*; Small regulatory RNAs; Nickel-regulated small RNA; Virulence factors; Cytotoxin associated antigen A; Gastric cancer

Freire de Melo F, Marques HS, Fellipe Bueno Lemos F, Silva Luz M, Rocha Pinheiro SL, de Carvalho LS, Souza CL, Oliveira MV. Role of nickel-regulated small RNA in modulation of *Helicobacter pylori* virulence factors. *World J Clin Cases* 2022; In press

**Core Tip:** This paper aims to review current information about the role of nickel-regulated small RNA (NikS) in the modulation of main *Helicobacter pylori* virulence factors, specially cytotoxin associated antigen A (CagA), which is crucial to gastric cancer development. Here we explore what is the most important about the epigenetic processes involved in the interaction between nickel levels, NikS, and CagA and their potential clinical repercussions.

**INTRODUCTION**

*Helicobacter pylori (H. pylori)* is a microaerophilic, Gram-negative, helical-shaped bacterium that inhabits the gastric environment of 60.3% of the world’s population[1,2]. The infection is associated with the development of chronic gastritis, gastric and duodenal peptic ulcer, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma[3]. In order to achieve a successful colonization, *H. pylori* must take advantage of some pathogenicity mechanisms, such as motility, adherence, manipulation of the gastric microenvironment, and virulence factors, of which we highlight cytotoxin associated antigen A (CagA), vacuolating cytotoxin A (VacA), and outer membrane proteins (OMPs). In this sense, the classification of this bacterium as a class I carcinogen is mostly due to the pro-oncogenic role of these virulence factors, especially CagA[4]. This oncoprotein is capable of inducing genetic, epigenetic, and morphological changes in gastric cells, including alterations of cell polarity and cytoskeleton, leading to "hummingbird" phenotype and promotion of genomic instability, which favor carcinogenesis[5-8]. In this regard, it has been recently discovered that nickel-regulated small RNA (NikS) plays a key role in gene expression during *H. pylori* infection, given that, through base pairing, it is able to repress CagA and VacA at the post-transcriptional level[9,10]. Notably, the expression of this sRNA is modulated by the nickel-responsive transcriptional regulator (NikR), consequently rendering *H. pylori* virulence factor expression dependent on nickel levels[10]. Therefore, considering that these virulence factors are associated with the onset of a carcinogenic process, the possible correlation between NikS expression and the development of gastric diseases secondary to *H. pylori* infection, including gastric carcinoma and MALT lymphoma, is indisputable. The present paper is a minireview that aims to gather, through an accessible perspective, important and current information regarding the role of a small regulatory RNA (sRNA), NikS, in the control of virulence factors of *H. pylori*, addressing the epigenetic processes involved and the potential clinical repercussions of this modulation.

**SMALL REGULATORY RNAs**

sRNAs are effective regulatory macromolecules that are able to modulate protein expression and function in response to environmental factors, such as pH, temperature, and metabolite concentration[11]. These post-transcriptional regulators of gene expression play a pivotal role in successful bacterial colonization and stress response, given that they enable metabolic adaptation to the host microenvironment and regulate the expression of virulence factors[12]. The three main classes of sRNAs comprise: (1) *Cis*-encoded antisense sRNAs; (2) *Trans*-encoded sRNAs; and (3) sRNAs that modify protein activity (Table 1)[13]. *Cis-*encoded antisense sRNAs are synthesized from the complementary strand of the mRNA that they modulate. Indeed, these regulators have been strongly associated with the repression of bacterial toxic proteins, through inhibition of primer maturation, transcriptional attenuation, and translational repression or promotion of RNA degradation[14,15]. In contrast, *trans*-encoded sRNAs are transcribed from a promoter somewhere else on the bacterial chromosome and are only partly complementary to their target mRNAs[16]. In general, this class of sRNAs mainly interfere with translational initiation and/or elongation, *e.g.*, by pairing to ribosome binding sites or translational enhancers. The translation impairment frequently leads to degradation of the mRNA, since it can be more easily targeted by ribonucleases (RNases)[17]. Lastly, sRNAs that modify protein activity are known to modulate protein activity by a mimicking mechanism and thus compete with RNA and DNA targets[13]. These mechanisms are described to utilize several auxiliary proteins, including RNases and ribosome-binding proteins. The Hfq RNA chaperon protein, for example, is strongly associated with the base-pairing between *trans*-encoded RNAs and their target mRNAs, hence acting in the regulation of virulence factors in Gram-negative bacteria[18].

Thus, as mentioned above, post-transcriptional regulatory macromolecules known as sRNAs can stimulate or inhibit gene expression, playing a key role in bacterial infection through its three distinct groups, ranging from preventing ribosomal binding to modifying protein activities.

