Gastric carcinogenesis is a multistep process orchestrated by aberrancies in the genetic and epigenetic regulation of oncogenes and tumor suppressor genes. Chronic infection with *Helicobacter pylori* is the strongest known risk factor for the development of gastric cancer. *H. pylori* expresses a spectrum of virulence factors that dysregulate host intracellular signaling pathways that lower the threshold for neoplastic transformation. In addition to bacterial determinants, numerous host and environmental factors increase the risk of gastric carcinogenesis. Recent discoveries have shed new light on the involvement of microRNAs (miRNAs) in gastric carcinogenesis. miRNAs represent an abundant class of small, non-coding RNAs involved in global post-transcriptional regulation and, consequently, play an integral role at multiple steps in carcinogenesis, including cell cycle progression, proliferation, apoptosis, invasion, and metastasis. Expression levels of miRNAs are frequently altered in malignancies, where they function as either oncogenic miRNAs or tumor suppressor miRNAs. This review focuses on miRNAs dysregulated by *H. pylori* and potential etiologic roles they play in *H. pylori*-mediated gastric carcinogenesis.

**Keywords:** gastric cancer, *Helicobacter pylori*, microRNA, cell cycle, proliferation, apoptosis

**INTRODUCTION**

Microbial infections are among the most significant causes of cancer worldwide with nearly one in five malignancies resulting from infectious agents (Parkin, 2006). Gastrointestinal cancers represent a major global health concern and account for nearly 20% of all cancer-related deaths (Ferlay et al., 2007). Despite the decreasing incidence of gastric cancer in developed countries, it remains the second leading cause of cancer-related death throughout the world, with ~700,000 deaths attributed to this malignancy annually (Parkin et al., 2005). The major contributing factor to the development of gastric cancer is colonization and chronic infection by the bacterial pathogen, *Helicobacter pylori*. *H. pylori* selectively colonizes the gastric epithelium of over 50% of the world’s population and typically persists for the lifetime of the host. Among colonized individuals, however, only a fraction develop gastric adenocarcinoma, emphasizing the importance of understanding the pathogenic mechanisms by which *H. pylori* promotes chronic inflammation and the progression to gastric cancer.

**VIRULENCE FACTORS THAT MEDIATE HELICOBACTER PYLORI PATHOGENESIS**

Chronic gastric inflammation induced by the bacterial pathogen, *H. pylori*, is the strongest known risk factor for the development of atrophic gastritis, metaplasia, dysplasia, and ultimately gastric adenocarcinoma (Figure 1). *H. pylori* is a Gram-negative, helical-shaped bacterium specifically adapted to persist within the human gastric niche. *H. pylori* possesses numerous elements to successfully colonize the gastric mucosa, establish chronic infection, and induce gastric pathology. *In vivo*, approximately 20% of *H. pylori* adhere to the gastric epithelium (Hessey et al., 1990). The large repertoire of adhesins expressed by *H. pylori* likely contribute to its specific adaptation to the gastric niche, allowing flexibility to target specific host cells and to exert a dynamic range of effector functions on host cells. *H. pylori* expresses a number of adhesins that have been linked to virulence. SabA (sialic acid-binding adhesin), which binds host sialyl-Lewis^a^, contributes to *H. pylori* persistence and mediates chronic gastric inflammation and injury (Mahdavi et al., 2002). The presence of blood group antigen-binding adhesin (BabA), which binds the host Lewis^b^ blood group antigen, increases the risk of gastric cancer in a synergistic fashion with other virulence factors, such as CagA (Ilver et al., 1998; Gerhard et al., 1999).

Following adherence and colonization of the gastric mucosa, *H. pylori* induces chronic gastritis and gastric injury, which are characterized by both neutrophilic and lymphocytic inflammation (Marshall et al., 1985; Goodwin et al., 1986). *H. pylori* expresses a number of factors capable of modulating the host immune system and eliciting proinflammatory immune responses. Some of these virulence factors include vacuolating cytotoxin (VacA) and the *cag* (cytotoxin associated gene) pathogenicity island. VacA is coded by the gene *vacA*, which is present in all strains of *H. pylori*, and which exhibits vacuolating activity (Leunk et al., 1988; Cover and Blaser, 1992; Cover et al., 1994; Phadnis et al., 1994; Schmitt and Haas, 1994; Telford et al., 1994). Additionally, VacA can induce apoptosis of host cells (Kuck et al., 2001; Xia and Talley, 2001) and suppress proliferation of T and B lymphocytes (Boncristiano et al., 2003; Gebert et al., 2003; Sundrud et al., 2004), which may contribute to the persistence of *H. pylori* through dysregulation of the host immune response. The *cag* pathogenicity island is present in
outcomes that facilitate the development of gastric cancer. Cumulatively, these bacterial factors contribute to adherence, persistence, host immune modulation, and virulence of \textit{H. pylori} within the gastric niche, ultimately resulting in \textit{H. pylori}-mediated chronic inflammation and a series of pathological outcomes that facilitate the development of gastric cancer.

\section*{HOST FACTORS THAT CONTRIBUTE TO GASTRIC CARCINOGENESIS}

In addition to microbial factors that potentiate gastric disease, there are a number of host factors that contribute to chronic gastritis and the progression to gastric adenocarcinoma. Cyclooxygenases (COX) are key enzymes that catalyze prostaglandin synthesis. Of the three isoforms identified, COX-2 is upregulated in gastric epithelial cells upon co-culture with \textit{H. pylori} \cite{Romano, Juttner, Meyer, Wu} and within the gastric mucosa of \textit{H. pylori}-infected individuals \cite{Sawaoka, Fu, McCarthy}. In \textit{vivo} studies show that COX-2 is further upregulated in \textit{H. pylori}-mediated adenocarcinoma \cite{Ristimaki, McCarthy}. COX-2 expression levels are considered an independent factor for poor prognosis and correlate with reduced patient survival, suggesting that \textit{H. pylori}-induced COX-2 overexpression is a risk factor for the development of gastric cancer.

Other host factors that increase the propensity for chronic inflammation and gastric adenocarcinogenesis are polymorphisms within human IL-1β \cite{El-Omar, Machado}, TNF-α \cite{El-Omar, Machado}, and IL-8 \cite{El-Omar, Machado} promoters \cite{El-Omar, Machado, Machado, Furuta, Meyer, Wu}, which lead to increased expression of proinflammatory cytokines IL-1β, TNF-α, and IL-8 \cite{Ristimaki} (Figure 1). These polymorphisms in combination with \textit{H. pylori} virulent genotypes increase the risk of gastric cancer up to 87-fold over baseline \cite{Figueiredo}, emphasizing the importance of microbial–host interactions in the development of gastric cancer. Collectively, data demonstrate that \textit{H. pylori} virulence factors, host genetics, and environmental factors interact to induce and maintain the persistent inflammatory immune response that initiates the multistep process leading to gastric cancer.

\section*{miRNAs and \textit{H. pylori}-mediated carcinogenesis}

Recent discoveries have shed new light on the involvement of host microRNAs (miRNAs) in gastric carcinogenesis. miRNAs are small, non-coding RNAs \cite{Lewis}–20–25 nucleotides in length, which function as critical post-transcriptional regulators of gene expression \cite{Bartel}. miRNAs were first characterized in 1993 \cite{Lee}, but their distinct role in transcriptional regulation was not recognized until the early 2000s. Most miRNAs are found in intergenic regions and contain their own promoter and regulatory units. Processed miRNAs function by binding to the 3′ untranslated region (3′UTR) of messenger RNAs (mRNAs), typically resulting in mRNA degradation and gene silencing or translational repression \cite{Bartel}. It is estimated that the human genome encodes thousands of miRNAs, targeting \sim 30–60% of all protein-coding genes \cite{Lewis}. miRNAs are involved in many biological processes, including development, differentiation, angiogenesis, cell cycle progression, proliferation, apoptosis, and signal transduction pathways \cite{Ambros}. Dysregulation of miRNA expression with subsequent disruption of these biological processes can result in disease states. There is an increasing body of evidence regarding the regulatory roles of miRNAs in immune and inflammatory disorders \cite{Wu, Sonkoly}, and aberrant expression of miRNAs is observed in many cancers \cite{Lu, Volinia}. Thus, recent studies have begun to dissect the mechanisms by which miRNAs function as either oncogenic miRNAs (oncomiRs) or tumor suppressors to promote or prevent tumorigenesis.

\section*{Dysregulation of miRNAs in \textit{H. pylori}-induced gastric carcinogenesis}

The number of studies analyzing miRNA expression profiles in gastric cancer is rapidly increasing and a comprehensive list of...
miRNAs dysregulated in gastric cancer, confirmed mRNA targets, and the biological processes affected is shown in Tables A1 and A2 in the Appendix. The first study to address miRNA expression profiles in various cancers, including gastric cancer, was performed in 2005 (Lu et al., 2005). Subsequent studies have not only focused on miRNA expression profiles in gastric cancer, but also those that are altered in response to *H. pylori*.

Matsushima et al. (2011) conducted a study to characterize miRNA expression signatures in *H. pylori*-infected human gastric mucosa. Using high throughput profiling analysis, 31 miRNAs were identified as being differentially expressed between *H. pylori*-infected and *H. pylori*-uninfected gastric mucosa. The relationship between miRNA expression levels and *H. pylori*-induced acute inflammation, characterized by neutrophil infiltration, and chronic inflammation, characterized by mononuclear cell infiltration were also determined. Expression levels of many miRNAs correlated with either the degree of acute or chronic inflammation and in some cases both (Tables 1 and 2). The relationship between miRNA expression and extent of glandular atrophy, and intestinal metaplasia was also assessed, but no significant correlations were found (Matsushima et al., 2011). A comprehensive list of miRNAs dysregulated by *H. pylori*, confirmed mRNA targets, and biological processes affected is shown in Tables 1 and 2. These data suggest that *H. pylori* infection affects global miRNA expression in human gastric mucosa, and this effect is, in part, linked to *H. pylori*-induced host inflammatory immune responses.

miRNAs that are dysregulated in response to *H. pylori* infection may not be the same miRNAs that are dysregulated in later stages of gastric disease. A comprehensive review of the literature, however, revealed that there is a select subset of miRNAs dysregulated both following *H. pylori* infection as well as gastric cancer. These include downregulated miRNAs, let-7a, miR-31, miR-101, miR-141, miR-203, miR-210, miR-218, miR-375, and miR-449 as well as upregulated miRNAs, miR-17, miR-20a, miR-21, miR-146a, miR-155, and miR-223. These miRNAs may be more biologically relevant to *H. pylori*-induced gastric inflammation and carcinogenesis and represent fruitful targets for studies focused on cancer that develops in the context of *H. pylori* infection.

The next sections will discuss miRNA dysregulation in *H. pylori*-induced disease and how specific miRNAs control various biological processes related to (1) host inflammatory immune response, (2) cell cycle progression, and (3) apoptosis and proliferation.

**HELCICOBACTER PYLORI-INDUCED miRNA DYSREGULATION TO CONTROL HOST INFLAMMATORY RESPONSES**

Host cells recognize invading pathogens and/or pathogen-associated molecular patterns (PAMPs) through membrane-associated or cytoplasmic pathogen recognition molecules known as Toll-like receptors (TLRs) and Nod-like receptors (NLRs), respectively. PAMPs activate adapter proteins and transcription factors that mediate host innate immunity through activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling (Figure 2). Gastric epithelial cells are the initial host element encountered by *H. pylori*. The innate immune response induced in epithelial cells is characterized by NOD1-dependent activation of NF-κB in response to *H. pylori* peptidoglycan (PGN), which is injected into host cells via the cag T4SS (Viala et al., 2004). Activation of NF-κB by *H. pylori* leads to induction of the pro-inflammatory cytokine IL-8 and likely contributes to carcinogenesis through activation of downstream targets that mediate inflammation, cell cycle progression, proliferation, and apoptosis. Myeloid cells constitute a second line of defense and secrete proinflammatory cytokines such as IL-6, IL-1, and TNF-α to establish T and B lymphocyte-mediated adaptive immunity.

