Research Article

Construction and Investigation of MicroRNA-mRNA Regulatory Network of Gastric Cancer with Helicobacter pylori Infection

Ping Yang, Junjie Liu, Tianci Yang, Lei Zhang, Peiyou Gong, Boqing Li, and Xiuzhi Zhou

1Department of Pathology, Yantai Yuhuangding Hospital of Qingdao University, Yantai 264000, Shandong Province, China
2School of Basic Medical Sciences, Binzhou Medical University, Yantai 264003, Shandong Province, China
3School of Clinical Medicine, Binzhou Medical University, Yantai 264003, Shandong Province, China
4Department of Infectious Diseases, Yantai Affiliated Hospital of Binzhou Medical University, Yantai 264100, Shandong Province, China
5Department of Medicine, Yantai Yuhuangding Hospital of Qingdao University, Yantai 264000, Shandong Province, China

Correspondence should be addressed to Boqing Li; sdliboqing@163.com and Xiuzhi Zhou; zxz7810@163.com

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Background. Helicobacter pylori (H. pylori) is a common human pathogen, which is closely related to gastric cancer (GC). However, the mechanism of H. pylori-related GC has not been elucidated. This study aimed to explore the role of H. pylori infection in GC and find biomarkers for early diagnosis of H. pylori-related GC.

Methods. We identified differentially expressed microRNAs (DEMs) and genes (DEGs) from the Gene Expression Omnibus (GEO) dataset, constructed microRNA-(miRNA-) mRNA expression networks, analyzed the function and signal pathway of cross-genes, analyzed the relations between cross-genes and GC prognosis with the Cancer Genome Atlas (TCGA) data, and verified the expression of cross-genes in patients with H. pylori infection.

Results. 22 DEMs and 68 DEGs were identified in GSE197694 and GSE27411 dataset. 16 miRNAs and 509 genes were involved in the expression network, while the cross-genes of the network were mainly enriched in MAP kinase (MAPK) signaling pathway and TGF-beta signaling pathway. Patients with higher expression of hsa-miR-196b-3p, CALML4, or SMAD6 or lower expression of PITX2 or TGFB2 had better outcomes than those with lower expression of hsa-miR-196b-3p, CALML4, or SMAD6 or higher expression of PITX2 or TGFB2 (P < 0.05). Patients with H. pylori infection had a higher expression of hsa-miR-196b-3p and CALML4 than those without H. pylori infection (P < 0.05).

Conclusion. The study of miRNA-mRNA expression network would provide molecular support for early diagnosis and treatment of H. pylori-related GC.

1. Introduction

Gastric cancer (GC) is one of the most common cancers in China, with half a million deaths annually [1]. Because of population growth and life extension, the incidence and mortality rates of GC are increasing [2]. Therefore, it is urgent and important to identify the key genes in its pathogenesis. Helicobacter pylori (H. pylori) is acknowledged as a class 1 carcinogen [3], which colonizes in the gastric mucosa and causes chronic gastric, atrophic gastric, and GC [4]. However, how H. pylori infection is involved in the pathogenesis and development of GC is unknown [4–6]. Therefore, elucidating the molecular mechanism of H. pylori-related GC is of great importance for its early diagnosis and targeted therapy.

MicroRNAs (miRNAs) are 19–25-nucleotide-long endogenous noncoding RNAs, which negatively regulate the expression of their targets at the posttranscription level and play significant roles in many biological processes, such as proliferation, differentiation, and apoptosis [7–9]. Evidence has been increasing that abnormal miRNAs expression is involved in the pathogenesis and development of many cancers, which suggests the promising biomarkers for early diagnosis and therapy of tumors [5, 10]. Now, a high-throughput platform combined with bioinformatics analysis has become a new way to identify biomarkers of disease [11, 12].
In this study, differentially expressed miRNAs (DEMs) and differentially expressed genes (DEGs) of gastric biopsy with *H. pylori* infection from the Gene Expression Omnibus (GEO) database [13, 14] were identified by R software. After predicting the potential targets of DEMs, we constructed the coexpression network of miRNA-mRNA to identify hub genes. The hub genes were identified using bioinformatics methods including gene ontology (GO) annotation [15] and Kyoto Encyclopedia of Genes and Genomes (KEGG) [16] signal pathway enrichment analysis, while the prognostic value of hub genes was analyzed from the Cancer Genome Atlas (TCGA) database, and the expression of hub genes was confirmed in 69 gastric specimens with or without *H. pylori* infection. We hope this study will provide new information for the molecular mechanism of *H. pylori*-related GC.