**ROLE OF SRNAS IN BACTERIAL PATHOGENS**

Hosts have evolved refined techniques to sense and react against pathogens, such as recognition of pathogen-associated molecular patterns that promotes activation of Toll-like receptors[19]. In this sense, the decisive pathogen’s actions for the infection's success are a faster response and efficient adjustment to a continuously changing hostile environment. Those responses are regulated by sRNAs, due to their flexibility to target a plethora of genes or transcription factors, influencing many ambits of expression and responses to environmental stress[20]. Besides this, sRNAs do not require translation, which means a lower energy consumption for the pathogen[21].

As mentioned above, when entering the host, the bacterium faces diverse innate immunity barriers including: Temperature, pH, changes in nutrient availability, and physical barriers. It is during these circumstances when the varied toolkit of activities of sRNAs perform their roles for pathogen’s survival[22]. These functions can be grouped in two main related fields: Management of biological processes, such as temperature response, biofilm formation, quorum sensing and virulence, and regulation of responses *vs* host barriers to infection, *e.g*., acidic pH, inflammation, and nutritional immunity[21].

Regarding the temperature response, it is known that pathogens have to evade the hyperthermia feedback during inflammation[23]. According to studies, an intense involvement of sRNAs in temperature adaptation has been noticed, helping the bacteria to regulate faster their physiology facing environmental thermal disorders[6]. For example, in analysis of *Borrelia burgdorferi*, responsible for Lyme disease, it was observed that a large set of sRNAs were entangled in regulation of genes involved in adaptation to pyrexia and identification of the molecular scheme to trigger according to environment[24].

Concerning biofilm formation, it is established that it requires coordination of quorum sensing mechanisms to succeed. In *P. aeruginosa*, researchers found a group of sRNAs, specially RhlS, that bind to the 5’ untranslated region (UTR) of *rhlI* mRNA and stabilizes it, which is Hfq dependent, resulting in the activation of biofilm genes according to the state of infection and offering additional protection against the host immune system[25].

The role of sRNAs in pathogen’s virulence is also well-represented in *P. aeruginosa.* The gene *RpoS* commands a diverse number of virulence related genes, and its translation has been observed to be regulated by the sRNA ReaL, also a Hfq dependent base pairing apparatus, refining the bacterial virulence factors[26].

In the second category group, one of the first barriers to infection is the acidic pH. To overcome the acidic environment of the human stomach and to reach out host cells, for example, it involves several colonization factors like motility and chemotaxis[14]. In this context, *H. pylori* has sRNAs like RepG and 5’*ureB* that regulate expression of chemotaxis receptors contributing to stomach colonization[27,28] and linking urease production to surrounding pH[29].

A recent study reported that extreme conditions related to the stress caused by the host inflammatory response during oxidative burst, induces a heavy expression of RsaC, a sRNA of *Staphylococcus aureus,* avoiding the synthesis of an ineffective enzyme (sodA)[30]. The RsaC attaches to the start codon of the *sodA* mRNA, committed in protection against reactive oxygen species, leading to repression of this enzyme and allowing the transcription of a second enzyme, sodM, that uses iron as cofactor instead of manganese, recovering the oxidative protection[21]. Therefore, it is firmly established that sRNAs are key players in the adjustment to unstable environmental conditions and response to distinct cellular stresses.

**POST-TRANSCRIPTIONAL REGULATION OF *H. pylori* VIRULENCE FACTORS BY NikS**

Recently, it was reported that the post-transcriptional regulation of *H. pylori* virulence factors depends on NikS. NikS has been described to act through base pairing in the 5′ UTR or coding sequence (CDS) of target mRNAs to repress gene expression, including the CagA oncoprotein[31]. In the past, NikSwas believed to act as a *cis*-acting sRNA, however, Eisenbart *et al*[10] analyzed nucleotides upstream of transcriptional start sites of putative sRNAs and antisense RNAs and observed that NikS expression changed according to the length of a stretch of thymines (T) in the promoter region and these findings contrasted with the premise that NikS acted as a *cis*-acting sRNA[32]. Once it has been clarified that *H. pylori* also has *trans* sRNAs, it is important to highlight that they usually form a base pairing in the 5' UTR or RNA encoding target mRNAs modulating gene expression at the post-transcriptional level[18]. Eisenbart *et al*[10] also demonstrated in their NikS study that the thymine stretch of the *NikS*-10 box varies in different strains of *H. pylori* and this in turn has the potential to alter the spacing between box-10 and other promoter elements. Subsequently, the authors employed Northern blot analysis in the study which revealed differences in NikS expression from 16 to 7 Ts with the lowest expression at 12 Ts. This finding further corroborated the idea that NikS transcription suffers effects from the length variation of hypermutable single sequence repeats[10].