The involvement of miRNAs in modulating both the innate and adaptive immune responses is well established (Chen et al., 2004) and *H. pylori* can dysregulate miRNA expression to evade host defenses and successfully persist in the gastric niche. *mir-146a* and *mir-155* are specifically involved in *H. pylori*-induced negative regulation of the proinflammatory immune response (Figure 2). Changes in *mir-146a* expression occur in the development of gastric cancer and in the negative regulation of the innate inflammatory immune response. Single-nucleotide polymorphisms (SNPs) in *mir-146a* are associated with an increased susceptibility to gastric cancer (Okubo et al., 2010) and *H. pylori* upregulates *mir-146a* in vitro and in vivo in a CagA-independent and an NF-κB-dependent manner (Liu et al., 2010; Li et al., 2011a). *mir-146a* targets the TLR-signaling adaptor molecules IRAK1 (interleukin-1 receptor-associated kinase) and TRAF6 (TNF receptor-associated factor), resulting in negative regulation of TLR and downstream proinflammatory signaling (Figure 2; Liu et al., 2010; Li et al., 2011a). As a result, *mir-146a* overexpression negatively regulates *H. pylori*-induced IL-8, TNF-α, IL-1β, GRO-α (CXCL1, chemokine (C-X-C motif) ligand), and MIP-3α (macrophage inflammatory protein) expression, all key components to the proinflammatory innate and adaptive immune responses (Li et al., 2010; Li et al., 2011a).

The second miRNA involved in *H. pylori*-induced downregulation of the host inflammatory immune response, *mir-155*, plays a critical role in regulating lymphocyte homeostasis and tolerance (Thai et al., 2007). *mir-155* is increased in many malignancies of B cell or myeloid origin (Volinia et al., 2006). In transgenic murine models of *mir-155* overexpression, mice develop spontaneous B cell lymphomas (Costinean et al., 2006). *mir-155* is induced during both bacterial and viral infections in myeloid cells through activation of TLR-signaling pathways. *H. pylori* upregulates *mir-155* expression in vitro and in vivo, which occurs in an NF-κB-dependent manner, and ultimately results in decreased induction of the proinflammatory cytokines, IL-8, and GRO-α (Xiao et al., 2009b; Tang et al., 2010). *mir-155* targets MyD88 (myeloid differentiation primary response gene), the universal adapter protein used by TLRs to activate NF-κB (Figure 2; Xiao et al., 2009b; Tang et al., 2010). Decreased levels of MyD88 subsequently result in decreased NF-κB activation and dampening of the host inflammatory response (Xiao et al., 2009b; Tang et al., 2010). Therefore, these data demonstrate that *H. pylori* dysregulates host miRNA expression to manipulate the host inflammatory immune response, which may promote bacterial survival and persistence within the gastric mucosa. Because these miRNAs have established roles in carcinogenesis as well as innate immunity, they could serve as an important link between *H. pylori*-induced inflammation and carcinogenesis.
Table 1 | miRNAs downregulated in response to *H. pylori*.

| miRNAs | Target mRNAs                  | Biological process targeted                  | Reference                                                                 |
|--------|-------------------------------|-----------------------------------------------|---------------------------------------------------------------------------|
| let-7a | RAB40C                        | Cell cycle progression                        | Matsushima et al. (2011), Motoyama et al. (2008), Yang et al. (2011)       |
|        |                               | Proliferation                                 |                                                                           |
|        | HMGA2                         | Invasion                                       |                                                                           |
| let-7b | HMGA2                         | Invasion                                       | Matsushima et al. (2011), Motoyama et al. (2008)                          |
| let-7d | HMGA2                         | Invasion                                       | Matsushima et al. (2011), Motoyama et al. (2008)                          |
| let-7e | HMGA2                         | Invasion                                       | Matsushima et al. (2011), Motoyama et al. (2008)                          |
| let-7f | HMGA2                         | Invasion                                       | Matsushima et al. (2011), Motoyama et al. (2008)                          |
| miR-1  | ND                            | Proliferation                                 | Saito et al. (2011)                                                      |
| miR-31-| ND                            | ND                                            | Matsushima et al. (2011)                                                 |
| miR-32 | ND                            | ND                                            | Matsushima et al. (2011)                                                 |
| miR-34b| ND                            | ND                                            | Suzuki et al. (2010)                                                     |
| miR-34c| ND                            | ND                                            | Suzuki et al. (2010)                                                     |
| miR-101| COX-2, FOS                    | Proliferation                                 | Matsushima et al. (2011), Varambally et al. (2008), Wang et al. (2010)   |
|        | MCL1                          | Apoptosis                                     |                                                                           |
|        | EZH2                          | Invasion migration                             |                                                                           |
| miR-103| ND                            | ND                                            | Matsushima et al. (2011)                                                 |
| miR-106b| p21                          | Cell cycle progression                        | Kan et al. (2009), Matsushima et al. (2011), Petrocca et al. (2008)       |
|        |                               | Proliferation                                 |                                                                           |
|        | BIM                           | Apoptosis                                     |                                                                           |
| miR-125a| ERBB2                        | Proliferation                                 | Matsushima et al. (2011), Nishida et al. (2011)                          |
| miR-130a| ND                           | ND                                            | Matsushima et al. (2011)                                                 |
| miR-133| ND                            | ND                                            | Saito et al. (2011)                                                      |
| miR-141| FGF2                          | Proliferation                                 | Du et al. (2009), Matsushima et al. (2011)                               |
| miR-200a+| ZEB1, ZEB2                   | Epithelial to mesenchymal transition (EMT)    | Ahn et al. (2011), Matsushima et al. (2011), Shinozaki et al. (2010)     |
| miR-200b+| BCL2, XIAP                    | Apoptosis                                     | Ahn et al. (2011), Matsushima et al. (2011), Shinozaki et al. (2010),    |
|        | ZEB1, ZEB2                    | EMT                                           | Zhu et al. (2011a)                                                       |
| miR-200c+| BCL2, XIAP                    | Apoptosis                                     | Matsushima et al. (2011), Shinozaki et al. (2010), Zhu et al. (2011a)   |
|        |                               | EMT                                           |                                                                           |
| miR-203| ABL1                          | Proliferation                                 | Craig et al. (2011b), Matsushima et al. (2011)                          |
|        |                               | Invasion                                       |                                                                           |
| miR-204| EZR                           | Proliferation                                 | Lam et al. (2011), Matsushima et al. (2011)                              |
| miR-210| ND                            | ND                                            | Matsushima et al. (2011)                                                 |
| miR-214| ND                            | ND                                            | Matsushima et al. (2011)                                                 |
| miR-218| ECOP                          | Proliferation                                 | Gao et al. (2010), Tie et al. (2010)                                     |
|        |                               | Apoptosis                                     |                                                                           |
|        | ROBO1                         | Invasion and metastasis                        |                                                                           |
| miR-320+| ND                           | ND                                            | Matsushima et al. (2011)                                                 |
| miR-372| LATS2                         | Cell cycle progression                        | Belair et al. (2011)                                                     |
| miR-373| LATS2                         | Cell cycle progression                        | Belair et al. (2011)                                                     |
| miR-375+| PDK1, 14-3-3, JAK2            | Apoptosis                                     | Ding et al. (2010), Matsushima et al. (2011), Tsukamoto et al. (2010)   |
|        |                               | Proliferation                                 |                                                                           |
| miR-377| ND                            | ND                                            | Matsushima et al. (2011)                                                 |
| miR-379| ND                            | ND                                            | Matsushima et al. (2011)                                                 |
| miR-429+| BCL2, XIAP                    | Apoptosis                                     | Matsushima et al. (2011), Sun et al. (2011), Zhu et al. (2011a)           |
|        | MYC                           | Proliferation                                 |                                                                           |
| miR-449| GMNN, CCNE2                   | Cell cycle progression                        | Bou Kheir et al. (2011), Lize et al. (2011)                              |
|        | MET, SIRT1                     | Proliferation                                 |                                                                           |
| miR-455| ND                            | ND                                            | Matsushima et al. (2011)                                                 |
| miR-491-5p| ND                         | ND                                            | Matsushima et al. (2011)                                                 |
| miR-500| ND                            | ND                                            | Matsushima et al. (2011)                                                 |

(Continued)
Table 1 | Continued

| miRNAs     | Target mRNAs | Biological process targeted | Reference                  |
|------------|--------------|----------------------------|----------------------------|
| miR-532#   | ND           | ND                         | Matsushima et al. (2011)   |
| miR-652#   | ND           | ND                         | Matsushima et al. (2011)   |

Target criteria included (1) reduced protein expression upon miRNA transfection in gastric cells or expression inversely correlated with miRNA in gastric tissue or (2) presence of miRNA binding site on the 3' UTR of target mRNA confirmed by luciferase reporter assay. ND, target mRNA or biological process not determined. *, miRNA expression correlates with acute inflammation. #, miRNA expression correlates with chronic inflammation. +, miRNA expression correlates with both acute and chronic inflammation. Bold indicates miRNA also downregulated in gastric cancer.

Table 2 | miRNAs upregulated in response to *H. pylori* infection.

| miRNAs     | Target mRNAs | Biological process targeted | Reference                  |
|------------|--------------|----------------------------|----------------------------|
| miR-17*    | p21          | Cell cycle progression     | Saito et al. (2010)        |
| miR-20a*   | p21          | Cell cycle progression     | Saito et al. (2010)        |
| miR-21     | PDCD4, RECK, PTEN | Proliferation, Invasion | Zhang et al. (2008)       |
| miR-146a   | IRAK1, TRAF6 | Immune response, Proliferation | Li et al. (2011a), Liu et al. (2010), Xiao et al. (2011) |
| miR-155    | SMAD4, IKK-ε, SMAD2 | Immune response, Apoptosis | Fassi Fehri et al. (2010), Oertli et al. (2011), Tang et al. (2010), Xiao et al. (2009b) |
| miR-223*   | EPB41L3      | Invasion and metastasis    | Li et al. (2011b), Matsushima et al. (2011) |

Target criteria included (1) reduced protein expression upon miRNA transfection in gastric cells or expression inversely correlated with miRNA in gastric tissue or (2) presence of miRNA binding site on the 3' UTR of target mRNA confirmed by luciferase reporter assay. ND, target mRNA or biological process not determined. *, miRNA expression correlates with acute inflammation.

**HELICOBACTER PYLORI AND miRNAs REGULATE CELL CYCLE PROGRESSION**

Disruption of cell cycle progression and increased cellular proliferation are common features of malignancies. Cell cycle progression requires coordinated expression of cyclins, which results in sequential activation of cyclin-dependent kinases (CDKs). miRNA dysregulation promotes cell cycle progression by upregulating cyclin expression and/or downregulating expression of CDK inhibitors (p15, p16, p18, p19, p21, p27, p28, p57) in various cancers, including gastric cancer (Figure 3). miR-449, a miRNA downregulated in *H. pylori*-infected gastric tissue and in gastric cancer, targets *GMNN* (geminin) and *CCNE2* (cyclin E2; Figure 3). Both geminin and cyclin E2 are overexpressed in numerous malignancies and promote M/G1 and G1/Scell cycle progression and cell proliferation (Bou Khier et al., 2011; Lize et al., 2011). Consequently, downregulation of miR-449, as occurs following *H. pylori* infection, promotes cell cycle progression and proliferation through upregulation of geminin and cyclin E2.

p42.3, a recently identified protein significantly upregulated in gastric cancer, regulates G2/M cell cycle progression and proliferation in gastric cancer cells (Xu et al., 2007). miR-29a, a miRNA significantly downregulated in gastric cancer, targets p42.3 (Cui et al., 2011; Figure 3). Thus, the downregulation of miR-29a results in a reciprocal increase in p42.3 expression, promoting increased cell cycle progression and proliferation.

The retinoblastoma protein (RB1) is a tumor suppressor dysregulated in many cancers. RB1 functions to prevent excessive cell proliferation by inhibiting G1/S cell cycle progression. RB1 binds and inhibits transcription factors of the E2F family. When RB1 is bound to E2F, the complex acts as a growth suppressor and prevents progression through cell cycle. A number of miRNAs target these factors. For instance, miR-106a is upregulated in gastric cancer and targets RB1 (Volinia et al., 2006), while miR-331-3p is downregulated in gastric cancer and targets E2F1 (Guo et al., 2010; Figure 3).