### 2. Materials and Methods

#### 2.1. Microarray Data. The miRNA and gene expression profiles were obtained from the GEO dataset (https://www.ncbi.nlm.nih.gov/geo/). The screening criteria for GEO were as follows: (1) human gastric mucosa samples with or without *H. pylori* infection; (2) datasets were raw or standardized. The miRNA expression profile (GSE19769) [7] and the gene expression profile (GSE27411) [17] were included in this study. The GSE19769 profile was from the platform of GPL9081 including 10 cases of *H. pylori*-negative and 9 cases of *H. pylori*-positive gastritis specimen, while the GSE27411 from the platform of GPL6255 containing 6 cases of *H. pylori*-negative and 6 cases of *H. pylori*-positive atrophic gastritis.

#### 2.2. Identification of DEMs and DEGs. The significance analysis of DEGs and DEMs in *H. pylori*-negative and -positive samples was performed using the R language software (version 3.6.0, https://www.r-project.org/) and a limma R package. The Benjamini and Hochberg false discovery rate (FDR) method was used to adjust the *P* value to reduce the false-positive risk. The raw data of miRNAs and mRNAs expression were averaged and normalized, and the data of miRNAs were also log2-transformed, while the data with a median expression value of zero or less than zero were removed. *P* value < 0.05 and |log2 fold change (FC)| > 1 were used as the filter threshold for identifying DEGs and DEMs. A pheatmap R package was used for hierarchical clustering analysis and for drawing heat maps of DEGs and DEMs, while a ggplot2 R package was used for drawing volcano plot [13, 14].

#### 2.3. Interactive Analysis and Construction Expression Network of miRNA-mRNA. miRNA-mRNA regulatory networks were constructed to predict the potential hub genes of DEMs and DEGs. First, TargetScan (http://www.targetscan.org/vert_72/), miRDB (http://mirdb.org/), PicTar (https://pictar.mdc-berlin.de/), and Miranda (http://miranda.org.uk/) were used to predict targets of DEMs, while different software programs with different algorithms and only genes predicted by at least 3 software programs were selected as the targets of DEMs. The overlapped genes of the targets of DEMs and differential expression genes of GSE27411 dataset were used to construct coexpression networks by Cytoscape software [18].

#### 2.4. GO and KEGG Analysis of Cross-Genes. GO analysis is a common approach for gene annotation and gene classification from three aspects of cellular component (CC), biological process (BP), and molecular function (MF) [15]. KEGG is a comprehensive database resource with 17 main databases, which are used to understand advanced gene functions and practical biological systems [16]. The org.Hs.eg.db, clusterProfiler, enrichplot, and ggplot2 R packages were used for GO and KEGG analysis with a cut-off criterion of a *P* value < 0.05 [19].

#### 2.5. TCGA Data Processing. The TCGA database is a comprehensive and open database, which contains a variety of human cancer types [20], and was used for validating the relations between the hub genes of the network and the GC prognosis. The inclusion criteria were as follows: (1) the primary site is the stomach; (2) the disease type is the adenomas and adenocarcinomas; (3) the data category is transcriptome profiling; (4) gene expression quantification is used for gene data type, while miRNA expression quantification is used for miRNA data type. Eventually, the mature miRNA expression and clinical data of 452 GC cases (42 normal and 410 having tumors) were downloaded from the TCGA database, and the gene expression and clinical data of 374 GC cases (30 normal and 344 having tumors) were obtained. Survival analysis was performed with a survival R package, while the original data were standardized by the log2 (x + 1) method. The prognostic value of cross-genes was determined by Kaplan–Meier analysis, and *P* < 0.05 was considered as a significant difference.