In this sense, Eisenbart *et al*[10]demonstrated that NikS represses the expression of the main virulence factors produced by *H. pylori* (CagA and VacA) and three additional factors (HofC, HorF, and HPG27\_1238) related to the pathogenicity of the G27 strain, through interactions of base pairing[6]. Completely, Kinoshita-Daitoku *et al*[32] were responsible for one of the main current studies on NikS. They identified eight factors downregulated by NikS including CagA, HofC, HELPY\_1262, HP0410, HorB, OMP14, HopE, and HP1227 and noted that the impact on the regulation of CagA expression stood out among the other factors[32]. Since the regulatory process performed by NikS acts on target mRNAs repressing or activating post-transcriptional gene expression, it is important to say that *H. pylori* resorts to endoribonucleases such as RNase III so that the sRNAs degrade the target mRNA leading to translation inhibition[18]. In this aspect, Kinoshita-Daitoku *et al*[32] also reported that NikS regulates the oncoprotein CagA by binding to multiple binding sequences present in its CDS region causing mRNA degradation by RNase III. Furthermore, the authors observed that NikS binding to CagA mRNA regulated the amount of interleukin-8 (IL-8) secreted in *H. pylori* infection, indicating that NikS acts in the functional control of CagA[32].

Moreover, it is known that VacA is a multifunctional toxin, which stands out mainly for cell vacuolation. In this sense, the repression of this virulence factor can impact the persistence of *H. pylori* infection[33]. The expression of OMPs in *H. pylori* strains, in turn, also contributes to bacterial pathogenicity through different mechanisms, such as adhesion, penetration of the defense barrier, and evasion of the immune system. In this sense, by repressing the biosynthesis of OMPs, such as HofC and HorF, the adhesion and colonization processes can be compromised[34].

Finally, it is important to mention that the integration between nickel availability and NikS expression is performed through the NikR[35]. When cytoplasmic nickel concentrations reach a certain threshold, the NikR protein represses nickel import mechanisms in order to control the availability of the metal and achieve the necessary homeostasis[36]. However, NikR also regulates the expression of other genes associated with nickel homeostasis by binding to NikR operators in the promoter or upstream regions[37]. For example, NikR has been shown to bind directly to the NikS promoter, being a key player in controlling NikS expression. In addition, researchers analyzed how strains with varying sizes of T stretch in the promoter region responded to changes in nickel concentration or NikR deletion. Their results showed that the addition of nickel caused a 2- to 10-fold decrease in NikS expression while the deletion of NikR led to a 2-fold increase in NikS levels[6]. In this way, NikS is transcriptionally repressed by nickel *via* NikR since NikR is able to ration nickel availability and reduced concentrations of this metal imply higher levels of NikS, thereby inhibiting the expression of *H. pylori* virulence factors (*e.g.* CagA) (Figure 1). Furthermore, NikS expression changed in nickel-added strains according to different T stretch lengths, but there was no direct correlation between these two factors[6].

**POTENTIAL CLINICAL REPERCUSSION OF MODULATION OF CagA EXPRESSION VIA POST-TRANSCRIPTIONAL CONTROL BY NikS**

CagA is a translocated effector protein that induces morphofunctional modifications in gastric epithelial cells and an inflammatory response, which lead, respectively, to increased bacterial adhesion and nutrient uptake[38,39] (Figure 2). This oncoprotein is encoded by the *Cag*A gene, which is a marker of the *cag* PAI, a 40 kb DNA fragment that contains about 31 genes and is present in more virulent strains of *H. pylori.* Some genes on this mobile region of the chromosome encode proteins that form a type IV secretion system, which is responsible for translocating the CagA protein into the cytoplasm of host cells[40-44]. The C-terminal region of CagA has a variable number of Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, which serve as tyrosine phosphorylation sites. Once it reaches the host cell cytosol, the EPIYA sites of the effector protein are phosphorylated by Src family kinases such as s-Src, Fyn, Lyn, and Yes or by Abl kinases[45,46]. Afterward, CagA acts as a promiscuous scaffold protein that simultaneously disturbs multiple intracellular signaling cascades, involved in regulation of a large range of cellular processes, including proliferation, differentiation, and apoptosis[47].