TGFβ suppresses gastric cancer cell proliferation via the transcriptional upregulation of the CDK inhibitor, p21 (Yoo et al., 1999). miR-93 and miR-106b directly target p21, resulting in its transcriptional silencing and impairment of the tumor-suppressing activity of TGFβ (Petrocchi et al., 2008; Kan et al., 2009; Figure 3). In addition, miR-25 targets the CDK inhibitor, p57, while miR-221 and miR-222 target the CDK inhibitors, p27 and p57 (Kim et al., 2009; Figure 3). These oncosgenic miRNA clusters are also significantly upregulated in gastric cancer (Volinia et al., 2006; Petrocca et al., 2008; Guo et al., 2009; Kim et al., 2009; Yao et al., 2009). Overexpression of these miRNAs results inactivation of CDK2, thereby promoting G1/S phase progression. Since numerous reports have described the role of *H. pylori* in the modulation of cyclins, CDKs, and CDK inhibitors and their link to gastric carcinogenesis (Shirin et al., 2001), these...
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**FIGURE 2 | TLR, NOD, and the NF-κB signaling pathways.** Host cells recognize invading pathogens through extracellular Toll-like receptors (TLRs) and intracellular Nod-like receptors (NOD). Pathogens, such as *H. pylori*, activate TLRs and adaptor molecules that ultimately lead to NF-κB activation and a proinflammatory immune response. The key adaptor molecule responsible for signaling by TLRs is MyD88. MyD88 and other adaptor proteins (IRAK-1 and TRAF6) in this signaling cascade are targeted by miRNAs to dampen the host immune response.

Data suggest that *H. pylori* modulates expression of cyclins, CDKs, and CDK inhibitors through dysregulation of host miRNAs, which may increase the propensity for gastric transformation.

**HELCOBACTER PYLORI AND miRNA DYSREGULATION INHIBIT APOPTOSIS AND PROMOTE CELL SURVIVAL**

Increased cellular proliferation and evasion of apoptosis are hallmarks of cellular transformation. Apoptosis can be classified as being dependent on either the intrinsic or extrinsic pathways. The intrinsic pathway is initiated within cells and hinges on the balance of activity between pro-apoptotic and anti-apoptotic members of the Bcl-2 (B cell lymphoma 2) superfamily of proteins, which act to regulate the permeability of the mitochondrial membrane. miRNAs regulate apoptosis by altering expression and balance of members of the pro-apoptotic (e.g., Bax, Bak, Bim, Bad, Bid, and BNIP3L) and anti-apoptotic (e.g., Bcl-2, Bcl-xL, and Mcl-1) Bcl-2 protein family (Figure 4).

Numerous miRNAs overexpressed in gastric cancer function as oncomiRs by targeting members of the pro-apoptotic Bcl-2 protein family. In addition to their role in regulating cell cycle progression, *miR-25*, *miR-93*, and *miR-106b* also inhibit apoptosis by preventing expression of the pro-apoptotic protein, Bim (Kan et al., 2009; Figure 4). Overexpression of *miR-130b* also contributes to suppression of Bim and apoptosis by targeting *RUNX3* (runt-related transcription factor; Lai et al., 2010), a known tumor suppressor frequently silenced in gastric cancer (Li et al., 2002). *miR-150* targets the *EGR2* (early growth response protein; Wu et al., 2010), a tumor-suppressive transcription factor that induces apoptosis by direct transactivation of pro-apoptotic factors, Bak, and BNIP3L (Unoki and Nakamura, 2003).

Numerous tumor suppressor miRNAs target members of the anti-apoptotic Bcl-2 protein family and are consequently downregulated in gastric cancer. *miR-15b*, *miR-16*, *miR-34*, *miR-181b*, *miR-181c*, and *miR-497* directly target anti-apoptotic BCL2 (Ji et al., 2008; Xia et al., 2008; Zhu et al., 2010b, 2011b; Figure 4). These miRNA clusters are downregulated in gastric cancer cells.
FIGURE 4 | Signaling cascades that regulate the intrinsic and extrinsic pathways of apoptosis. TNFα signaling leads to activation of NF-κB and the anti-apoptotic protein XIAP. Other receptors that detect survival factors, such as growth factors and cytokines, induce ERK1/2 and PI3K/Akt signaling cascades that ultimately result in the inhibition of the pro-apoptotic protein, Bad. In contrast, upon removal of survival factors, these receptors can signal via JNK to induce the pro-apoptotic protein, Bax. Pro-apoptotic and anti-apoptotic proteins govern the intrinsic pathway of cell death, which results in the release of cytochrome c from the mitochondria and induction of the caspase cascade. Signaling through death receptors initiate the extrinsic pathway of apoptosis, leading to the induction of caspases and cell death. There are numerous miRNAs that regulate each of these pathways and dysregulation of these miRNAs can lead to anti-apoptotic and tumorigenic responses.

(Guo et al., 2009), leading to increased expression of Bcl-2 and inhibition of apoptosis. The miR-200bc/429 cluster is downregulated in gastric cells, and these miRNAs directly target BCL2 and XIAP (x-linked inhibitor of apoptosis), leading to reduced expression and increased apoptosis (Zhu et al., 2011a; Figure 4). miR-101 and miR-512-5p target another anti-apoptotic member of the Bcl-2 family, MCL1 (myeloid leukemia cell differentiation protein; Saito et al., 2009; Wang et al., 2010; Figure 4). Both miR-101 and miR-512-5p are downregulated in gastric cancer, leading to increased levels of Mcl-1 and an anti-apoptotic phenotype. In addition, miR-101 is downregulated by H. pylori (Matsushima et al., 2011). miR-449 is also likely involved in mediating the intrinsic pathway of apoptosis and has been classified as a potent inducer of cell cycle arrest and cell death. miR-449 expression is reduced in H. pylori-infected gastric tissue, and its expression is lost in gastric tumors (Bou Kheir et al., 2011; Lize et al., 2011). Conversely, overexpression of miR-449 inhibits cellular proliferation and induces significant levels of apoptosis, and since miR-449 belongs to the family of p53-responsive miRNAs, its overexpression also results in activation of p53 and apoptosis-specific marker, caspase 3.

In contrast to the intrinsic pathway, the extrinsic pathway of apoptosis is initiated on the cell surface through the activation of specific pro-apoptotic, death receptors. Specific pro-apoptotic ligands are known to activate the extrinsic pathway of apoptosis via specific receptor binding. Ligand binding induces receptor clustering and the recruitment of the adaptor protein Fas-associated death domain (FADD), leading to induction of caspases and ultimately cell death. In addition to its role in regulating the host...
immune response, miR-155 targets FADD (Figure 4), leading to decreased expression of this key adaptor molecule (Xiao et al., 2009b). Therefore, the upregulation of miR-155 by H. pylori and during carcinogenesis results in downregulation of FADD and inhibition of apoptosis.

In addition to targeting proteins directly involved in the intrinsic and extrinsic pathways of cell death, miRNAs target other factors that ultimately lead to inhibition of apoptosis and increased proliferation. miR-375 targets 14-3-3 zeta, an anti-apoptotic protein that mediates cell survival by binding the pro-apoptotic protein Bad and sequestering it to the cytosol (Tsukamoto et al., 2002). Overexpression of miR-375 also targets PDK1 (3-phosphoinositide dependent protein kinase), a kinase that directly phosphorylates Akt, thereby regulating the PI3K/Akt signaling pathway (Figure 4). Overexpression of miR-375 was shown to substantially reduce cell viability through induction of the caspase-dependent apoptotic pathway. miR-375 is one of the most highly downregulated miRNAs in gastric cancer (Tsukamoto et al., 2010), suggesting its role as a potent tumor suppressor that contributes to the development of gastric carcinoma.

In contrast, miR-21, a known oncomir that targets many known tumor suppressors, is consistently upregulated in various human cancers, including gastric cancer (Volinia et al., 2006; Chan et al., 2008; Petrocca et al., 2008; Zhang et al., 2008; Guo et al., 2009), and miR-21 expression is increased in H. pylori-infected gastric tissues (Zhang et al., 2008). Overexpression of miR-21 shifts the balance between proliferation and apoptosis, increasing cellular proliferation and inhibiting apoptosis. Specifically, miR-21 targets PTEN (phosphatase and tensin homolog), a tumor suppressor and negative regulator of the PI3K/Akt signaling pathway (Yamanaka et al., 2009), which is involved in both apoptotic and proliferative pathways (Figure 4). Mutations in PTEN are important in the progression of many cancers, including gastric carcinoma (Kang et al., 2002). miR-21 likely also contributes to apoptosis by targeting PDCD4 (programmed cell death protein 4), which is localized to the nucleus of proliferating cells; however, its direct role in apoptosis has not been elucidated (Lu et al., 2008; Motoyama et al., 2010).

Similar to PI3K/Akt signaling, the NF-κB signaling pathway is important in the inhibition of apoptosis and cell survival. The NF-κB signaling cascade is activated during H. pylori-induced gastritis and is constitutively active in gastric cancer (Sasaki et al., 2001). miR-218 expression is reduced in numerous cancers, including gastric cancer. H. pylori infection also reduces the expression of miR-218 in vitro and in vivo. miR-218 induces apoptosis in gastric cancer cells through direct targeting of ECOP (epidermal growth factor receptor-co-amplified and overexpressed protein), a known positive regulator of NF-κB transcriptional activity. Downregulation of miR-218 leads to overexpression of ECOP, inhibition of NF-κB transcriptional activation, and transcription of a downstream target COX-2, ultimately inhibiting apoptosis, and inducing cell proliferation (Gao et al., 2010). Another miRNA important in regulating NF-κB signal transduction pathways is miR-9, which directly targets NF-κB1, thereby suppressing NF-κB transcriptional activity (Figure 4). miR-9 is downregulated in gastric cancer and in vitro studies have shown that restoration of miR-9 expression suppresses proliferation of gastric cancer cells (Luo et al., 2009; Wân et al., 2010). Cumulatively, these studies demonstrate that aberrant activation of NF-κB signaling as a result of H. pylori-induced miRNA dysregulation results in inhibition of apoptosis and increased proliferation, thereby sensitizing cells for subsequent mutagenesis.

miR-451, another downregulated miRNA in H. pylori-infected gastric mucosa and gastric cancer, targets MIF (macrophage migration inhibitory factor; Bandres et al., 2009), a lymphokine involved in cell-mediated immunity that is expressed in response to H. pylori infection and during gastric carcinogenesis (He et al., 2006). Overexpression of miR-451 results in targeted downregulation of MIF, which is accompanied by a decrease in cell proliferation and increased apoptosis (Figure 4). Furthermore, there is an inverse correlation between miR-451 and MIF expression in gastric cancer, suggesting that miR-451 functions as a tumor suppressor by silencing MIF expression, leading to a proliferative and anti-apoptotic phenotype (Bandres et al., 2009). miR-141, another miRNA significantly decreased in H. pylori-infected gastric tissue (Matsushima et al., 2011) as well as gastric carcinoma, targets FGFR2 (fibroblast growth factor receptor), and overexpression of miR-141 leads to decreased FGFR2 expression and inhibition of proliferation (Du et al., 2009; Figure 4). miR-23a functions as a growth-promoting and anti-apoptotic factor. It is significantly upregulated in gastric adenocarcinoma and targets IL-6R (interleukin-6 receptor), which promotes increased proliferation and decreased apoptosis in gastric adenocarcinoma cells (Zhu et al., 2010a; Figure 4).

