Helicobacter pylori and gastric cancer: possible role of microRNAs in this intimate relationship

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Abstract

Chronic infection by Helicobacter pylori is a major risk factor for gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. H. pylori possesses a set of virulence factors, including the CagA effector, which interferes with intracellular signalling pathways and mediates phenotypic alterations, strongly evoking neoplastic transformation. MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression involved in development, cell proliferation and immune responses. miRNAs are frequently altered in cancers, revealing their functions as oncogenes or tumour suppressors. However, the role, if any, that miRNAs play in the host cell responses to H. pylori remains unknown. This review considers the possible involvement of some miRNAs, including miR-146, miR-155, miR-21, miR-27a, miR-106-93-25 and miR-221-222 clusters and the miR-200 family in H. pylori-induced infection and gastric cancers. Further exploration of miRNA-mediated gene silencing, taking into account the relationship between host targets and bacterial effectors, will most certainly bring new insights into the control of gene expression in human gastric cells chronically infected by H. pylori.

Keywords: Cancer, gastric mucosa, Helicobacter pylori, microRNA, post-transcriptional gene regulation


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Gastric cancer is the second leading cause of cancer-related death worldwide [1]. The major risk factor associated with gastric adenocarcinoma is infection by Helicobacter pylori, a Gram-negative microaerophilic bacterium that colonizes the gastric mucosa of approximately 50% of the human population. It induces a chronic gastric inflammation, which evolves in approximately 10% of cases towards more severe forms of gastric diseases, such as peptic ulcer, gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Chronic inflammation represents a major pathological background for human malignancies. H. pylori possesses a set of virulence factors for colonizing the gastric mucosa and establishing a chronic infection. Notably, the most virulent strains harbour the cag pathogenicity island (cag PAI), which encodes a type IV secretion system (T4SS) and the protein CagA. Once translocated into the host cell cytoplasm, CagA is phosphorylated by Src kinases and can subsequently interfere with intracellular signalling pathways, leading to cell proliferation, cytoskeletal rearrangement, cell–cell adhesion loss, extracellular matrix remodelling and β-catenin pathway activation [2]. All of these CagA-mediated phenotypic alterations strongly suggest a role of this bacterial effector in cell transformation. Other virulence factors associated with H. pylori pathogenesis are adhesins (BabA, HopZ, HopH), the vacuolating cytotoxin VacA, tumour necrosis factor (TNF)-α-inducing protein [3], and the T4SS, which interacts with integrins and injects the CagA protein and components of the peptidoglycan into the host cells [4,5].

Discovered approximately 10 years ago, microRNAs (miRNAs) are currently considered as crucial post-transcriptional regulators of gene expression. Their roles in development, cell proliferation and differentiation are widely recognized, as is their importance in the regulation of immune responses [6]. Furthermore, miRNAs are frequently altered in cancer cells and reveal their functions as either oncogenes or tumour suppressors [7,8]. The emerging role of miRNAs in diverse and fundamental cellular mechanisms suggests that proper control of these regulatory elements is essential for the maintenance of a nonpathological state.
Do miRNAs play any role in the host cell responses to *H. pylori* infection? To date, only one miRNA has been reported to be up-regulated in *H. pylori*-infected cells [9]. However, the abundant literature on changes in gene expression in cells and tissues infected by this pathogen, the growing amount of data on miRNAs implicated in cancer, including gastric cancer and lymphomas, and in infection, make it worthwhile to study the possible involvement of other miRNAs in the response of the gastric mucosa to *H. pylori* infection. This could provide additional insights into the mechanisms by which this pathogen causes chronic inflammation and gastric pathologies. This review summarizes the currently available knowledge concerning the role of miRNAs in infection and carcinogenesis and raises the question of the possible involvement of some miRNA in *H. pylori*-induced infection and gastric cancer.