Phosphorylated CagA is able to stimulate cell proliferation through the activation of promitogenic signaling pathways. Among these, we highlight the activation of the ERK-MAPK pathway through binding to the Src-homology domain 2 and consequent activation of SHP-2[48]. This process also leads to alterations in the cytoskeleton, which induces host cell elongation and change to the recognized "hummingbird" phenotype[7,8,49]. In addition, CagA causes disruption of cell polarity by interaction with the serine-threonine kinase Par-1b and disturbs cell junction-mediated functions[8,47]. This virulence factor is also able to reduce apoptosis in gastric epithelial cells, through the inhibition of tumor suppressor factors such as p53 and RUNX3[50-53]. These direct effects of CagA on epithelial cells could be related to the development of precancerous lesions, since carcinoma development has been observed in animal models even in the absence of inflammation[54-56]. Nevertheless, this effector protein was reported to be able to induce the transcription factor NF-κB and IL-8, which are crucial determinants of chronic inflammation and thus of the pathogenesis of peptic ulcer and gastric cancer[43,57]. At last, CagA also induces genetic and epigenetic alterations in the host cells that lead to a pro-carcinogenic environment[7].

In this regard, some authors suggest that the modulation of CagA expression *via* post-transcriptional control by NikS favors a more delicate equilibrium between induction of morphofunctional changes and inflammatory response with its regulation, so as to establish a balance between eradication and nutrient uptake[54]. Using *in vitro* infection studies, Eisenbart *et al*[10] demonstrated that possibly due to increased CagA expression, G27 strains deficient in NikS show higher numbers of intracellular bacteria, greater “hummingbird” phenotype induction in host cells, as well as increased epithelial barrier disruption. From these findings, it is possible to infer that higher expression of NikS and, consequently, lower synthesis and translocation of the oncoprotein, would reduce the CagA-induced morphofunctional alterations in the host cell, such as apoptosis of epithelial cells, loss of cell polarity, and chronic NF-κB-dependent inflammatory response, along with carcinogenesis. Interestingly, it was further reported by Kinoshita-Daitoku *et al*[32] that NikS expression is lower in clinical isolates from gastric cancer patients than in isolates derived from non-cancer patients, while the expression of NikS-targeted virulence factors, including CagA, is higher in isolates from gastric cancer patients. Therefore, it is possible to suggest a possible correlation between NikS expression and the onset of peptic ulcer and gastric malignancies, such as gastric carcinoma and MALT lymphoma secondary to *H. pylori* infection.

**FUTURE PERSPECTIVES ON REGULATION OF NIKS OVER *H. pylori* VIRULENCE**

Considering that the regulatory role of NikS on *H. pylori* virulence factors is a recent discovery, there are still few studies on the subject. However, the broad action of NikS on these virulence factors may be strongly related to the risk of diseases derived from *H. pylori* infection. In this sense, one of the aims of our group is to evaluate whether the variation of the number of Ts in the promoter region of the *NikS* gene is associated with the risk of duodenal ulcer or gastric carcinoma in adults. However, further studies are still required for better understanding the role of NikS in the pathogenesis of *H. pylori*, as well as its possible relationship with other genes.

**CONCLUSION**

In summary, recent findings on sRNA-mediated regulation of *H. pylori* infection revealed that increased nickel concentrations lead to reduced NikS expression and this in turn up-regulates CagA levels. There is still much to be clarified about the regulatory properties involved in *H. pylori* infection. However, it is notable that CagA is the protagonist of gastric carcinogenesis and a deeper understanding of the interaction between this virulence factor and sRNAs such as the nickel-dependent NikS is of utmost importance for a broader understanding of the mechanisms involved in the control mediated by RNAs in *H. pylori* and their association with gastric malignancies and other clinical conditions. Finally, given the potential for heterogeneity of the bacterium, evolution of its strains, its pathogenicity, and the emergence of therapeutic resistance of this pathogen, it is essential to periodically reassess the molecular issues of the infection to achieve advances in the diagnosis and treatment of the disease.

**REFERENCES**

1 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186 [PMID: 12374879 DOI: 10.1056/NEJMra020542]

2 **Sjomina O**, Pavlova J, Niv Y, Leja M. Epidemiology of Helicobacter pylori infection. *Helicobacter* 2018; **23 Suppl 1**: e12514 [PMID: 30203587 DOI: 10.1111/hel.12514]

3 **Watari J**, Chen N, Amenta PS, Fukui H, Oshima T, Tomita T, Miwa H, Lim KJ, Das KM. Helicobacter pylori associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. *World J Gastroenterol* 2014; **20**: 5461-5473 [PMID: 24833876 DOI: 10.3748/wjg.v20.i18.5461]

4 **Alipour M**. Molecular Mechanism of Helicobacter pylori-Induced Gastric Cancer. *J Gastrointest Cancer* 2021; **52**: 23-30 [PMID: 32926335 DOI: 10.1007/s12029-020-00518-5]

5 **Higashi H**, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M. SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. *Science* 2002; **295**: 683-686 [PMID: 11743164 DOI: 10.1126/science.1067147]