**HELICOBACTER PYLORI AND miRNA DYSREGULATION PROMOTES CELL INVASION AND METASTASIS**

Invasion and metastasis are hallmarks of cancer cells. Several intracellular signaling pathways, such as those mediated by TGFβ and hepatocyte growth factor/Met signaling, promote metastasis. In addition to its role in regulating cell cycle progression, the H. pylori downregulated miR-449 also targets Met, a known proto-oncogene that encodes the hepatocyte growth factor receptor. Aberrant activation of Met triggers oncogenic processes, such as proliferation, angiogenesis, invasion, and metastasis (Bou Kheir et al., 2011; Lize et al., 2011). Thus, the targeted downregulation of miR-449 by H. pylori and during gastric carcinogenesis results in upregulation of Met, increased cell proliferation, and likely other oncogenic processes.

The metastatic potential of cancer cells is also regulated by mechanisms that control cell survival, cytoskeletal changes, as well as the activity of extracellular matrix-degrading proteinases (MMPs). Many miRNAs known to regulate cell cycle progression, proliferation, and apoptosis pathways are also involved in metastasis. For example, overexpression of miR-21 has been shown to increase the invasiveness of gastric cancer cells. In addition to its known tumor suppressor targets, miR-21 also targets RECK (reversion-inducing-cysteine-rich protein with kazal motifs), a tumor and metastasis suppressor that inhibits tumor metastasis and angiogenesis through modulation of matrix metalloproteinases (MMPs; Zhang et al., 2008). H. pylori induces expression of MMPs, including MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, which have been linked to H. pylori-induced disease and carcinogenesis (Elkington et al., 2005). These data suggest that H. pylori has the potential to modulate expression of MMPs through
disregulation of host miRNAs and these disruptions may increase the propensity for gastric transformation.

miR-106a is significantly upregulated in cancer cells (Volinia et al., 2006) and is known to correlate with increased lymphatic and distant metastasis (Xiao et al., 2009a). Conversely, miR-218, a tumor suppressor miRNA, is downregulated in gastric cancer (Volinia et al., 2006), which correlates with increased metastasis and invasion. This is thought to occur through direct targeting of ROBO1 (roundabout homolog), which leads to enhanced signaling through the ROBO1 receptor. The SLIT/ROBO signaling pathway has been implicated in many biological responses through regulation of cell migration (Tie et al., 2010). Thus, disruption of this signaling cascade can result in increased invasion and metastasis.

CONCLUSION
The discovery of miRNAs just over a decade ago has challenged the central dogma of genetic and epigenetic regulation. Although extensive work has been dedicated to identifying miRNAs, mRNA targets, and their contribution to accepted regulatory networks, we have only begun to scratch the surface. With thousands of miRNAs within the human genome, and the ability of each miRNA to target and regulate numerous protein-coding mRNAs, affected regulatory networks are likely to be modified by countless miRNA contributors and will continue to evolve.

Many questions arise when comparing miRNA expression profiles in different model systems in vitro and in vivo and when comparing miRNA expression profiles in H. pylori-infected gastric tissue and gastric cancer. For example, miR-106b, a known oncogenic miRNA, upregulated in various malignancies including gastric cancer, is decreased in H. pylori-infected gastric mucosa. Similarly, miR-34b, miR-34c, miR-103, miR-200a, miR-200b, miR-214, and miR-372 are all overexpressed in gastric cancer, while in H. pylori-infected gastric mucosa these miRNAs are significantly downregulated. In contrast, miR-146a is significantly decreased in gastric cancer, but upregulated in H. pylori-infected gastric tissue. However, a recent report has shown that miR-146a is upregulated in a subset of gastric cancers. Although examining miRNA expression signatures in gastric cancer is clearly important in understanding the disease, development of novel therapeutics requires greater insight into the miRNA profiles in precancerous gastric tissues. Since the majority of gastric cancers arise within the context of chronic inflammation, it will be particularly important to discriminate between preneoplastic and tumor-specific miRNA expression profiles. A more comprehensive understanding of the roles of miRNAs in normal biological processes and disease is needed to fully appreciate miRNA dysregulation by pathogens, such as H. pylori. Furthermore, the relationship between single miRNAs and their targets are important to consider, but many of these relationships are cell and context specific. Thus, it is critically important to dissect these intricate pathways and understand how host–pathogen interactions disrupt these encompassing regulatory pathways.

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REFERENCES

Ahn, S. M., Cha, J. Y., Kim, J., Kim, D., Trang, H. T., Kim, Y. M., Cho, Y. H., Park, D., and Hong, S. (2011). Smad3 regulates E-cadherin via miRNA-200 pathway. Oncogene. doi: 10.1038/onc.2011.484. [Epub ahead of print].

Akopyants, N. S., Clifton, S. W., Ker, S., Yerou, B. E., Reece, C. A., Bukanov, N. O., Drzak, E. S., Roe, B. A., and Berg, D. E. (1998). Analyses of the cag pathogenicity island of Helicobacter pylori. Mol. Microbiol. 28, 37–53.

Alm, R. A., Ling, L. S., Moir, D. T., King, B. L., Brown, E. D., Doig, P. C., Smith, D. R., Noonan, B., Guild, B. C., delonge, B. L., Carmel, G., Tummino, P. J., Caruso, A., Uria-Nickelsen, M., Mills, D. M., Ives, C., Gibson, R., Merbergh, D., Mills, S. D., Jiang, Q., Taylor, D. E., Vovis, G. E., and Trust, T. J. (1999). Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori. Nature 397, 176–180.

Ambros, V. (2004). The functions of animal microRNAs. Nature 431, 350–355.

Bandres, E., Bitarte, N., Arias, F., Agoretta, J., Fortes, P., Aguirre, X., Zarate, R., Diaz-Gonzalez, J. A., Ramirez, N., Sola, J. I., Jimenez, P., Rodriguez, J., and Garcia-Foncillas, J. (2009). miRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. Clin. Cancer Res. 15, 2281–2290.

Bartel, D. P. (2009). microRNAs: target recognition and regulatory functions. Cell 136, 215–233.

Belair, C., Baud, J., Chabas, S., Sharma, C. M., Vogel, I., Staedel, C., and Dar feulle., F. (2011). Helicobacter pylori interferes with an embryonic stem cell micro RNA cluster to block cell cycle progression. Silence 2, 7–23.

Boncristiano, M., Paccani, S. R., Barone, S., Olivieri, C., Patrussi, L., Ilver, D., Amedei, A., D’Elisio, M. M., Telford, J. L., and Bal dari, C. T. (2003). The Helicobacter pylori vacuolating toxin inhibits T cell activation by two independent mechanisms. J. Exp. Med. 198, 1887–1897.

Bou Kheir, T., Futoma-Kazmierczak, E., Jacobsen, A. K., Krog, A., Bardram, L., Høther, C., Gronback, K., Federspiel, B., Lund, A. H., and Friis-Hansen, L. (2011). miR-449 inhibits cell proliferation and is down-regulated in gastric cancer. Mol. Cancer 10, 29–40.

Ceminski, S., Lange, C., Xiang, Z., Crabtree, J. E., Ghiaia, P., Borodovsky, M., Rappuoli, R., and Covacci, A. (1996). cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. U.S.A. 93, 14648–14653.

Chan, S. H., Wu, C. W., Li, A. F., Chi, C. W., and Lim, W. C. (2008). miR-21 microRNA expression in human gastric carcinomas and its clinical association. Anticancer Res. 28, 907–911.

Chen, C. Z., Li, L., Lodish, H. F., and Bartel, D. P. (2004). microRNAs modulate hematopoietic lineage differentiation. Science 303, 83–86.

Costinean, S., Zanesi, N., Pekarsky, Y., Tili, E., Volinia, S., Heerema, N., and Croce, C. M. (2006). Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. Proc. Natl. Acad. Sci. U.S.A. 103, 7024–7029.

Covacci, A., Ceminski, S., Bugnoli, M., Petracca, R., Burroni, D., Macchia, G., Massone, A., Papini, E., Xiang, Z., Figura, N., and Rappuoli, R. (1993). Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. Proc. Natl. Acad. Sci. U.S.A. 90, 5791–5795.

Cover, T. L., and Blaser, M. J. (1992). Purification and characterization of the vacuolating toxin from Helicobacter pylori. J. Biol. Chem. 267, 10570–10573.

Cover, T. L., Tummuru, M. K., Cao, P., Thompson, S. A., and Blaser, M. J. (1994). Divergence of genetic sequences for the vacuolating cytotoxin among Helicobacter pylori strains. J. Biol. Chem. 269, 10566–10573.
Craig, V. J., Cogliatti, S. B., Imig, J., Renner, C., Neuenschwander, S., Rehrauer, H., Schlaphbach, R., Dinh, L. O., Tkatchov, A., and Muller, A. (2011a). Myc-mediated repression of microRNA-34a promotes high-grade transformation of B-cell lymphoma by dysregulation of FoxP1. Blood 117, 6272–6276.

Craig, V. J., Cogliatti, S. B., Rehrauer, H., Wundisch, T., and Muller, A. (2011b). Epigenetic silencing of microRNA-203 downregulates ABL1 expression and drives Helicobacter-associated gastric lymphomagenesis. Cancer Res. 71, 3616–3624.

Cui, Y., Su, W. Y., Xing, J., Wang, Y. C., Wang, P., Chen, X. Y., Shen, Z. Y., Cao, H., Lu, Y. Y., and Fang, J. Y. (2011). miR-29a inhibits cell proliferation and induces cell cycle arrest through the downregulation of p21/Cip1 in human gastric cancer. PLoS ONE 6, e25872. doi:10.1371/journal.pone.0025872

Ding, L., Xu, Y., Zhang, W., Deng, Y., Si, M., Du, Y., Yao, H., Liu, X., Ke, Y., Si, J., and Zhou, T. (2010). miR-375 frequently downregulated in gastric cancer inhibits cell proliferation by targeting JAK2. Cell. Res. 20, 784–793.

Du, Y., Xu, Y., Ding, L., Yao, H., Yu, H., Zhou, T., and Si, J. (2009). Down-regulation of miR-141 in gastric cancer and its involvement in cell growth. J. Gastroenterol. 44, 556–561.

Elkington, P. T., O’Kane, C. M., and Friedland, J. S. (2005). The paradox of matrix metalloproteinases in infectious disease. Clin. Exp. Immunol. 142, 12–20.

El-Omar, E. M., Carrington, M., Chow, W. H., McColl, K. E., Bream, J. H., Young, H. A., Herrera, J., Lisowska, J., Yuan, C. C., Rothman, N., Pharoah, D., Ogren, J., Frick, J. M., Lee, H. S., Incecik, E., Berg, D. E., Covacci, A., Engstrand, L., and Boren, T. (1998). Helicobacter pylori pylori adhesin binding fusocylated histo-blood group antigens revealed by retagging. Science 279, 373–377.

Furuta, T., El-Omar, E. M., Xiao, F., Shirai, N., Takashima, M., and Sugimura, H. (2002). Interleukin-1beta polymorphisms increase risk of duodenal ulcer recurrence in Japan. Gastroenterology 116, 1319–1329.

Guo, J., Zhang, Z., Liu, W., Xiao, S., Gu, W., and Lu, H. (2010). Reduced microRNA-218 expression is associated with high nuclear factor kappa B activation in gastric cancer. Cancer 116, 41–49.

Heath, A. C., Clendenen, C. B., J., James, S. M., Petzl, J., Wilson, M. D., and Vaisman, J. (1990). Bacterial infection with Helicobacter pylori mediates carcinogenesis. J. Med. Microbiol. 43, 1456–1463.

Kuck, D., Kolmerer, B., Iking-Konertz, C., Krammer, P. H., Streimmel, W., and Rudi, J. (2001). Vacuolating cytotoxin of Helicobacter pylori induces apoptosis in the human gastric epithelial cell line AGS. Infect. Immun. 69, 5080–5087.

Lai, K. W., Koh, K. K., Loh, M., Tada, K., Subramaniam, M. M., Lim, X. Y., Vafihlingam, A., Salto-Tellez, M., Lacopetis, B., Ito, Y., and Soong, R. (2010). microRNA-130b regulates the tumour suppressor RUNX3 in gastric cancer. Eur. J. Cancer 46, 1456–1463.

Lee, R. C., Feinbaum, R. L., and Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75, 843–854.