**Human miRNA Biogenesis**

miRNAs are small noncoding RNAs that regulate gene expression by post-transcriptional silencing, most usually after binding to the 3’ untranslated region (UTR) of mRNAs. miRNAs are generated from sequential processing of longer primary transcripts by Drosha and Dicer RNases (Fig. 1). Active mature miRNAs are single-stranded RNA, 20–25 nucleotides long, integrated in a RNA-induced silencing complex and associated with the 3’ UTR of specific target mRNAs to suppress their translation. RNA silencing could involve P-bodies, where the miRNA-targeted mRNAs would be relocalized [10,11].

Bioinformatic analyses lead to an estimation that as many as 30% of human genes are targets of miRNA [12]. Currently, there are 678 mature human miRNA sequences listed in the miRNA registry (http://www.microrna.sanger.ac.uk), with approximately 1000 predicted miRNAs, each potentially targeting approximately 200 genes.

**miRNAs in the Host Response to Pathogens**

Innate immunity and miRNAs

Cells recognize invading pathogens and/or their secreted effectors through ‘pathogen-recognition molecules’, known as Toll-like receptors (TLR) and Nod-like receptors (NLR), located on the cell membrane and in the cytoplasm, respectively. Pathogen-recognition molecules subsequently activate a set of adaptor proteins and transcription factors mediating...
host innate immunity such as the nuclear factor-κB (NF-κB) and the activator protein 1 (AP-1) pathways. NF-κB has been identified as a potential molecular bridge between inflammation and cancer because improper NF-κB activation transactivates several target genes harbouring inflammatory (e.g. cyclooxygenase 2, inducible nitric oxide synthase, TNF-α), anti-apoptotic (e.g. cIAP1 and 2, XIAP, Bcl-2, Bcl-3, Bcl-XL), cell cycle regulatory (e.g. cyclin D1) and proangiogenic (e.g. vascular endothelial growth factor, angiopoietin) functions, and/or down-regulates apoptosis-inducing genes (e.g. p53, Bax, Bad), thus contributing to tumourigenesis [13].

The involvement of miRNAs in the innate and adaptative immune responses is now established. MiR-155 and miR-146a are involved in innate immunity by regulating the acute inflammatory response in monocyte/macrophages after pathogen recognition by TLR. MiR-146a, induced by NF-κB via TLR2, 4, 5 signalling, targets the TLR-signalling adaptors IRAK1 and TRAF6, placing this miRNA into a negative-feedback loop, regulating TLR signalling in response to bacterial products [14]. MiR-155 is induced during both bacterial and viral infection in myeloid cells through the TLR pathway, as well as by pro-inflammatory cytokines. It down-regulates FADD, RIP and IKK, other components of the TLR signalling mechanism [15]. Conversely, miR-125b is decreased in parallel to miR-155 up-regulation. MiR-125b represses TNF-α synthesis, ensuring the suppression of this pro-inflammatory cytokine under noninfectious conditions, whereas its decrease allows for TNF-α production to occur after TLR stimulation [16]. MiR-155 is up-regulated in activated B and T cells and in the germinal B cell centre [17]. This miRNA is best known for its involvement in the development of B-cell malignancies; it has been found at high levels in human B cell lymphomas [18,19], and enforced miR-155 overexpression in mouse B cells is sufficient to trigger murine B cell lymphoma [20]. It is encoded in the BIC locus, which represents a common viral integration site in chickens. Because of its involvement in both the innate immune response and its oncogenic potential, miR-155 could comprise an additional bridge between inflammation and cancer [21].

**Exploitation of Host-cell miRNAs Pathways by Pathogens**

Microbes have evolved strategies to circumvent the host immune response with the aim of establishing a sustainable infection. This includes the development of effectors that are able to interfere with the miRNA pathway. Viruses provide several examples of proteins hijacking miRNAs, thus favouring virus replication: the tomato bushy stunt virus p19 [22], the mammalian Nodamura virus B2 [23], or the primate foamy virus I Tas proteins [24]. Pathogens can also take advantage of host cell miRNAs; for example, the liver-specific miR-122 enhances hepatitis C virus replication rate [25]. The parasite Plasmodium falciparum better survives in Anopheles gambia when miRNA maturation is impaired [26].