6 **Hatakeyama M**. Helicobacter pylori CagA and gastric cancer: a paradigm for hit-and-run carcinogenesis. *Cell Host Microbe* 2014; **15**: 306-316 [PMID: 24629337 DOI: 10.1016/j.chom.2014.02.008]

7 **Saadat I**, Higashi H, Obuse C, Umeda M, Murata-Kamiya N, Saito Y, Lu H, Ohnishi N, Azuma T, Suzuki A, Ohno S, Hatakeyama M. Helicobacter pylori CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. *Nature* 2007; **447**: 330-333 [PMID: 17507984 DOI: 10.1038/nature05765]

8 **Zeaiter Z**, Cohen D, Müsch A, Bagnoli F, Covacci A, Stein M. Analysis of detergent-resistant membranes of Helicobacter pylori infected gastric adenocarcinoma cells reveals a role for MARK2/Par1b in CagA-mediated disruption of cellular polarity. *Cell Microbiol* 2008; **10**: 781-794 [PMID: 18005242 DOI: 10.1111/j.1462-5822.2007.01084.x]

9 **Sharma CM**, Hoffmann S, Darfeuille F, Reignier J, Findeiss S, Sittka A, Chabas S, Reiche K, Hackermüller J, Reinhardt R, Stadler PF, Vogel J. The primary transcriptome of the major human pathogen Helicobacter pylori. *Nature* 2010; **464**: 250-255 [PMID: 20164839 DOI: 10.1038/nature08756]

10 **Eisenbart SK**, Alzheimer M, Pernitzsch SR, Dietrich S, Stahl S, Sharma CM. A Repeat-Associated Small RNA Controls the Major Virulence Factors of Helicobacter pylori. *Mol Cell* 2020; **80**: 210-226.e7 [PMID: 33002424 DOI: 10.1016/j.molcel.2020.09.009]

11 **Gripenland J**, Netterling S, Loh E, Tiensuu T, Toledo-Arana A, Johansson J. RNAs: regulators of bacterial virulence. *Nat Rev Microbiol* 2010; **8**: 857-866 [PMID: 21079634 DOI: 10.1038/nrmicro2457]

12 **Gimpel M**, Brantl S. Dual-function small regulatory RNAs in bacteria. *Mol Microbiol* 2017; **103**: 387-397 [PMID: 27750368 DOI: 10.1111/mmi.13558]

13 **Gottesman S**, Storz G. Bacterial small RNA regulators: versatile roles and rapidly evolving variations. *Cold Spring Harb Perspect Biol* 2011; **3** [PMID: 20980440 DOI: 10.1101/cshperspect.a003798]

14 **Fozo EM**, Hemm MR, Storz G. Small toxic proteins and the antisense RNAs that repress them. *Microbiol Mol Biol Rev* 2008; **72**: 579-589, Table of Contents [PMID: 19052321 DOI: 10.1128/MMBR.00025-08]

15 **Brantl S**. Regulatory mechanisms employed by cis-encoded antisense RNAs. *Curr Opin Microbiol* 2007; **10**: 102-109 [PMID: 17387036 DOI: 10.1016/j.mib.2007.03.012]

16 **Brantl S**, Müller P. *Cis*- and *Trans*-Encoded Small Regulatory RNAs in *Bacillus subtilis*. *Microorganisms* 2021; **9** [PMID: 34576762 DOI: 10.3390/microorganisms9091865]

17 **Svensson SL**, Sharma CM. Small RNAs in Bacterial Virulence and Communication. *Microbiol Spectr* 2016; **4** [PMID: 27337442 DOI: 10.1128/microbiolspec.VMBF-0028-2015]

18 **Melamed S**, Peer A, Faigenbaum-Romm R, Gatt YE, Reiss N, Bar A, Altuvia Y, Argaman L, Margalit H. Global Mapping of Small RNA-Target Interactions in Bacteria. *Mol Cell* 2016; **63**: 884-897 [PMID: 27588604 DOI: 10.1016/j.molcel.2016.07.026]

19 **Ausubel FM**. Are innate immune signaling pathways in plants and animals conserved? *Nat Immunol* 2005; **6**: 973-979 [PMID: 16177805 DOI: 10.1038/ni1253]

20 **González Plaza JJ**, Hulak N, Zhumadilov Z, Akilzhanova A. Fever as an important resource for infectious diseases research. *Intractable Rare Dis Res* 2016; **5**: 97-102 [PMID: 27195192 DOI: 10.5582/irdr.2016.01009]

21 **González Plaza JJ**. Small RNAs as Fundamental Players in the Transference of Information During Bacterial Infectious Diseases. *Front Mol Biosci* 2020; **7**: 101 [PMID: 32613006 DOI: 10.3389/fmolb.2020.00101]