Leuk, R. D., Johnson, P. T., David, B. C., Kraft, W. G., and Morgan, D. R. (1988). Cytoxic activity in broth culture filtrates of Campylobacter pylori. J. Med. Microbiol. 26, 93–99.

Lewis, B. P., Shi, I. H., Jones-Rhoades, M. W., Bartel, D. P., and Burge, C. B. (2003). Prediction of mammalian microRNA targets. Cell 115, 787–798.

Li, N., Xu, X., Xiao, B., Zhu, E. D., Li, B. S., Liu, Z., Tang, B., Bao, Q. M., Liang, H. P., and Mao, X. H. (2011a). Hel. pylori related proinflammatory cytokines contribute to the induction of miR-146a in human gastric epithelial cells. Mol. Biol. Rep. doi:10.1007/s11033-011-1257-5. [Epub ahead of print].

Li, X., Zhang, Z., Zhang, H., Liu, X., Gong, T., Li, M., Sun, L., Ji, G., Shi, Y., Han, Z., Han, S., Nie, Y., Chen, X., Zhao, Q., Ding, J., Wu, K., and Daiming, F. (2011b). miRNA-223 promotes gastric cancer invasion and metastasis by targeting tumor suppressor EBP41L3. Mol. Cancer Res. 9, 824–833.

Li, Q. L., Ito, K., Sakakura, C., Fukamachi, H., Inoue, K., Chi, X. Z., Lee, K. Y., Nomura, S., Lee, C. W., Han, S. B., Kim, H. M., Kim, W J., Yamamoto, H., Yasumita, N., Yano, T., Ikeda, T., Itohara, S., Inazawa, J., Abe, T., Hagihara, A., Yamagishi, H., Ooe, A., Kaneda, A., Sugimura, T., Ushijima, T., Bae, S. C., and Ito, Y. (2002). Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell 109, 113–124.

Liu, Z., Xiao, B., Tang, B., Li, B., Li, N., Zhu, E., Guo, G., Gu, J.,
Zhuyang, Y., Liu, X., Ding, H., Zhao, X., Guo, H., Mao, X., and Zou, Q. (2010). Up-regulated microRNA-146a negatively modulates Helicobacter pylori-induced inflammatory response in human gastric epithelial cells. Microbes Infect. 12, 854–863.

Lize, M., Klimek, A., and Dobbelstein, M. (2011). microRNA-449 in cell fate determination. Cell Cycle 10, 2874–2882.

Lu, J., Gerz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B. L., Mak, R. H., Ferrando, A. A., Downing, J. R., Jacks, T., Horvitz, H. R., and Golub, T. R. (2005). microRNA expression profiles classify human cancers. Nature 435, 834–838.

Lu, Z., Liu, M., Strahlbinski, V., Klinge, C. M., Ramos, K. S., Colburn, N. H., and Li, Y. (2008). microRNA-21 promotes cell transformation by targeting the programmed cell death 4 gene. Oncogene 27, 4373–4379.

Luo, H., Zhang, H., Zhang, Z., Zhang, X., Ning, B., Guo, J., Nie, N., Liu, B., and Wu, X. (2009). Down-regulated miR-9 and miR-433 in human gastric carcinoma. J. Exp. Clin. Cancer Res. 28, 82–91.

Machado, J. C., Pharoah, P., Sousa, S., Carvalho, R., Oliveira, C., Figuereido, A., Amorim, A., Seruca, R., Caldas, C., Carneiro, F., and Sobrinho-Simoes, M. (2001). Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology 121, 823–829.

Mahdavi, J., Sonden, B., Hurtig, M., Olaf, F. O., Forsberg, L., Roche, N., Angstrom, J., Larsson, T., Tenenberg, S., Karlsson, K. A., Altraja, S., Wadstrom, T., Kreuzt, D., Berg, D. E., Dubois, A., Petersson, C., Magnusson, K. E., Norberg, T., Lindh, F., Lundskog, B. A., Arnyost, A., Hammarstrom, L., and Boren, T. (2002). Helicobacter pylori Saba adhesin in persistent infection and chronic inflammation. Science 297, 573–578.

Marshall, B. J., Armstrong, J. A., McCleachie, D. B., and Glancy, K. J. (1985). Attempt to fulfill Koch's postulates for pyloric Campylobacter. Med. J. Aust. 142, 436–439.

Matsushita, K., Isohoto, H., Inoue, N., Nakayama, T., Hayashi, T., Nakayama, M., Nakao, K., Hirayama, T., and Kohno, S. (2011). microRNA signatures in Helicobacter pylori-infected gastric mucosa. Int. J. Cancer 128, 361–370.

McCarthy, C. J., Crofford, L. J., Greenen, J., and Scheiman, J. M. (1999). Cyclooxygenase-2 expression in gastric antral mucosa before and after eradication of Helicobacter pylori infection. Am. J. Gastroenterol. 94, 1218–1223.

Meyer, F., Ramanujam, K. S., Gobert, A. P., James, S. P., and Wilson, K. T. (2003). Cutting edge: cyclooxygenase-2 activation suppresses Tfh polarization in response to Helicobacter pylori. J. Immunol. 171, 3913–3917.

Motomuya, K., Inoue, H., Mimori, K., Tanaka, F., Koyama, K., Uetake, H., Sugihara, K., and Mori, S. (2010). Clinicopathological and prognostic significance of PDCD4 and microRNA-21 in human gastric cancer. Int. J. Oncol. 36, 1089–1095.

Motomuya, K., Inoue, H., Nakamura, Y., Uetake, H., Sugihara, K., and Mori, S. (2011). Clinical significance of high mobility group A2 in human gastric cancer and its relationship to gastric antral mucosa before and after eradication of Helicobacter pylori. Cell Cycle 10, 403–414.

Ristimaki, A., Honkanen, N., Jankila, H., Sipponen, P., and Harkonen, M. (1997). Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res. 57, 1276–1280.

Romano, M., Ricci, V., Memoli, A., Tuccillo, C., Donolo, A., and P., Acquaviva, A. M., Del Vecchio Blanco, C., Bruni, C. B., and Zarrilli, R. (1998). Helicobacter pylori up-regulates cyclooxygenase-2 mRNA expression and prostaglandin E2 synthesis in MKN28 gastric mucosal cells in vitro. J. Biol. Chem. 273, 28560–28563.

Saito, Y., Murata-Kamiya, N., Hirayama, T., Ohba, Y., and Hatakeyama, M. (2010). Conversion of Helicobacter pylori CAGA from senescence inducer to oncogenic driver through polarity-dependent regulation of p21. J. Exp. Med. 207, 2157–2174.

Saito, Y., Suzuki, H., Tsugawa, H., Naka- gawa, I., Matsuoka, J., Kanai, Y., and Hibi, T. (2009). Chromatin remodeling at Alu repeats by epigenetic treatment activates silenced microRNA-512-5p with downregulation of Mcl-1 in human gastric cancer cells. Oncogene 28, 2728–2744.

Saito, Y., Suzuki, H., Tsugawa, H., Suzuki, S., Matsuoka, J., Hira, K., and Hibi, T. (2011). Dysfunctional gastric emptying with down-regulation of muscle-specific microRNAs in Helicobacter pylori-infected mice. Gastroenterology 140, 189–198.

Sasaki, N., Morisaki, T., Hashizume, K., Yao, T., Tsuneyoshi, M., Noshiro, H., Nakamura, K., Yamanaka, T., Uchiyama, A., Tanaka, M., and Katano, M. (2001). Nuclear factor-kappaB p65 (RelA) transcription factor is constitutively activated in human gastric carcinoma tissue. Clin. Cancer Res. 7, 4136–4142.

Sawaoaka, H., Kawano, S., Tsuji, S., Suji, M., Sun, W., Gunawan, E. S., and Hori, M. (1998). Helicobacter pylori infection induces cyclooxygenase-2 expression in human gastric mucosa. Prostaglandins Leukot. Essent. Fatty Acids 59, 313–316.

Schmitt, W., and Haas, R. (1994). Genetic analysis of the Helicobacter pylori vacuolating cytotoxin: structural similarities with the IgA protease type of exported protein. Mol. Microbiol. 12, 307–319.

Shinohara, A., Sakatani, T., Ushiku, T., Hino, R., Isoagi, M., Ishikawa, S., Uozaki, H., Takada, K., and Fukayama, M. (2010). Downregulation of microRNA-200 in EBV-associated gastric cancer. Cancer Res. 70, 4197–4217.

Shirin, H., Weinstein, I. B., and Moss, S. F. (2001). Effects of H. pylori infection of gastric epithelial cells on cell cycle control. Front. Biosci. 6, E104–E118.

Senkoly, E., and Pivarcsi, A. (2009). Advances in microRNA: implications for immunity and inflammatory diseases. J. Cell. Mol. Med. 13, 24–38.

Sun, T., Wang, C., Xing, J., and Wu, D. (2011). miR-429 modulates the expression of c-myc in human gastric carcinomas. Eur. J. Cancer. doi: 10.1016/j.ejca.2011.05.021. [Epub ahead of print].

Sundrud, M. S., Torres, V. J., Unutmaz, D., and Cover, T. L. (2004). Inhibition of primary human T cell proliferation by Helicobacter pylori vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. Proc. Natl. Acad. Sci. U.S.A. 101, 7727–7732.

Sung, J. J., Leung, W. K., Go, M. Y., Y., Cheng, A., Ng, E. K., and Chan, F. K. (2000). Cyclooxygenase-2 expression in Helicobacter pylori-associated premalignant and malignant gastric lesions. Am. J. Pathol. 157, 729–735.

Suzuki, H., Yamamoto, E., Nojima, M., K., M., Yamano, H. O., Yoshikawa, K., Kimura, T., Uchimori, A., and Imai, K., Toyota, M., and Shinomura, Y. (2010). Methylation-associated silencing of microRNA-34b/c in gastric cancer and its involvement in an epigenetic field defect. Carcinogene- sis 31, 2066–2073.

Tang, B., Xiao, B., Liu, Z., Li, N., Zhu, E. D., Li, B. S., Xie, Q. H., Zhuang, Y., Zou, Q. M., and Mao, X. H. (2010). Identification of MyD88 as a novel target of mir-155, involved in negative regulation of Helicobacter pylori-induced inflammation. FEBS Lett. 584, 1481–1486.

Telford, L. J., Ghia, P., Dell’Orco, M., Comanducci, M., Burrone, D., Bugnoli, M., Tecce, M. F., Censini, S., Covacci, A., Xiang, Z., Papini, E.,
Varambally, S., Cao, Q., Mani, R. S., Shankar, S., Wang, X., Ateeq, B., Laxman, B., Cao, X., Jing, X., Ramnarayan, K., Brenner, J. C., Yu, J., Kim, J. H., Han, B. T., Pan, K. Murumkar-Shina, C., Lonigro, R. J., Palanisamy, N., Maher, C. A., and Chinnaiyan, A. M. (2008). Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 322, 1695–1699.

Viala, J., Chaput, C., Boneca, I. G., Carmona, A., Girardin, S. E., Moran, A. P., Athman, R., Memet, S., Huere, M. R., Coyle, A. J., DiStefano, P. S., Sansonetti, P. J., Labigne, A., Bertin, J., Philpott, D. J., and Ferrero, R. L. (2004). Nod1 responds to peptido-glycan delivered by the Helicobacter pylori cag pathogenicity island. Nat. Immunol. 5, 1166–1174.

Volinia, S., Calin, G. A., Liu, C. G., Ambs, S., Cimmino, A., Petrocca, F., Viorati, R., Mancini, G., Ferracin, M., Prueitt, R. L., Yanaihara, N., Lanza, G., Scarpa, A., Vecchione, A., Negrini, M., Harris, C. C., and Croce, C. M. (2006). A microRNA expression signature of human solid tumors defines cancer gene targets. Proc. Natl. Acad. Sci. U.S.A. 103, 2379–2384.

Wang, H. Y., Guo, L. M., Liu, T., Liu, M., Li, X., and Tang, H. (2010). Regulation of the transcription factor NF-kappaB1 by microRNA-9 in human gastric adenocarcinoma. Mol. Cancer 9, 16.