#### 2.6. Patient Data. A total of 431 patients, who were confirmed as gastritis or primary GC from March 2019 to October 2019 at the Affiliated Hospital of Binzhou Medical University (Yantai, China) and the Yuhuangding Hospital (Yantai, China), were enrolled in this study. Blood urease was used to detect the *H. pylori* infection. However, 362 patients were excluded because of the lacking of accurate classification or detecting of the *H. pylori* infection. Finally, 69 patients, including 18 negative and 51 positive for *H. pylori* infection, were included in this study. The related clinical and pathological characteristics are listed in Table 1. The samples involved in this study have been approved by the ethics society of Binzhou Medical University and by the patients themselves or their families. None of the patients has received prior radiotherapy or chemotherapy.

#### 2.7. Detecting DEMs by SYBR Green Real-Time PCR (RT-PCR). Total RNA was extracted from paraffin-embedded tissue by miRNeasy FFPE Kit (no. 217504, Qiagen, Germany), and the RNA concentration and purity were measured by NanoDrop® ND-2000, while adjusting the A260/A280 ratio
of RNA solution from 1.8 to 2.1. The cDNA synthesis was performed with the miRNA First Strand cDNA Synthesis (tailing reaction) (no. B342451, Sangon, China). The expression of hsa-miR-196b-3p and hsa-miR-196b-5p was detected in the ABI7500 quantitative PCR system (Applied Biosystems, USA) instrument by SYBR Green Premix Ex Taq II (Tli RNase H Plus, Takara, Japan). U6 small nuclear RNA was used as the internal controls. The 20 μl reaction mixture included 10.0 μl SYBR Green Master Mix, 4 μl cDNA template, 0.4 μl ROX Reference Dye, 1.0 μl primer pairs (10 μm), and 4.6 μl deionized water. PCR cycle was performed as follows: initial degeneration for 30 s at 95°C, followed by 40 cycles of 5 s at 95°C and 34 s at 60°C. The relative expression of hsa-miR-196b-3p and hsa-miR-196b-5p was calculated by comparing the cycle threshold (CT) method with U6 expression according to [21]. The primers of hsa-miR-196b-3p were 5′-TGCAGACGACGACACTGCCTC-3′ (sense) and 5′-GACACGGACCCACAGACA-3′ (antisense), while the hsa-miR-196b-5p primers were 5′-GCACACGCTAGG-TAGTITTCC-3′ (sense) and 5′-TATGTGGTTTCTC-GTCTCTGTTGTC-3′ (antisense).

### 2.8. Detecting Cross-Genes by Immunohistochemistry

Immunohistochemistry (IHC) was performed in paraffin-embedded sections on the basis of the standardized protocol. Briefly, paraffin-embedded sections (2–4 μm) were deparaffinized with xylene and rehydrated in series gradient ethanol (100%, 95%, and 85% for 1 min, respectively) at room temperature. Heat antigen repair was performed in an ethanol (100%, 95%, and 85% for 1 min, respectively) at 95°C. After antigen repair, sections were incubated in xylene and rehydrated in series gradient ethanol. Briefly, paraffin-embedded sections (2–4 μm) were placed in the microwave and heated at 121°C for 1.5 min in a citrate sodium buffer (0.01 M), and then endogenous peroxidase was blocked using 3% hydrogen peroxide for 10 min. Sections were incubated with anti-human antibody SMAD6 (ab80049, Abcam), TGFβ2 (ab53778, Abcam), PITX2 (ab98297, Abcam), CALML4 (A5086, Zhongshan Golden Bridge, Beijing), and NRP1 (ab25998, Abcam) for 1 h at 37°C and then incubated with a rabbit polyclonal antibody for 20 minutes. After staining with dianobenzidine for 5–10 min at room temperature, sections were sealed with neutral balsa, respectively. The sections were evaluated with semiquantitative method. Briefly, more than 400 cells were counted in each section, while some necrotic cells and peripheral-colored cells were elided. More than 10% of cells were nuclear; staining in all cells was defined as protein-positive expression, while less than 10% was protein-negative expression.