Bacterial suppressors of the miRNA network have been recently identified in plants [27]. It was reported that Pseudomonas syringae, a Gram-negative bacterium, injected bacterial effectors through a type III secretion system to suppress PAMP-responsive miRNAs. This repression took place at multiple levels: transcription, miRNA biogenesis, stability and activity. This is the first example of a bacterium hijacking the miRNA system, leading to the possibility that human pathogenic bacteria, including H. pylori, have also evolved to suppress miRNA silencing to ensure persistent infection.

**miRNAs and Gastric Cancers**

**MiR-21-induced expression upon H. pylori infection**

MiR-21 is consistently up-regulated in solid human cancers, including the stomach, as compared with matching noncancerous tissue [19]. Direct targets of miR-21 have been identified, with all of them being tumour suppressors: the PTEN phosphatase [28], the actin-binding protein tropomyosin 1 [29] and the reversion-inducing-cystein-rich protein with Kazal motifs [30]. Thus, this single miRNA provides a significant survival advantage to cells upon deregulation.

In a recent study, Zhang et al. [9] found that miR-21 was over-expressed in gastric cancer tissue samples and cell lines, as well as in chronically H. pylori-infected gastric epithelium tissue, as opposed to noninfected tissue. MiR-21 was also up-regulated in cultured gastric epithelial cells upon co-culture with H. pylori. This is the first evidence of miRNA modulation upon H. pylori infection. Zhang et al. [9] showed that over-expression of miR-21 promoted cell proliferation and migration and inhibited apoptosis in this cell line. AP-1 and the signal transducer and activator of transcription 3 (STAT3) are able to induce miR-21 [31,32]. NF-κB activation and interleukin (IL)-6 secretion in the gastric mucosa, which activate AP-1 and STAT3, respectively, could explain miR-21 up-regulation during H. pylori infection.

**Specific over-expression of miR-106b,-93,-25 in gastric cancers**

In patients suffering from H. pylori-induced gastritis, a relationship was found among cagA-positive strains, epithelial proliferation and inflammatory reaction, mostly located at the antral lesser curvature, an area where most carcinoma
arise. The intricate balance of pro- and anti-inflammatory cytokines in chronic inflammation may mediate the outcome of *H. pylori* infection by affecting cell proliferation and apoptosis. Among the anti-inflammatory cytokines, transforming growth factor (TGF)β is involved in mucosal immunity and morphogenetic programming, comprising a cross-talk between the epithelial and stromal compartments that guides gastrointestinal cells toward proliferation, differentiation and apoptosis, thus controlling the physiological turnover of epithelial cells [33].

The downstream effectors of TGFβ-dependent cell cycle arrest and apoptosis are the cyclin-dependent kinase inhibitor p21CIP1/WAF1 and the pro-apoptotic factor Bim, respectively. TGFβ induced a marked down-regulation of the E2F1 protein and Mcm7 mRNA as cells physiologically underwent G1/S cell cycle arrest. Furthermore, gastric tumours of diffuse type are characterized by E2F1 up-regulation and TGFβ resistance. In a microarray analysis of miRNAs associated with gastric mucosa inflammation, preneoplastic lesions or carcinomas, Petrocca et al. [35] identified several miRNAs that are associated with chronic inflammation, including up-regulated miRNAs (miR-1, miR-155) and down-regulated miRNAs (miR-205, -303, -202, -20 and 26b), as well as others that were up-regulated in human gastric adenocarcinomas, namely miR-106b, miR-93 and miR-25. Petrocca et al. [35] provide evidence that miR-106b, miR-93 and miR-25 alter the physiological response of gastric cancer cells to TGFβ, affecting both the cell cycle and apoptosis [35,36]. E2F1 is a transcriptional factor required for the G1/S transition. In resting cells, it is sequestered by the unphosphorylated retinoblastoma protein pRb. Although E2F1 self-activates its own promoter through a positive-feedback loop, it also transactivates a cluster of intronic miRNAs, miR-106b, miR-93 and miR-25, hosted in the Mcm7 gene, inducing their accumulation in gastric primary tumours and gastric epithelial cell lines [35,36]. Conversely, miR-106b and miR-93 decrease E2F1 expression, establishing a negative-feedback loop preventing E2F1 self-activation. MiR-106b, miR-93 and miR-25 also silence p21CIP1/WAF1 in addition to E2F1, leading to a decreased response of gastric cells to TGFβ (i.e. cells continue to grow despite a high level of the cytokine).