Wang, H. J., Ruan, H. J., Li, L. D., Wang, F., Wu, Y., Tong, W. D., Guo, H., Mao, X. H., and Zou, Q. M. (2009b). Induction of microRNA-155 during Helicobacter pylori infection and its negative regulatory role in the inflammatory response. J. Infect. Dis. 200, 916–925.

Wang, B., Zhu, E. D., Li, N., Lu, D. S., Li, W., Li, B. S., Zhao, Y. L., Mao, X. H., Guo, G. Y., Gu, P. W., and Zou, Q. M. (2011). Increased miR-146a in gastric cancer directly targets SMAD4 and is involved in modulating cell proliferation and apoptosis. Oncol. Rep. doi: 10.3892/or.2011.1514. [Epub ahead of print].

Xiao, B., Zhu, E., Dong, N., Chen, H., Shi, Y., Wang, F., Wu, Y., Tong, W. D., Guo, H., Mao, X. H., and Zou, Q. M. (2009a). Detection of miR-106a in gastric cancer and its clinical significance. Clin. Chim. Acta 400, 97–102.

Xia, B., Liu, Z., Li, B. S., Tang, B., Li, W., Guo, R., Shi, Y., Wang, F., Wu, Y., Tong, W. D., Guo, H., Mao, X. H., and Zou, Q. M. (2009b). Induction of microRNA-155 during Helicobacter pylori infection and its negative regulatory role in the inflammatory response. J. Infect. Dis. 200, 916–925.

Xiao, B., Zhu, E. D., Li, N., Lu, D. S., Li, W., Li, B. S., Zhao, Y. L., Mao, X. H., Guo, G. Y., Gu, P. W., and Zou, Q. M. (2011). Increased miR-146a in gastric cancer directly targets SMAD4 and is involved in modulating cell proliferation and apoptosis. Oncol. Rep. doi: 10.3892/or.2011.1514. [Epub ahead of print].

Xu, X., Li, W., Fan, X., Liang, Y., Zhao, M., Zhang, J., Tong, W., Wang, J., Yang, W., and Lu, Y. (2007). Identification and characterization of a novel p24.3 gene as tumor-specific and mitosis phase-dependent expression gene in gastric cancer. Oncogene 26, 7371–7379.

Yamamoto, Y., Tagawa, H., Takahashi, N., Watanabe, A., Guo, Y. M., Iwamoto, K., Yamashita, J., Saitoh, H., Kameoka, Y., Shimizu, N., Ichinohasama, R., and Sawada, K. (2009). Aneberr overexpression of microRNAs activate AKT signaling via down-regulation of tumor suppressors in natural killer-cell lymphoma/leukemia. Blood 114, 3265–3275.

Yang, Q., Jie, Z., Cao, H., Greenlee, A. R., Yang, C., Zou, F., and Jiang, Y. (2011). Low-level expression of let-7a in gastric cancer and its involvement in tumorigenesis by targeting RAB40C. Carcinogenesis 32, 713–722.

Yao, X., Sun, A. L., Li, Z. F., Liu, L. Y., Tian, N., Li, Z., Zhang, W. G., Nan, K. J., Song, T. S., and Huang, C. (2009). microRNA profiling of human gastric cancer. Mol. Med. Report 2, 963–970.

Yoo, Y. D., Choi, J. Y., Lee, S. J., Kim, J. S., Min, B. R., Lee, Y. I., and Kang, Y. K. (1999). TGF-beta-induced cell-cycle arrest through the p21(WAF1/CIP1)-G1 cyclin/Cdksp-130 pathway in gastric-carcinoma cells. Int. J. Cancer 83, 512–517.

Zhang, Z., Li, Z., Gao, C., Chen, P., Chen, J., Liu, W., Xiao, S., and Lu, H. (2008). miR-21 plays a pivotal role in gastric cancer pathogenesis and progression. Lab. Invesit. 88, 1358–1366.

Zhu, L. H., Liu, T., Tang, H., Tian, R. Q., Su, C., Liu, M., and Li, X. (2010a). microRNA-23a promotes the growth of gastric adenocarcinoma cell line MGC803 and decreases its clinical significance. J. Gastrointestinal Cancer 5, 325–330.

Zhu, W., Shan, X., Wang, T., Shu, Y., and Liu, P. (2010b). miR-181b modulates multidrug resistance by targeting BCL2 in human cancer cell lines. Int. J. Cancer 127, 2520–2529.

Zhu, W., Xu, H., Zhu, D., Zhi, H., Wang, T., Wang, J., Jiang, B., Shu, Y., and Liu, P. (2011a). miR-200bc/200 cluster modulates multidrug resistance of human cancer cell lines by targeting BCL2 and XIAP. Cancer Chemother. Pharmacol. doi: 10.1007/s00280-011-1752-5. [Epub ahead of print].

Zhu, W., Zhu, D., Lu, S., Wang, T., Wang, J., Jiang, B., Shu, Y., and Liu, P. (2011b). miR-200bc/200 cluster modulates multidrug resistance of human cancer cell lines by targeting BCL2. Mol. Oncol. doi: 10.1007/s12032-010-9797-4. [Epub ahead of print].

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## APPENDIX

Table A1 | miRNAs downregulated in gastric cancer.

| miRNAs | Target mRNAs* | Biological processes targeted | Reference |
|--------|--------------|------------------------------|-----------|
| let-7a  | RAB40C       | Cell cycle progression       | Li et al. (2010, 2011c), Motoyama et al. (2008), Tsujiura et al. (2010), Yang et al. (2011), Zhang et al. (2007), Zhu et al. (2010c) |
|         | HMGA2        | Proliferation                |           |
| miR-9   | CDX2         | Cell cycle progression       | Luo et al. (2009), Rotkrua et al. (2011), Tsai et al. (2011a), Wan et al. (2010) |
|         | NFκB1        | Invasion                     |           |
| miR-15b | BCL2         | Apoptosis                    | Xia et al. (2008) |
| miR-16  | BCL2         | Apoptosis                    | Shin et al. (2011), Xia et al. (2008) |
| miR-29a | p24.3        | Cell cycle progression       | Cui et al. (2011), Lang et al. (2010) |
|         | CDC42        | Proliferation                |           |
| miR-29b | CDC42        | Proliferation                | Lang et al. (2010) |
| miR-29c | CDC42        | Invasion                     |           |
| miR-30a | ND           | ND                           | Li et al. (2010) |
| miR-30b | ND           | ND                           | Ueda et al. (2010) |
| miR-30c | ND           | ND                           | Ueda et al. (2010) |
| miR-31  | ND           | ND                           | Guo et al. (2009), Zhang et al. (2010b) |
| miR-33b | ND           | ND                           | Volinia et al. (2006) |
| miR-34  | BCL2         | Apoptosis                    | Ji et al. (2008) |
| mir-96  | ND           | ND                           | Volinia et al. (2006) |
| miR-101 | COX-2, FOS   | Proliferation                | Varambally et al. (2008), Wang et al. (2010) |
| MCL1    | Apoptosis    |                              |           |
| EZH2    | Invasion migration |                          |           |
| miR-126 | CRK          | Cell cycle progression       | Feng et al. (2010), Li et al. (2010, 2011c), Otsubo et al. (2011) |
|         | SOX2         | Proliferation                |           |
|         |              | Invasion and metastasis      |           |
| miR-128b| ND           | ND                           | Katada et al. (2009) |
| miR-129 | CDK6         | Cell cycle progression       | Katada et al. (2009), Shen et al. (2010), Tsai et al. (2011b), Wu et al. (2010a) |
|         | SOX4         | Apoptosis                    |           |
| miR-133b| ND           | ND                           | Guo et al. (2009), Wu et al. (2011a) |
| miR-136 | ND           | ND                           | Ueda et al. (2010) |
| miR-138 | ND           | ND                           | Volinia et al. (2006) |
| miR-139-5p| ND          | ND                           | Guo et al. (2009) |
| miR-141 | FGFR2        | Proliferation                | Du et al. (2009) |
| miR-143 | ND           | Proliferation                | Li et al. (2011a), Takagi et al. (2009), Wu et al. (2011a) |
| miR-145 | ND           | Proliferation                | Li et al. (2011a), Takagi et al. (2009), Tchernitsa et al. (2010) |
| miR-146a| IRAK1, TRAF6 | Immune response              | Hou et al. (2011), Kogo et al. (2011), Li et al. (2011a,d), Okubo et al. (2010), Tchernitsa et al. (2010) |
|         |              | Proliferation                |           |
|         |              | Apoptosis                    |           |
| miR-148 | ND           | ND                           | Katada et al. (2009) |
| miR-148a| CCXBR        | Proliferation                | Chen et al. (2010), Guo et al. (2011), Zheng et al. (2011) |
| p27     |              | Cell cycle progression       |           |
| ROCK1   |              | Invasion and metastasis      |           |
| miR-148b| CCXBR        | Proliferation                | Song et al. (2011) |
| miR-152 | CCXBR        | Proliferation                | Chen et al. (2010), Ueda et al. (2010) |

(Continued)
Table A1 | Continued

| miRNAs | Target mRNAs* | Biological processes targeted | Reference |
|--------|---------------|-------------------------------|-----------|
| miR-181b | BCL2 | Apoptosis | Jiang et al. (2011), Li et al. (2011c), Zhu et al. (2010b) |
| miR-181c | NOTCH4, KRAS, BCL2 | Proliferation, Apoptosis | Hashimoto et al. (2010), Zhu et al. (2010b) |
| miR-195 | ND | ND | Guo et al. (2009), Wu et al. (2011a) |
| miR-197 | ND | ND | Li et al. (2011c) |
| miR-203 | ABL1 | Proliferation | Chiang et al. (2011), Craig et al. (2011b) |
| miR-210 | ND | ND | Li et al. (2011c) |
| miR-212 | MECP2, MYC | Proliferation | Volinia et al. (2006), Wada et al. (2010), Wu et al. (2011a), Xu et al. (2010) |
| miR-218 | ECOP | Proliferation | Gao et al. (2010), Tie et al. (2010), Ueda et al. (2010), Volinia et al. (2006) |
| miR-331-3p | E2F1 | Cell cycle progression, Apoptosis | Guo et al. (2010) |
| miR-339 | ICAM-1 | Immune response | Ueda et al. (2009) |
| miR-375 | PDK1, 14-3-3, JAK2 | Apoptosis, Proliferation | Ding et al. (2010), Tsukamoto et al. (2010), Ueda et al. (2010), Xu et al. (2011) |
| miR-378 | ND | ND | Guo et al. (2009), Yao et al. (2009) |
| miR-433 | GRB2 | Proliferation | Luo et al. (2009) |
| miR-449 | GMNN, CCNE2, MET, SIRT1 | Cell cycle progression, Proliferation | Bou Kheir et al. (2011), Lize et al. (2011) |
| miR-451 | MIF | Proliferation, Apoptosis | Bandres et al. (2009) |
| miR-497 | BCL2 | Apoptosis | Guo et al. (2009), Zhu et al. (2011b) |
| miR-512-5p | MCL1 | Apoptosis | Saito et al. (2009) |
| miR-638 | ND | ND | Yao et al. (2009) |
| miR-768-3p | ND | ND | Guo et al. (2009) |

*Target criteria included (1) reduced protein expression upon miRNA transfection in gastric cells or expression inversely correlated with miRNA in gastric tissue or (2) presence of miRNA binding site on the 3’ UTR of target mRNA confirmed by luciferase reporter assay. ND, target mRNA or biological process not determined. Bold indicates miRNA also downregulated following H. pylori infection.
### Table A2 | miRNAs upregulated in gastric cancer.