22 **Chakravarty S**, Massé E. RNA-Dependent Regulation of Virulence in Pathogenic Bacteria. *Front Cell Infect Microbiol* 2019; **9**: 337 [PMID: 31649894 DOI: 10.3389/fcimb.2019.00337]

23 **Casadevall A**, Pirofski LA. The damage-response framework of microbial pathogenesis. *Nat Rev Microbiol* 2003; **1**: 17-24 [PMID: 15040176 DOI: 10.1038/nrmicro732]

24 **Popitsch N**, Bilusic I, Rescheneder P, Schroeder R, Lybecker M. Temperature-dependent sRNA transcriptome of the Lyme disease spirochete. *BMC Genomics* 2017; **18**: 28 [PMID: 28056764 DOI: 10.1186/s12864-016-3398-3]

25 **Thomason MK**, Voichek M, Dar D, Addis V, Fitzgerald D, Gottesman S, Sorek R, Greenberg EP. A *rhlI* 5' UTR-Derived sRNA Regulates RhlR-Dependent Quorum Sensing in Pseudomonas aeruginosa. *mBio* 2019; **10** [PMID: 31594819 DOI: 10.1128/mBio.02253-19]

26 **Thi Bach Nguyen H**, Romero A D, Amman F, Sorger-Domenigg T, Tata M, Sonnleitner E, Bläsi U. Negative Control of RpoS Synthesis by the sRNA ReaL in *Pseudomonas aeruginosa*. *Front Microbiol* 2018; **9**: 2488 [PMID: 30420839 DOI: 10.3389/fmicb.2018.02488]

27 **Pernitzsch SR**, Tirier SM, Beier D, Sharma CM. A variable homopolymeric G-repeat defines small RNA-mediated posttranscriptional regulation of a chemotaxis receptor in Helicobacter pylori. *Proc Natl Acad Sci U S A* 2014; **111**: E501-E510 [PMID: 24474799 DOI: 10.1073/pnas.1315152111]

28 **Baldwin DN**, Shepherd B, Kraemer P, Hall MK, Sycuro LK, Pinto-Santini DM, Salama NR. Identification of Helicobacter pylori genes that contribute to stomach colonization. *Infect Immun* 2007; **75**: 1005-1016 [PMID: 17101654 DOI: 10.1128/IAI.01176-06]

29 **Wen Y**, Feng J, Sachs G. Helicobacter pylori 5'ureB-sRNA, a cis-encoded antisense small RNA, negatively regulates ureAB expression by transcription termination. *J Bacteriol* 2013; **195**: 444-452 [PMID: 23104809 DOI: 10.1128/JB.01022-12]

30 **Lalaouna D**, Baude J, Wu Z, Tomasini A, Chicher J, Marzi S, Vandenesch F, Romby P, Caldelari I, Moreau K. RsaC sRNA modulates the oxidative stress response of Staphylococcus aureus during manganese starvation. *Nucleic Acids Res* 2019; **47**: 9871-9887 [PMID: 31504767 DOI: 10.1093/nar/gkz728]

31 **Pernitzsch SR**, Sharma CM. Transcriptome complexity and riboregulation in the human pathogen Helicobacter pylori. *Front Cell Infect Microbiol* 2012; **2**: 14 [PMID: 22919606 DOI: 10.3389/fcimb.2012.00014]

32 **Kinoshita-Daitoku R**, Kiga K, Miyakoshi M, Otsubo R, Ogura Y, Sanada T, Bo Z, Phuoc TV, Okano T, Iida T, Yokomori R, Kuroda E, Hirukawa S, Tanaka M, Sood A, Subsomwong P, Ashida H, Binh TT, Nguyen LT, Van KV, Ho DQD, Nakai K, Suzuki T, Yamaoka Y, Hayashi T, Mimuro H. A bacterial small RNA regulates the adaptation of Helicobacter pylori to the host environment. *Nat Commun* 2021; **12**: 2085 [PMID: 33837194 DOI: 10.1038/s41467-021-22317-7]

33 **Xu C**, Soyfoo DM, Wu Y, Xu S. Virulence of Helicobacter pylori outer membrane proteins: an updated review. *Eur J Clin Microbiol Infect Dis* 2020; **39**: 1821-1830 [PMID: 32557327 DOI: 10.1007/s10096-020-03948-y]

34 **Thi Huyen Trang T**, Thanh Binh T, Yamaoka Y. Relationship between vacA Types and Development of Gastroduodenal Diseases. *Toxins (Basel)* 2016; **8** [PMID: 27294955 DOI: 10.3390/toxins8060182]