### 2.9. Statistical Analysis

All data were expressed as mean ± standard deviation (SD) of 3 independent experiments, and statistical analyses were performed with SPSS 17.0 (SPSS Ins., Chicago, IL, USA). The difference of miRNA and protein expression was analyzed with two-tail unpaired t-test with P < 0.05 considered as significant difference.

### 3. Results

#### 3.1. Identification of DEMs and DEGs

The dataset GSE19769 was selected to screen DEMs including 10 H. pylori-negative samples and 9 H. pylori-positive samples. 470 human miRNAs were analyzed in this dataset, and 22 DEMs have met the filtration criteria of logFC > 1 and P value < 0.05, including 11 upregulated and 11 downregulated miRNAs (Table 2). Volcano map and heat map for the hierarchical clustering of the DEMs were carried out by a heatmap R package (Figures 1(b) and 1(d)). 6 H. pylori-negative and 6 H. pylori-positive specimens of GSE27411 were analyzed in this study, while there are 18 samples in the dataset. A total of 18187 human mRNAs were expressed, and 68 genes reached the filtration criteria, among which 56 genes showed upregulated and 12 genes showed downregulated expression (Table S1). Volcano map and heat map for the hierarchical clustering of the DEGs were drawn by the heatmap R package (Figures 1(a) and 1(c)).

#### 3.2. Network Construction of the miRNA-mRNA

The miRNAs could bind to the 3′ UTR of their targets, resulting in the posttranscriptional suppression of these genes [22, 23]. The biological targets of DEMs were predicted by 4 different software programs, while gene targets of hsa-miR-455, hsa-miR-411, hsa-miR-551b, hsa-miR-509, and hsa-miR-520e were not predicted in the PicTar software. For network construction, the targets were selected in at least 3 databases. The numbers of targets of hsa-miR-455, hsa-miR-223, hsa-miR-200a-5p, hsa-miR-146b, hsa-miR-200a-3p, hsa-miR-155, hsa-miR-411, hsa-miR-551b, hsa-miR-142-3p, hsa-miR-203, hsa-miR-142-5p, hsa-miR-153, hsa-miR-204, hsa-miR-196b, hsa-miR-509, hsa-miR-326, hsa-miR-146a, hsa-miR-299-5p, hsa-miR-520e, and hsa-miR-138 were 88, 43, 9, 18, 131, 60, 36, 2, 65, 89, 65, 151, 69, 48, 189, 25, 18, 18, 20, and 61, respectively (Table S2 and Figures S1 and S2). Intersected genes were obtained with the target genes and all expressed mRNAs in the dataset GSE27411. And all the intersected genes were imported into the Cytoscape software to conduct the miRNAs-mRNA expression network (Figure 2). A total of 16 miRNAs and 509 genes were involved in the network.

#### 3.3. GO and KEGG Analysis of Cross-Genes in the Network

To explore the biological functions of the cross-genes of the network, we used an enrichplot and a ggplot2 R package to...
analyze the GO categories and KEGG signal pathways of the cross-genomes. In the BP, the cross-genomes were concentrated in outflow tract septum morphogenesis, epithelial cell migration, and endothelial cell migration. In the CC, the cross-genomes were enriched in transcription factor complex, synaptic membrane, and adherence junction, but RNA polymerase II proximal promoter sequence-specific DNA binding, and enhancer binding in the MF (Table 3 and Table S3) in GC tissue was significantly higher than that in normal tissue. The expression of hsa-miR-196b-5p and hsa-miR-196b-5p was also detected by qPCR in 69 GC and gastritis patient specimens, which included 18 \( H. pylori \) negative and 51 positive. The results showed that hsa-miR-196b-3p and hsa-miR-196b-5p were overexpressed in the 69 patients with log2FC values of 2.01665 and 1.8458, respectively (Figure 4(d)). Further analysis showed that the expression level of hsa-miR-196b-3p in \( H. pylori \)-positive patients was significantly higher than that in the negative group \( (P < 0.05, \text{Figure 4(e)}) \); however, there was no significant difference in hsa-miR-196b-5p expression between the \( H. pylori \)-negative and -positive groups \( (P > 0.05, \text{Figure 4(f)}) \). The protein expression of CALML4, SMAD6, PITX2, and TGFβ signaling pathway was detected by immunohistochemistry in 69 specimens. The positive rate of CALML4 in \( H. pylori \)-positive samples (84.3%) was significantly higher than that in the negative samples (55%, \( P < 0.01 \)), while there was no significant difference in SMAD6, PITX2, and TGFβ2 between the two groups \( (P > 0.05, \text{Figures 4(a) and 4(b)}) \).