| miRNAs     | Target mRNAs* | Biological processes targeted         | Reference                                                                 |
|------------|---------------|---------------------------------------|---------------------------------------------------------------------------|
| mir-7      | ND            | ND                                    | Volinia et al. (2006), Wu et al. (2011b)                                  |
| mir-17     | ND            | ND                                    | Guo et al. (2009), Volinia et al. (2006), Yao et al. (2009), Zhou et al. (2010) |
| mir-17-5p  | ND            | ND                                    | Petrocca et al. (2008), Tsujiura et al. (2010), Ueda et al. (2010), Volinia et al. (2006) |
| mir-18a    | ND            | ND                                    | Guo et al. (2009), Yao et al. (2009)                                       |
| mir-18b    | ND            | ND                                    | Guo et al. (2009)                                                          |
| mir-19a    | ND            | ND                                    | Guo et al. (2009), Ueda et al. (2010)                                      |
| mir-20a    | ND            | ND                                    | Guo et al. (2009), Volinia et al. (2006)                                  |
| mir-20b    | ND            | ND                                    | Guo et al. (2009), Katada et al. (2009), Ueda et al. (2010)                |
| mir-21     | PDCD4         | Proliferation                         | Chan et al. (2008), Guo et al. (2009), Li et al. (2010, 2011c), Lu et al. (2008), Motoyama et al. (2010), Petrocca et al. (2008), Shin et al. (2011), Tsujiura et al. (2010), Ueda et al. (2010), Volinia et al. (2006), Zhang et al. (2008) |
|            | RECK          | Apoptosis                             |                                                                             |
|            | PTEN          | Invasion                              |                                                                             |
| miR-23a    | IL-6R         | Proliferation                         | Li et al. (2011c), Volinia et al. (2006), Zhu et al. (2010a)               |
| miR-23b    | ND            | ND                                    | Li et al. (2011c)                                                          |
| miR-24     | AE1           | Proliferation                         | Chan et al. (2010), Volinia et al. (2006), Wu et al. (2010b)              |
|            |               | Apoptosis                             |                                                                           |
| miR-25     | p57 BIM      | Cell cycle progression                | Kan et al. (2009), Kim et al. (2009), Li et al. (2011c), Petrocca et al. (2008), Ueda et al. (2010), Volinia et al. (2006) |
| miR-27     | APC           | Apoptosis                             | Zhang et al. (2011)                                                       |
| miR-27a    | PHB ZBTB10   | Proliferation                         | Katada et al. (2009), Li et al. (2011a), Liu et al. (2009), Sun et al. (2010), Zhao et al. (2011) |
| miR-34a    | SIRT1 FOXP1  | Cell cycle progression                | Craig et al. (2011a), Yamanouchi and Lowenstein (2009), Yao et al. (2009) |
| miR-34b    | ND            | ND                                    | Katada et al. (2009), Suzuki et al. (2010), Tsai et al. (2011b)            |
| miR-34c    | ND            | ND                                    | Katada et al. (2009), Suzuki et al. (2010)                                |
| miR-92     | ND            | ND                                    | Li et al. (2011c), Petrocca et al. (2008), Ueda et al. (2010), Volinia et al. (2006) |
| miR-93     | p21 BIM      | Cell cycle progression                | Kim et al. (2009), Petrocca et al. (2008), Ueda et al. (2010)              |
| miR-98     | ND            | ND                                    | Yao et al. (2009)                                                          |
| miR-99a    | ND            | ND                                    | Li et al. (2011c)                                                          |
| miR-99b    | ND            | ND                                    | Volinia et al. (2006)                                                     |
| miR-103    | ND            | ND                                    | Li et al. (2011c), Tchernitsa et al. (2010), Volinia et al. (2006)          |
| miR-106a   | RB1           | Cell cycle progression                | Guo et al. (2009), Petrocca et al. (2008), Tsujiura et al. (2010), Ueda et al. (2010), Volinia et al. (2006), Xiao et al. (2009a), Yao et al. (2009) |
| miR-106b   | p21 BIM      | Cell cycle progression                | Guo et al. (2009), Kim et al. (2009), Petrocca et al. (2008), Tsujiura et al. (2010), Ueda et al. (2010), Yao et al. (2009) |
| miR-107    | CDK6 DICER   | Proliferation                         | Feng et al. (2011), Li et al. (2011b,c), Volinia et al. (2006)            |
| miR-125b   | ND            | ND                                    | Li et al. (2011c), Ueda et al. (2010), Volinia et al. (2006)               |
| miR-128a   | ND            | ND                                    | Katada et al. (2009)                                                      |
| miR-130b   | RUNX3         | Apoptosis                             | Li et al. (2002), Lai et al. (2010), Yao et al. (2009)                     |
| miR-135a   | ND            | ND                                    | Ueda et al. (2010)                                                        |
| miR-138    | ND            | ND                                    | Yao et al. (2009)                                                         |
| mir-146a   | SMAD4         | Proliferation                         | Xiao et al. (2011)                                                        |
| mir-147    | ND            | ND                                    | Yao et al. (2009)                                                         |
| mir-150    | EGR2          | Apoptosis                             | Katada et al. (2009), Wu et al. (2010c)                                  |

(Continued)
Table A2 | Continued

| miRNAs | Target mRNAs* | Biological processes targeted | Reference |
|--------|---------------|--------------------------------|-----------|
| miR-155 | IKK-ε, SMAD2 | Immune response | Fassi Fehri et al. (2010), Oertli et al. (2011), Tang et al. (2010), Thai et al. (2007), Volinia et al. (2006), Xiao et al. (2009b), Yao et al. (2011) |
|        | FADD, PKi | Apoptosis | | |
| miR-181a-2 | ND | ND | Yao et al. (2009) |
| miR-185 | ND | ND | Yao et al. (2009) |
| miR-191 | NdST1 | Proliferation | Li et al. (2011c), Shi et al. (2011), Ueda et al. (2010), Volinia et al. (2006) |
| miR-192 | ALCAM | Proliferation | Jin et al. (2011), Volinia et al. (2006) |
| miR-196a | ND | ND | Okubo et al. (2010), Yao et al. (2009) |
| miR-200a | ZEB1, ZEB2 | Epithelial to mesenchymal transition (EMT) | Ahn et al. (2011) |
| miR-200b | ZEB1, ZEB2 | EMT | Ahn et al. (2011), Zhu et al. (2011a) |
|         | BCL2, XIAP | Apoptosis | | |
| miR-214 | ND | ND | Li et al. (2011c), Ueda et al. (2010), Volinia et al. (2006) |
| miR-215 | ALCAM | Proliferation | Jin et al. (2011), Volinia et al. (2006) |
| miR-221 | p27, p57 | Cell cycle progression | Chun-Zhi et al. (2010), Kim et al. (2009), Li et al. (2011c), Volinia et al. (2006), Yao et al. (2009) |
|         | PTEN | Proliferation | | |
| miR-222 | p27, p57 | Cell cycle progression | Chun-Zhi et al. (2010), Kim et al. (2009), Li et al. (2011c), Ueda et al. (2009), Volinia et al. (2006) |
|         | PTEN | Proliferation | | |
|         | ICAM-1 | Immune response | | |
| miR-223 | EPB41L3 | Invasion and metastasis | Li et al. (2011c), Petrocca et al. (2008), Volinia et al. (2006), Yao et al. (2009) |
| miR-302f | ND | ND | Yao et al. (2009) |
| miR-337-3p | ND | ND | Yao et al. (2009) |
| miR-340 | ND | ND | Guo et al. (2009), Yao et al. (2009) |
| miR-345 | ND | ND | Ueda et al. (2010) |
| miR-372 | LATS2 | Cell cycle progression | Cho et al. (2009) |
|         | | Apoptosis | | |
| miR-421 | CBX7, RBMXL1 | Proliferation | Guo et al. (2009), Jiang et al. (2010) |
| miR-520c-3p | ND | ND | Yao et al. (2009) |
| miR-575 | ND | ND | Yao et al. (2009) |
| miR-601 | ND | ND | Yao et al. (2009) |
| miR-616 | ND | ND | Yao et al. (2009) |
| miR-650 | ING4 | Apoptosis | Zhang et al. (2010a) |
| miR-658 | ND | ND | Guo et al. (2009) |
| miR-1259 | ND | ND | Yao et al. (2009) |

*Target criteria included (1) reduced protein expression upon miRNA transfection in gastric cells or expression inversely correlated with miRNA in gastric tissue or (2) presence of miRNA binding site on the 3′ UTR of target mRNA confirmed by luciferase reporter assay. ND, target mRNA or biological process not determined. Bold indicates miRNA also upregulated following H. pylori infection.
REFERENCES

Ahn, S. M., Cha, J. Y., Kim, J., Kim, D., Trang, H. T., Kim, Y. M., Cho, Y. H., Park, D., and Hong, S. (2011). Smad3 regulates E-cadherin via miRNA-200 pathway. Oncogene. doi: 10.1038/onc.2011.484. [Epub ahead of print].

Bandres, E., Bitarte, N., Arias, F., Agorreta, I., Fortes, P., Agirre, X., Zarate, R., Diaz-Gonzalez, J. A., Ramirez, N., Sola, J. J., Jimenez, P., Rodriguez, I., and Garcia-Foncillas, J. (2009). microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. Clin. Cancer Res. 15, 2281–2290.

Bou Kheir, T., Futoma-Kazmierzczak, E., Jacob, I., Sebti, S. M., and Lai, C. (2010). Reduced microRNA-218 expression is associated with high nuclear factor kappa B activation in gastric cancer. Cancer 116, 41–49.

Cho, J., Miao, Y., Xiao, B., Huan, R., Jiang, Z., Meng, D., and Wang, Y. (2009). Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues. J. Gastroenterol. Hepatol. 24, 652–657.

Guo, S. L., Peng, Z., Yang, X., Fan, K. J., Ye, H., Li, Z. H., Wang, Y., Xu, X. L., Li, J., Wang, Y. L., and Teng, Y. (2011). miR-148a promoted cell proliferation by targeting p27 in gastric cancer cells. Int. J. Biol. Sci. 7, 567–574.

Guo, X., Guo, L., Ji, J., Zhang, J., Chen, X., Cai, Q., Li, J., Gu, Q., Liu, B., Zhu, Z., and Yu, Y. (2010). miRNA-331-3p downregulates LATS2. Biochem. Biophys. Res. Commun. 398, 1–6.

Hashimoto, Y., Akiyama, Y., Otsubo, T., Shimada, S., and Yusa, Y. (2010). Involvement of epigenetically silenced microRNA-181c in gastric carcinogenesis. Carcinogenesis 31, 777–784.

Hou, Z., Xie, L., Yu, L., Qin, X., and Liu, R. (2011). microRNA-146a is downregulated in gastric cancer and regulates cell proliferation and apoptosis. Med. Oncol. doi: 10.1186/1300-0118-7-90. [Epub ahead of print].

Ji, Q., Hao, X., Meng, Y., Zhang, M., Desano, J., Fan, D., and Xu, L. (2008). Restoration of tumor suppressor miR-34 inhibits human p53 mutant gastric cancer tumour spheres. BMC Cancer 8, 266–278. doi:10.1186/1471-2407-8-266.

Jiang, J., Zheng, X., Xu, X., Zhou, Q., Yan, H., Zhang, X., Lu, B., Wu, C., and Ju, J. (2011). Prognostic significance of miR-181b and miR-21 in gastric cancer patients treated with S-1/oxaplatin or doxil/durixil/oxaplatin. PLoS ONE 6, e23271. doi:10.1371/journal.pone.0023271.

Jiang, Z., Guo, J., Xiao, B., Miao, Y., Jiang, X., Hu, J., and Zhang, Y. (2010). Increased expression of miR-421 in human gastric carcinoma and its clinical association. J. Gastroenterol. 45, 17–23.

Jin, Z., Selaru, F. M., Cheng, Y., Kan, T., Agarwal, R., Mori, Y., Olaru, A. V., Yang, J., David, S., Hamilton, J. P., Yang, J., Abraham, J. M., Mori, Y., and Melzer, S. J. (2009). The miR-106b-25 polycistron, activated by genomic amplification, functions as an oncogene by suppressing p21 and Bim. Gastroenterology 136, 1689–1700.

Katada, T., Ishiguro, H., Kubawara, Y., Kimura, M., Mitui, A., Mori, Y., Ogawa, R., Harata, K., and Fujii, Y. (2009). microRNA expression profile in undifferentiated gastric cancer. Int. J. Oncol. 34, 537–542.