35 **Chivers PT,** Sauer RT. Regulation of high affinity nickel uptake in bacteria. Ni2+-Dependent interaction of NikR with wild-type and mutant operator sites. *J Biol Chem* 2000; 275: 19735-19741 [PMID: 10787413 DOI:10.1074/jbc.m002232200]

36 **Stoof J**, Kuipers EJ, van Vliet AH. Characterization of NikR-responsive promoters of urease and metal transport genes of Helicobacter mustelae. *Biometals* 2010; **23**: 145-159 [PMID: 19894125 DOI: 10.1007/s10534-009-9275-7]

37 **Vannini A**, Pinatel E, Costantini PE, Pelliciari S, Roncarati D, Puccio S, De Bellis G, Peano C, Danielli A. Comprehensive mapping of the Helicobacter pylori NikR regulon provides new insights in bacterial nickel responses. *Sci Rep* 2017; **7**: 45458 [PMID: 28393877 DOI: 10.1038/srep45458]

38 **Montecucco C**, Rappuoli R. Living dangerously: how Helicobacter pylori survives in the human stomach. *Nat Rev Mol Cell Biol* 2001; **2**: 457-466 [PMID: 11389469 DOI: 10.1038/35073084]

39 **Backert S**, Tegtmeyer N, Selbach M. The versatility of Helicobacter pylori CagA effector protein functions: The master key hypothesis. *Helicobacter* 2010; **15**: 163-176 [PMID: 20557357 DOI: 10.1111/j.1523-5378.2010.00759.x]

40 **Censini S**, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci U S A* 1996; **93**: 14648-14653 [PMID: 8962108 DOI: 10.1073/pnas.93.25.14648]

41 **Backert S**, Ziska E, Brinkmann V, Zimny-Arndt U, Fauconnier A, Jungblut PR, Naumann M, Meyer TF. Translocation of the Helicobacter pylori CagA protein in gastric epithelial cells by a type IV secretion apparatus. *Cell Microbiol* 2000; **2**: 155-164 [PMID: 11207572 DOI: 10.1046/j.1462-5822.2000.00043.x]

42 **Odenbreit S**, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. *Science* 2000; **287**: 1497-1500 [PMID: 10688800 DOI: 10.1126/science.287.5457.1497]

43 **Selbach M**, Moese S, Hauck CR, Meyer TF, Backert S. Src is the kinase of the Helicobacter pylori CagA protein *in vitro* and in vivo. *J Biol Chem* 2002; **277**: 6775-6778 [PMID: 11788577 DOI: 10.1074/jbc.C100754200]

44 **Stein M**, Bagnoli F, Halenbeck R, Rappuoli R, Fantl WJ, Covacci A. c-Src/Lyn kinases activate Helicobacter pylori CagA through tyrosine phosphorylation of the EPIYA motifs. *Mol Microbiol* 2002; **43**: 971-980 [PMID: 11929545 DOI: 10.1046/j.1365-2958.2002.02781.x]

45 **Tammer I**, Brandt S, Hartig R, König W, Backert S. Activation of Abl by Helicobacter pylori: a novel kinase for CagA and crucial mediator of host cell scattering. *Gastroenterology* 2007; **132**: 1309-1319 [PMID: 17408661 DOI: 10.1053/j.gastro.2007.01.050]

46 **Poppe M**, Feller SM, Römer G, Wessler S. Phosphorylation of Helicobacter pylori CagA by c-Abl leads to cell motility. *Oncogene* 2007; **26**: 3462-3472 [PMID: 17160020 DOI: 10.1038/sj.onc.1210139]

47 **Hatakeyama M**. Oncogenic mechanisms of the Helicobacter pylori CagA protein. *Nat Rev Cancer* 2004; **4**: 688-694 [PMID: 15343275 DOI: 10.1038/nrc1433]

48 **Segal ED**, Cha J, Lo J, Falkow S, Tompkins LS. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by Helicobacter pylori. *Proc Natl Acad Sci U S A* 1999; **96**: 14559-14564 [PMID: 10588744 DOI: 10.1073/pnas.96.25.14559]

49 **Backert S**, Moese S, Selbach M, Brinkmann V, Meyer TF. Phosphorylation of tyrosine 972 of the Helicobacter pylori CagA protein is essential for induction of a scattering phenotype in gastric epithelial cells. *Mol Microbiol* 2001; **42**: 631-644 [PMID: 11722731 DOI: 10.1046/j.1365-2958.2001.02649.x]