### 4. Discussion

Although \( H. pylori \) infection is closely related to GC, the pathogenesis of \( H. pylori \)-related GC has not been clarified [7, 24–26]. Therefore, a comprehensive study of the molecular mechanism of \( H. pylori \)-related GC may be helpful to understand the disease and get better diagnosis and treatment methods. As an important part of bioinformatics, gene expression microarray, which has been widely used in tumor research [27, 28], can analyze the expression of thousands of genes simultaneously, providing a valuable tool for the study of gene expression in GC.
genes. In this study, the miRNA and mRNA expression profile data of H. pylori-infected gastric tissue from GEO database were analyzed, DEMs and DEGs were analyzed with R software, DEMs targets were predicted, miRNA-mRNA expression network was constructed, prognostic value of hub genes for GC was verified, and hub genes expression was detected in the clinical sample. We screened 1 miRNA (hsa-miR-196b-3p) and 4 important nodal genes.

Figure 1: Identification of DEMs and DEGs between H. pylori-negative and -positive patients from GEO. Heatmap (a) and volcano map (c) for DEGs between H. pylori-negative (Hp−) and -positive (Hp+) groups from GSE27411. Heatmap (b) and volcano map (d) for DEMs from GSE19769. Red and green colors indicate significant gene overexpression and underexpression, respectively.
miRNA participates in biological, pathological processes and infections by downregulating target expression [29]. The ectopic expression of miRNAs plays an important role in the pathogenesis of multiple cancers, including *H. pylori*-related GC which can be used as biomarkers for GC prognosis.

Table 3: Top 10 GO functions of the cross-genes in the network.

| Category                  | Gene terms                                                      | Count | P value   |
|---------------------------|-----------------------------------------------------------------|-------|-----------|
| Biological process        | GO:0003148, outflow tract septum morphogenesis                   | 10    | 1.24E−09  |
|                           | GO:0003151, outflow tract morphogenesis                          | 15    | 3.98E−09  |
|                           | GO:0001655, urogenital system development                        | 30    | 6.76E−09  |
|                           | GO:0003279, cardiac septum development                           | 17    | 1.22E−08  |
|                           | GO:000411, cardiac septum morphogenesis                          | 13    | 1.60E−07  |
|                           | GO:0010632, regulation of epithelial cell migration               | 26    | 1.74E−07  |
|                           | GO:0010631, epithelial cell migration                             | 29    | 1.75E−07  |
|                           | GO:0061564, axon development                                     | 33    | 1.77E−07  |
|                           | GO:0043542, endothelial cell migration                            | 25    | 1.77E−07  |
|                           | GO:0090132, epithelium migration                                 | 29    | 2.09E−07  |
|                           | GO:0005667, transcription factor complex                          | 23    | 4.28E−07  |
|                           | GO:0097060, synaptic membrane                                    | 26    | 9.71E−06  |
|                           | GO:0045211, postsynaptic membrane                                | 21    | 2.70E−05  |
|                           | GO:0099699, integral component of synaptic membrane               | 14    | 3.31E−05  |
|                           | GO:0005912, adherens junction                                    | 28    | 7.12E−05  |
|                           | GO:0099240, intrinsic component of synaptic membrane              | 14    | 7.62E−05  |
|                           | GO:0003267, axon part                                            | 22    | 0.000108619|
|                           | GO:000790, nuclear chromatin                                     | 20    | 0.000128584|
|                           | GO:0099055, integral component of postsynaptic membrane           | 11    | 0.000162968|
|                           | GO:0000151, ubiquitin ligase complex                              | 16    | 0.000198327|
| Cellular component        | GO:0000978, RNA polymerase II proximal promoter sequence-specific DNA binding | 38    | 5.75E−10  |
|                           | GO:0000987, proximal promoter sequence-specific DNA binding       | 39    | 5.92E−10  |
|                           | GO:0035326, enhancer binding                                     | 19    | 1.24E−09  |
|                           | GO:000980, RNA polymerase II distal enhancer sequence-specific DNA binding | 15    | 1.89E−08  |
|                           | GO:0001158 enhancer sequence-specific DNA binding                 | 16    | 5.18E−08  |
|                           | GO:0003682, chromatin binding                                    | 34    | 9.92E−08  |
|                           | GO:001228, DNA-binding transcription activator activity, RNA polymerase II-specific | 29    | 2.20E−06  |
|                           | GO:0004714, transmembrane receptor protein tyrosine kinase activity | 9     | 7.94E−06  |
|                           | GO:0003712, transcription coregulator activity                    | 32    | 1.19E−05  |
|                           | GO:00199, transmembrane receptor protein kinase activity          | 10    | 1.30E−05  |