Prolonged exposure to TGFβ leads to apoptosis. MiR-25, but not miR-106b or miR-93, negatively influences Bim in gastric cell lines through a post-transcriptional regulatory mechanism. Anti-apoptotic and pro-apoptotic responses associated with miR-106b, -93 and -25 emerge in the late phase of TGFβ stimulation, when cell cycle arrest is revoked and apoptosis becomes the dominant process characterizing the response of a gastric cell to TGFβ. This is relevant in a gastric cancer model because impairment of the TGFβ-mediated tumour suppressor pathway is a critical step in the development of gastric tumours.

A paralogue to the miR-106b-25 cluster is the miR-17-92 cluster at chromosome 13q31, encoding miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92-1. The miR-17-92 cluster also harbours an oncogenic potential; indeed, miR-17-92 transcripts are elevated in B cell lymphomas [37] as well as in lung, colon and pancreas carcinomas [19], and ectopic expression of miR-17-92 causes lymphoproliferative disease and autoimmunity [38]. miRNAs encoded by that cluster are up-regulated in gastric tumour samples compared to the normal counterpart [39].

In addition to the miR-106b-25 cluster, the miR-221-222 cluster also has highly increased gastric tumour samples and, interestingly, the expression patterns of the two clusters correlate with each other [39]. Although p21CIP1/WAF1 was confirmed as miR-106b and miR-93 targets, there was evidence that miR-25, miR-221 and miR-222 silenced p53 and p27Kip1, which are other cyclin-dependent-kinase inhibitors of the cip/kip family. Thus, these two clusters appear to be functionally co-regulated to facilitate G1/S transition in gastric tumour cells.

**Putative Role of miRNAs in *H. pylori* Infection and Gastric Cancer**

**MiR-146, miR-155 and immunity**

In *H. pylori* infection, the gastric epithelial cells provide the first line of defence against this noninvasive pathogen. In these cells, the innate immune response is characterized by the activation of the NF-κB pathway in a NLR Nod1-dependent manner in response to *H. pylori* peptidoglycan, which is injected into the host cell cytoplasm via the T4SS [5]. This activation leads to IL-8 secretion. Other pathogen-recognition molecules, such as TLR2, TLR4 and TLR5, which sense specific Gram-negative bacterial components, including lipopolysaccharides, lipopolysaccharide, and flagellin, respectively, appear to play a minor role in the recognition of *H. pylori* by gastric epithelial cells [40]. However, miR-146a and miR-155 are not expressed in gastric epithelial cells during infection, raising the question of which miRNAs (if any) are involved in feedback regulation of NF-κB in those cells.

Myeloid cells (monocyte/macrophage and dendritic cells) constitute the second line of defense, sensing *H. pylori* components via TLR2, TLR4 or Nod1 signalling, and secrete pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α in order to establish T and B lymphocyte-mediated adapta-
tive immunity [40]. MiR-146a and miR-155 are likely to be induced in myeloid or lymphocytes in response to the bacterium. Notably, the oncogenic miR-155 [16] and miR-17-92 [37] may deserve special attention in gastric MALT lymphoma, resulting from H. pylori-induced uncontrolled B cell proliferation.

**Oncogenic miRNAs and H. pylori**

Because specific miRNAs have been found up-regulated in gastric carcinomas, the question of their deregulation in gastric epithelium during H. pylori infection is obvious. As mentioned above, Zhang et al. [9] demonstrated a link between H. pylori infection and miR-21. That study should now be extended to other miRNAs implicated in gastric cancer, such as the miR-106b-25 and miR-221-222 clusters. A clue could be TGF-β, which is significantly and specifically increased by H. pylori in cultured gastric epithelial cells and monocytes. This effect was attributed to a soluble protein of the bacteria [34], which may, in this way, modulate the immune response, contribute to the persistence of H. pylori infection, and dysregulate the physiological turnover of gastric epithelial cells.