50 **Wei J**, Nagy TA, Vilgelm A, Zaika E, Ogden SR, Romero-Gallo J, Piazuelo MB, Correa P, Washington MK, El-Rifai W, Peek RM, Zaika A. Regulation of p53 tumor suppressor by Helicobacter pylori in gastric epithelial cells. *Gastroenterology* 2010; **139**: 1333-1343 [PMID: 20547161 DOI: 10.1053/j.gastro.2010.06.018]

51 **Mimuro H**, Suzuki T, Nagai S, Rieder G, Suzuki M, Nagai T, Fujita Y, Nagamatsu K, Ishijima N, Koyasu S, Haas R, Sasakawa C. Helicobacter pylori dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. *Cell Host Microbe* 2007; **2**: 250-263 [PMID: 18005743 DOI: 10.1016/j.chom.2007.09.005]

52 **Buti L**, Spooner E, Van der Veen AG, Rappuoli R, Covacci A, Ploegh HL. Helicobacter pylori cytotoxin-associated gene A (CagA) subverts the apoptosis-stimulating protein of p53 (ASPP2) tumor suppressor pathway of the host. *Proc Natl Acad Sci U S A* 2011; **108**: 9238-9243 [PMID: 21562218 DOI: 10.1073/pnas.1106200108]

53 **Tsang YH**, Lamb A, Romero-Gallo J, Huang B, Ito K, Peek RM Jr, Ito Y, Chen LF. Helicobacter pylori CagA targets gastric tumor suppressor RUNX3 for proteasome-mediated degradation. *Oncogene* 2010; **29**: 5643-5650 [PMID: 20676134 DOI: 10.1038/onc.2010.304]

54 **Amieva M**, Peek RM Jr. Pathobiology of Helicobacter pylori-Induced Gastric Cancer. *Gastroenterology* 2016; **150**: 64-78 [PMID: 26385073 DOI: 10.1053/j.gastro.2015.09.004]

55 **Brandt S**, Kwok T, Hartig R, König W, Backert S. NF-kappaB activation and potentiation of proinflammatory responses by the Helicobacter pylori CagA protein. *Proc Natl Acad Sci U S A* 2005; **102**: 9300-9305 [PMID: 15972330 DOI: 10.1073/pnas.0409873102]

56 **Matos JI**, de Sousa HA, Marcos-Pinto R, Dinis-Ribeiro M. Helicobacter pylori CagA and VacA genotypes and gastric phenotype: a meta-analysis. *Eur J Gastroenterol Hepatol* 2013; **25**: 1431-1441 [PMID: 23929249 DOI: 10.1097/MEG.0b013e328364b53e]

57 **Tan S**, Noto JM, Romero-Gallo J, Peek RM Jr, Amieva MR. Helicobacter pylori perturbs iron trafficking in the epithelium to grow on the cell surface. *PLoS Pathog* 2011; **7**: e1002050 [PMID: 21589900 DOI: 10.1371/journal.ppat.1002050]

**Footnotes**

**Conflict-of-interest statement:** All authors declare no potential conflicts of interest for this article.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review started:** July 7, 2022

**First decision:** July 31, 2022

**Article in press:**

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Brazil

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): 0

Grade C (Good): C, C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Deng K, China; Mi Y, China **S-Editor:** Wang DM **L-Editor:** Wang TQ **P-Editor:** Wang DM

 **Figure Legends**



**Figure 1 Nickel-regulated small RNA regulates the expression of cytotoxin associated antigen A depending on nickel availability.** NikR: Nickel-responsive transcription factor; NikS: Nickel-regulated sRNA; *CagA*: Cytotoxin-associated gene A.



**Figure 2 Simplified molecular mechanisms of cytotoxin associated antigen A mediated carcinogenesis.** After the phosphorylation process, cytotoxin associated antigen A acts as a promiscuous scaffold or hub protein that simultaneously disturbs multiple host signaling pathways, involved in regulation of a large range of cellular processes, including proliferation, differentiation, and apoptosis. Moreover, cytotoxin associated antigen A is also able to induce NF-kB-mediated chronic inflammation. Ultimately, the disharmonic interaction between cytotoxin associated antigen A and host proteins leads to pre-cancerous cellular alterations.*CagA*: Cytotoxin-associated gene A; *H. pylori*: *Helicobacter pylori*; IL-8: Interleukin-8.

**Table 1 Regulatory bacterial sRNA groups and their characteristics**

|  |  |  |
| --- | --- | --- |
| **sRNA group** | **Characteristics** | **Ref.** |
| *Cis*-encoded sRNAs | Repress genes encoding toxic proteins | Brantl[15] |
| *Trans*-encoded sRNAs | Modulate mRNA stability and translation | Brantl[15], Brantl and Müller[16] |
| sRNAs that modify protein activity | Mimic proteins and compete with RNA and DNA targets | Svensson and Sharma[17] |