(CALML4, SMAD6, PITX2, and TGFB2) in *H. pylori*-related GC which can be used as biomarkers for GC prognosis.
was significantly lower than that in EBV negative [33]. However, the role of hsa-miR-196b in GC, especially in EBV or *H. pylori* infection, is still unknown.

Our study showed that the expression level of hsa-miR-196b in *H. pylori*-positive group was significantly higher than that in *H. pylori*-negative group. Analyzing TCGA data showed that the hsa-miR-196b-3p expression, rather than hsa-miR-196b-5p, could be used as a better biomarker for GC prognosis, which was consistent with the previous report [31]. And we also verified the hsa-miR-196b-3p and hsa-miR-196b-5p expression in clinical samples with or without *H. pylori* infection. The results showed that the expression of

| KEGG terms                                | Count | P value      |
|-------------------------------------------|-------|--------------|
| MAPK signaling pathway                    | 25    | 3.70E − 06   |
| Axon guidance                             | 17    | 3.89E − 05   |
| Ras signaling pathway                     | 19    | 9.06E − 05   |
| TGF-beta signaling pathway                | 11    | 0.00134809   |
| cGMP-PKG signaling pathway                | 15    | 0.000184067  |
| Human cytomegalovirus infection           | 18    | 0.000186779  |
| Adrenergic signaling in cardiomyocytes    | 14    | 0.00018806   |
| Hepatocellular carcinoma                  | 15    | 0.000196653  |
| Rap1 signaling pathway                    | 17    | 0.000244921  |
| ErbB signaling pathway                    | 10    | 0.000259955  |
| Growth hormone synthesis, secretion, and action | 12    | 0.000281056  |

Figure 3: Prognostic value of hub genes for GC patients from TCGA. Overall survival time of GC patients with over- or underexpression of (a) CALML4, (b) PITX2, (c) hsa-miR-196b-3p, (d) SMAD6, (e) TGFB2, and (f) hsa-miR-196b-5p. CALML4: calmodulin-like protein 4; PITX2: paired-like homeodomain 2; SMAD6: SMAD family member 6; TGFB2: transforming growth factor beta 2.
hsa-miR-196b-3p in H. pylori-positive group was significantly higher than that in the negative group \((P < 0.05)\), while there was no significant difference in hsa-miR-196b-5p expression \((P > 0.05)\). miRNA target analysis showed that hsa-miR-196b and hsa-miR-326 could regulate the expression of SMAD6 involved in many biological activities through phosphorylation of the TGF-β signaling pathway \([34, 35]\). The results suggested that H. pylori may be involved in the pathogenesis of GC with hsa-miR-196b by regulating the expression of many genes and activating the infectious immune pathways. However, the molecular mechanism of hsa-miR-196b in infection and GC needs further experimental study.