MiR-27a was also identified as an oncogenic miRNA in a gastric adenocarcinoma cell line, in which it targets the tumour suppressor prohibitin, an evolutionary conserved and ubiquitous protein interacting with pRb and its family members [41]. Another miR-27a target, the zinc finger ZBTB10, a transcriptional repressor of specificity protein (Sp) transcription factors, has been identified in breast carcinoma cells [42]. Sp factors are over-expressed in tumours, notably in gastric tumours, and contribute to the proliferative and angiogenic phenotype associated with cancer cells. Inhibition of miR-27a resulted in increased expression of ZBTB10 mRNA, decreased expression of Sp factors, and cell cycle arrest. This latter response was associated with the induction of Myt-1, another miR-27a target, which blocks G2/M transition through inactivation of the cdc2 cyclin-dependent kinase. Thus, the oncogenic activity of miR-27a is a result, at least in part, to the suppression of ZBTB10 and Myt-1. This mechanism may be relevant to H. pylori-induced, CagA-dependent progression to G2/M in gastric epithelial cells [43].

It is expected that multiple miRNAs may contribute to tumour development and that some miRNAs may have cooperative or redundant functions.

**MiR-200 family and the epithelial-to-mesenchymal transition (EMT)**

H. pylori induces an invasive phenotype in epithelial cells that resembles an EMT. Indeed, CagA, once injected into the host cell, changes the epithelial cell morphology by disrupting cell–cell junctions and inducing loss of apical–basolateral polarity [2]. By its interaction with several junction proteins, including ZO-1, JAM and E-cadherin, CagA disturbs the assembly and function of both tight and adherent junctions [2]. Its interaction with E-cadherin leads to the release of β-catenin complexed to E-cadherin and the subsequent activation of the Wnt/β-catenin signalling pathway [51,52]. Deregression of β-catenin plays a crucial role in gastrointestinal cancers. Numerous targets of this transcription factor have been described, including genes implicated in proliferation, tumour invasion and metastasis. Regarding the miRNAs, a correlation between miR-375 repression and β-catenin-activating mutation in hepatocellular adenoma and carcinoma has been described [53]. However, the mechanism of this repression remains to be elucidated. In addition, a direct connection between E-cadherin and gastric carcinogenesis has been demonstrated through the finding that genetic mutations in CDH1, the gene encoding E-cadherin, are associated with hereditary diffuse gastric cancer [44]. The miR-200 family, which includes miR-200a, b, c, miR-141 and miR-429, inhibits EMT and invasion in cancer cells [45–48]. All of these members are implicated in a negative regulation loop, in which they repress the E-cadherin transcriptional repressors ZEB1 and ZEB2/SIP1 (Zinc-finger E-box Binding homebox 1 and 2), themselves down-regulating the miR-200 family transcription. The expression of these miRNAs is lost in invasive cancer cells harbouring a mesenchymal phenotype. Moreover, ZEB2/SIP1 also represses cyclin D1 transcription [49]. This cyclin, which promotes G1/S transition, is induced via AP-1 and the cAMP-response element in gastric epithelial cells during H. pylori infection and under CagA dependence [50]. Therefore, a role of the miR-200 family and ZEB repressors is likely in the EMT-like phenotype observed in H. pylori-infected cells.

**Conclusions**

The miRNA family has recently emerged to challenge the accepted paradigm of gene regulation by the combinatorial action of transcriptional factors activating or repressing specific genes. It has been proposed that they can act as developmental switches and fail-safe regulators of transcriptional programmes. Regulatory loops can be established between genes coding for classical transcriptional factors and genes coding for miRNAs. It should be noted that, for a comprehensive understanding of the role of a given miRNA, the direct relationship between a miRNA and the expression of a particular target may be more important in some situations than in others, depending on the cellular context. Consequently, the therapeutic potential of miRNAs may be strictly
associated with the occurrence of specific miRNA-dependent function alterations. Further exploration of miRNA-mediated gene silencing, taking into account the host miRNA/host target/bacterial effector relationship, most certainly will bring new insights into how these small regulatory RNAs modulate gene expression in human cells that chronically harbour H. pylori.

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References