Kim, Y. K., Yu, J., Han, T. S., Park, S. Y., Namkoong, B., Kim, D. H., Hur, K., Yoo, M. W., Lee, H. J., Yang, K. H., and Kim, V. N. (2009). Functional links between clustered microRNAs and repression of cell-cycle inhibitors by microRNA clusters in gastric cancer. Nucleic Acids Res. 37, 1672–1681.

Kogo, R., Mimori, K., Tanaka, F., Komune, S., and Mori, M. (2011). Significant clinical significance of miR-146a in gastric cancer cases. Clin. Cancer Res. 17, 4277–4284.

Lai, K. W., Koh, K. X., Loh, M., Tada, K., Subramaniam, M. M., Lim, X., Vaitilingham, A., Salto-Tellez, M., Lacopeta, B., Ito, Y., and Soong, R. (2010). microRNA-130b regulates the tumour suppressor RUNX3 in gastric cancer. Eur. J. Cancer 46, 1456–1463.

Lang, N., Liu, M., Tang, Q. L., Chen, X., Liu, Z., and Bi, F. (2010). Effects of microRNA-29 family members on proliferation and invasion of gastric cancer cell lines. Chin. J. Cancer 29, 603–610.

Li, Q. L., Ito, K., Sakakura, C., Fukamachi, H., Inoue, K., Chi, X. Z., Lee, K. Y., Nomura, S., Lee, C. W., Han, S. B., Kim, H. M., Kim, W. J., Yamamoto, H., Yamashita, N., Yano, T., Ikeda, T., Itohara, S., Inazawa, J., Abe, T., Hagihara, A., Yamagishi, H., Ooe, A., Kaneda, A., Sugimura, T., Ushijima, T., Bae, S. C., and Ito, Y. (2002). Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell 109, 113–124.

Li, X., Luo, F., Li, Q., Xu, M., Feng, D., Zhang, G., and Wu, W. (2011a). Identification of new aberrantly expressed miRNAs in intestinal type. Oncol. Rep. 26, 1431–1439.

Li, X., Zhang, Y., Shi, Y., Dong, G., Liang, J., Han, Y., Wang, X., Zhao, Q., Ding, J., Wu, K., and Fan, D. (2011b). microRNA-107, an oncogenic microRNA that regulates tumour invasion and metastasis by targeting DICER1 in gastric cancer. J. Cell. Mol. Med. 15, 1887–1895.
Okubo, M., Tahara, T., Shibata, T., Yamashita, H., Nakamura, M., Yoshioha, D., Yonemura, J., Ishizuka, T., Arisawa, T., and Hrita, I. (2010). Association between common genetic variants in pre-microRNAs and gastric cancer risk in Japanese population. Helicobacter 15, 524–531.

Otsubo, T., Akiyama, Y., Hashimoto, Y., Shimada, S., Goto, K., and Yusa, Y. (2011). microRNA-126 inhibits SOX2 expression and contributes to gastric carcinogenesis. PLoS ONE 6, e16617. doi:10.1371/journal.pone.0016617

Petrucco, F., Visone, R., Onelli, M. R., Shah, M. H., Niccolò, M. S. de, Martino, I., Iliopoulos, D., Pilozi, E., Liu, C. G., Negrini, M., Cavazzini, L., Volinia, S., Alder, H., Ruo, L. P., Baldassarre, G., Groce, C. M., and Vecchione, A. (2008). E2F1-regulated microRNAs impair TGFβeta-dependent cell-cycle arrest and apoptosis in gastric cancer. Cancer Cell 13, 272–286.

Rotkrua, P., Akiyama, Y., Hashimoto, Y., Otsubo, T., and Yusa, Y. (2011). miR-9 downregulates CDX2 expression in gastric cancer cells. Int. J. Cancer 129, 2611–2620.

Saito, Y., Suzuki, H., Tsugawa, H., Naka- gawa, I., Matsuizaki, J., Kanai, Y., and Hibi, T. (2009). Chromatin remodelling at Alu repeats by epigenetic treat- ment activates silenced microRNA-512-5p with downregulation of Mcl-1 in human gastric cancer cells. Oncogene 28, 2738–2744.

Shen, K., Fan, S., Qi, S., Lin, X., and Chen, S. (2010). Epigenetic repression of microRNA-129-2 leads to overexpression of SOX4 in gastric cancer. Biochem. Biophys. Res. Commun. 394, 1047–1052.

Shi, X., Su, S., Long, J., Mei, B., and Chen, Y. (2011). microRNA-191 targets N-desethylase/N-sulfotransferase 1 and promotes cell growth in human gastric carcinoma cell line MGC803. Acta Biochim. Biophys. Sin. doi: 10.1093/abbs/gmr084. [Epub ahead of print]

Shin, V. Y., Jin, H., Ng, E. K., Cheng, A. S., Chong, W. W., Wong, Y. M., Leung, W. K., Sung, J., and Chu, K. M. (2011). NF-kappab regulates miR-16-5p and miR-21 in gastric cancer: involvement of prostaglandin E receptors. Carcinogenesis 32, 240–245.

Song, Y. X., Yue, Z. Y., Wang, Z. N., Xu, Y. Y., Luo, Y., Xu, H. M., Zhang, X., Jiang, L., Xing, C. Z., and Zhang, Y. (2011). microRNA-148b is frequently down-regulated in gastric cancer and acts as a tumor suppressor by inhibiting cell proliferation. Mol. Cancer 10, 1–13.

Sun, Q., Gu, H., Zeng, Y., Xia, Y., Wang, Y., Jing, Y., Yang, L., and Wang, B. (2010). Isa-mir-27a genetic variant contributes to gastric cancer susceptibility through affecting mir-27a and target gene expression. Cancer Sci. 101, 2241–2247.

Suzuki, H., Yamamoto, E., Nojima, M., Kai, M., Yamano, H. O., Yoshikawa, K., Kimura, T., Kudo, T., Harada, E., Sugai, T., Taka- maru, H., Niinuma, T., Maruyama, R., Yamamoto, H., Tokino, T., Imai, K., Toyota, M., and Shinomura, Y. (2010). Methylation-associated silencing of microRNA-34b/c in gas- tric cancer and its involvement in an epigenetic field. Cancergenesis 31, 2066–2073.

Takagi, T., Iio, A., Nakagawa, Y., Naoe, T., Tanigawa, N., and Akao, Y. (2009). Decreased expression of microRNA-143 and -145 in human gastric can- cers. Oncology 77, 12–21.

Tang, B., Xiao, B., Liu, Z., Li, N., Zhu, E. D., Li, B. S., Xie, Q. H., Zhuang, Y., Zou, Q. M., and Mao, X. H. (2010). Identification of MyD88 as a novel target of miR-153, involved in negative regulation of Helicobacter pylori-induced inflammation. FERS LET 584, 1481–1486.

Tchernitsa, O., Kajimura, A., Takagi, T., Iio, A., Nakagawa, Y., Naoe, T., Tanigawa, N., and Akao, Y. (2009). Decreased expression of microRNAs and -mediated carcinogenesis. PLoS ONE 6, e16617. doi:10.1371/journal.pone.0016617

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Y. (2010). miR-212 is downregulated and suppresses methyl-CpG-binding protein McCP2 in human gastric cancer. *Int. J. Cancer* 127, 1106–1114.

Wan, H. Y., Guo, L. M., Liu, T., Liu, M., Li, X., and Tang, H. (2010). Regulation of the transcription factor NF-kappaB1 by microRNA-9 in human gastric adenocarcinoma. *Mol. Cancer* 9, 16–26.

Wang, H. J., Ruam, H. J., He, X. J., Ma, Y. Y., Jiang, X. T., Xia, Y. J., Ye, Z. Y., and Tao, H. Q. (2010). microRNA-101 is down-regulated in gastric cancer and involved in cell migration and invasion. *Eur. J. Cancer* 46, 2295–2303.

Wu, J., Qian, J., Li, C., Kwock, L., Cheng, F., Liu, P., Perdono, C., Kotton, D., Vaziri, C., Anderlind, C., Spira, A., and Zhang, L. F. (2008). miR-21 plays a pivotal role in modulation of NF-kappaB and its negative regulatory role in the inflammatory response. *J. Invest. Dis.* 200, 916–925.

Xiao, B., Yuan, X. J., Sun, H., Wu, Y., Zhang, L., Jiang, P., and Zhang, J. (2009a). Detection of miR-106a in gastric carcinoma and its clinical significance. *Clin. Chim. Acta* 400, 97–102.

Xiao, B., Liu, Z., Li, B. S., Tang, B., Li, W., Guo, G., Shi, Y., Wang, F., Wu, Y., Tong, W. D., Guo, H., Mao, X. H., and Zou, Q. M. (2009b). Induction of microRNA-155 during *Helicobacter pylori* infection and its negative regulatory role in the inflammatory response. *J. Invest. Dis.* 200, 916–925.

Xiao, B., Zhou, E. D., Li, N., Lu, D. S., Li, W., Li, B. S., Zhao, Y. L., Mao, X. H., Guo, G., Yu, P. W., and Zou, Q. M. (2011). Increased miR-146a in gastric cancer directly targets SMAI3 and is involved in modulating cell proliferation and apoptosis. *Oncol. Rep.* doi: 10.3892/or.2011.1514. [Epub ahead of print].

Xu, L., Wang, F., Xu, X. F., Mo, W. H., Xia, Y. J., Yan, R., Wang, X. P., and Guo, C. Y. (2010). Down-regulation of miR-212 expression by DNA hypermethylation in human gastric cancer cells. *Med. Oncol.* doi: 10.1007/s12032-010-0961-0. [Epub ahead of print].

Xu, Y., Zheng, Y., Su, C., Liu, M., and Li, Y. M., Zhong, Z. X., and Liu, Z. (2010). Overexpression of microRNA-29a promotes cell proliferation and invasion of human gastric cancer cell line. *Cell. Mol. Immunol.* 7, 1106–1114.

Yao, Y., Yao, R., Ma, Y., Du, Y., Liao, M., Li, H., Jiang, W., Yuan, J., Zhi, H., Xiong, Y., Xiao, H., and Liao, Y. (2011). The altered expression of proliferation-related microRNAs with microRNA-155 expression correlates with Th17 differentiation in patients with acute coronary syndrome. *Cell. Mol. Immunol.* doi: 10.1038/cmi.2011.22. [Epub ahead of print].

Zhou, H., Guo, J. M., Lou, Y. R., Zhang, X. J., Zhong, F. D., Jiang, Z., Cheng, J., and Xiao, B. X. (2010). Detection of circulating tumor cells in peripheral blood from patients with gastric cancer using microRNA as a marker. *J. Mol. Med.* 88, 709–717.

Zhu, L. H., Liu, T., Tang, H., Tian, R. Q., Su, C., Liu, M., and Li, X. (2010a). microRNA-23a promotes the growth of gastric adenocarcinoma cell line MGC803 and downregulates interleukin-6 receptor. *FEBS J.* 277, 3736–3734.

Zhu, W., Shan, X., Wang, T., Shu, Y., and Liu, P. (2010b). miR-181b modulates multidrug resistance by targeting BCL2 in human gastric cancer cells. *Tumor Biol.* 31, 1275–1280.

Zhu, Y. M., Zheng, Z. X., and Liu, Z. M. (2010c). Relationship between let-7a and gastric mucosa carcinomatization and its significance. *World J. Gastroenterol.* 16, 3325–3329.

Zhu, W., Xu, H., Zhu, D., Zhu, H., Wang, T., Wang, J., Shu, Y., and Liu, P. (2011a). miR-200bc/429 cluster modulates multidrug resistance of human cancer cell lines by targeting BCL2 and XIAP. *Cancer Chemother. Pharmacol.* doi: 10.1007/s00280-011-1752-3. [Epub ahead of print].

Zhu, W., Zhu, D., Lu, S., Wang, T., Wang, J., Jiang, B., Shu, Y., and Liu, P. (2011b). miR-497 modulates multidrug resistance of human cancer cell lines by targeting BCL2. *Mol. Oncol.* doi: 10.1016/j.socm.2011-09-003.