Studies have shown that the hsa-miR-200 family, including hsa-miR-200a, participates in the negative feedback loop formed by ZEB1, ZEB2, and SIP1, in which hsa-miR-200 suppresses the expression of ZEB1, ZEB2, and, SIP1 and then downregulates their expression \([36, 37]\). ZEB2 and SIP1 have also been suggested to inhibit the transcription of cyclin D1 \([38]\). Some studies showed that, in H. pylori infection, the CagA gene can promote transformation from G1 into S/G2 in the cell cycle through activating AP-1 and cAMP, which suggested that hsa-miR-200 was involved in the transformation from gastric epithelial cells to EMT through ZEB loop \([39]\). Our study showed that hsa-miR-200a-3p showed low expression in H. pylori-related GC, and it could inhibit the expression of PITX2 and TGFB2, which participate in the TGF-β pathway. Analysis of the relationship between cross-genes and GC prognosis showed that PITX2 and TGFB2 were related to the prognosis of GC. The survival time of GC patients with overexpression of PITX2 and TGFB2 was remarkably shorter than those with underexpression, while hsa-miR-200a-3p had no prognostic value for GC.

Some results have shown that hsa-miR-223 was abnormally overexpressed in GC and was significantly upregulated in H. pylori-infected tissues, which could be involved in the pathogenesis of GC by targeting FBXW7 \([40]\).
studies showed that hsa-miR-411 showed low expression in GC, and overexpression of hsa-miR-411 in GC cell led to decreasing proliferation and increasing apoptosis [40], while hsa-miR-411 could not be used as an independent predictor of GC prognosis [41]. However, the role of hsa-miR-411 in H. pylori infection has not been reported. Our results showed that the expression level of hsa-miR-223 was high in H. pylori-positive GC, while that of hsa-miR-411 was low, and both of them can regulate CALML4, which can activate the CGMP-PKG signaling pathway. Besides, patients with overexpression of CALML4 had better outcomes than those with underexpression. However, the mechanism of CALML4 regulated by hsa-miR-223 and hsa-miR-411 in the pathogenesis of H. pylori-related GC needs further experimental study.

In conclusion, we constructed a coexpression network of miRNA-mRNA and identified the key genes of hsa-miR-196b, CALML4, PITX2, TGFB2, and SMAD6 in miRNA-mRNA and identified the key genes of hsa-miR-223 and hsa-miR-411 in the pathogenesis of H. pylori-related GC, which may provide a new way for the diagnosis and treatment of H. pylori-related GC.

Abbreviations

H. pylori: Helicobacter pylori
miRNAs: MicroRNAs
DEM:s: Differentially expressed miRNAs
DEGs: Differentially expressed genes
GC: Gastric cancer
GEO: Gene expression omnibus
BP: Biological process
CC: Cellular component
MF: Molecular function
qPCR: Quantitative polymerase chain reaction
KEGG: Kyoto Encyclopedia of Genes and Genomes
GO: Gene ontology
TCGA: The Cancer Genome Atlas
EBV: Epstein–Barr virus
FC: Fold change
SD: Standard deviation
FDR: False discovery rate.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions

Ping Yang, Junjie Liu, and Tianci Yang contributed equally to this work.

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Supplementary Materials

Figure S1: Venn diagram of potential targets of DEMs predicted by 4 software programs. Venn for hsa-miR-455 (A), hsa-miR-223 (B), hsa-miR-200a-5p (C), hsa-miR-146b (D), hsa-miR-200a-3p (E), hsa-miR-155 (F), hsa-miR-411 (G), hsa-miR-551b (H), hsa-miR-142-3p (I), hsa-miR-203 (J), hsa-miR-142-5p (K), and hsa-miR-153 (L). Figure S2: Venn diagram of potential targets of DEMs predicted by 4 software programs. Venn for hsa-miR-204 (A), hsa-miR-196b (B), hsa-miR-509 (C), hsa-miR-326 (D), hsa-miR-146a (E), hsa-miR-299-5p (F), hsa-miR-520e (G), and hsa-miR-138 (H). Figure S3: GO and KEGG function analysis of the cross-genes. Circle diagram of (A) GO clusters and (B) KEGG pathway clusters. Table S1: differential expression genes of H. pylori-negative and -positive patients. Table S2: targets of DEMs in the network. Table S3: expression of hsa-miR-196b-3p and hsa-miR-196b-5p in TCGA. (Supplementary Materials)

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