Short Communication

Genome-Wide Analysis of Long Noncoding RNA Profile in Human Gastric Epithelial Cell Response to *Helicobacter pylori*

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SUMMARY: Long noncoding RNAs (lncRNAs) are an important class of pervasive genes, and their misregulation has been shown in various types of diseases. However, the relationship between lncRNAs and the immune response to pathogen infection has been rarely reported. *Helicobacter pylori* is a major human pathogenic bacterium that causes gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric cancer. The regulatory mechanism of the *H. pylori*-induced immune response is not yet clear. In the present study, we identified nonoverlapping signatures of a small number of lncRNAs that were aberrantly expressed in *H. pylori*-infected gastric epithelial cells using microarray analysis followed by bioassays. From microarray data, we found that 23 lncRNAs were up-regulated and 21 were downregulated. Five lncRNAs, XLOC_004562, XLOC_005912, XLOC_000620, XLOC_004122, and XLOC_014388, were further evaluated using quantitative reverse transcription-PCR, and the results matched well with microarray data. In addition, XLOC_004122 and XLOC_014388 were decreased in gastric mucosal tissues of *H. pylori*-positive patients. Differentially expressed lncRNAs may play a partial or key role in the immune response to *H. pylori*, and this may provide potential targets for the future treatment of *H. pylori*-related diseases.

Helicobacter pylori is the causative agent of gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric cancer (1). A remarkable feature of *H. pylori* infection is that it results in the induction of a complex and fascinating immune response. During its long coexistence with humans, *H. pylori*, the host, and environmental factors have constituted a complex network that precisely regulates the immune response. Scientists believe that better understanding of this complex network may help to explore the diagnostic and treatment methods. Recently, certain techniques such as gene chip and microarray analysis have been used to analyze global transcriptional changes in host genes in an attempt to describe the systemic response to *H. pylori* and to identify novel targets or biomarkers (2,3).

The human transcriptome comprises not only large numbers of protein-coding messenger RNAs but also a large set of nonprotein-coding transcripts that have structural, regulatory, or unknown functions. Noncoding RNAs are divided into long noncoding RNAs (lncRNAs) and short noncoding RNAs (microRNAs, small interfering RNAs, small nucleolar RNAs, and PIWI-interacting RNAs) based on their length. lncRNAs, tentatively defined as noncoding RNAs more than 200 nt in length, are characterized by the complexity and diversity of their sequences and mechanisms of action. A few recent publications have reported

that lncRNA may play important roles during cellular development, and their misregulation has also been shown in various types of disease (4). However, reports on the potential role of lncRNAs in the immune response to pathogens are rare. In this study, we identified nonoverlapping signatures of a small number of lncRNAs that were aberrantly expressed in *H. pylori*-infected gastric epithelial cells using microarray analysis followed by bioassays.

The nonmalignant gastric epithelial cell line GES-1 was routinely cultured in the RPMI 1640 medium (Invitrogen, Grand Island, NY, USA) supplemented with 10% fetal bovine serum and 100 U/ml penicillin in a humidified incubator containing 5% CO₂ at 37°C. The wild-type *H. pylori* strain 26695 was obtained from the American Type Culture Collection (Manassas, VA, USA) and grown as previously described (5). Subsequently, cells were seeded in a 12-well culture plate and grown to 80% confluence. Then, the medium was replaced with an antibiotic-free medium. *H. pylori* was added to cells at a multiplicity of infection (MOI) of 100:1. The infection model was monitored by measuring the release of interleukin 8 (IL-8) and growth-related oncogene α (GRO- α) using DuoSet ELISA Development System (R&D Systems, Minneapolis, MN, USA).

Clinical samples were obtained from a total of 57 patients in PLA 324 Hospital with *H. pylori*-induced chronic gastritis and 22 *H. pylori*-negative healthy control subjects. The *H. pylori*-infection status was confirmed by bacterial culture, a ¹³C-urea breath test, and a histologic test. Patients were regarded as being *H. pylori*-positive if one of these tests yielded positive results. None of the patients received nonsteroidal anti-inflammatory drugs or had taken antibiotics or proton pump inhibitors in the preceding 4 weeks. Patients with

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H. pylori infection received a 1-week triple therapy with omeprazole, amoxicillin, and clarithromycin (20 mg, 1 g, and 500 mg, respectively), all taken twice daily for 1 week. One month after treatment was completed, the ¹³C-urea breath test was conducted to detect *H. pylori* infection. A total of 13 patients were followed-up with endoscopy 1 year later. All the experiments were conducted according to the principles of the Helsinki Declaration. The Ethics Committee of Dalian Medical University Hospital approved the study.

Total RNA was extracted from cells using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and then reverse transcribed using the Fermentas RT reagent kit according to the manufacturer's instructions. Levels of lncRNAs and β -actin were measured using quantitative reverse transcription (qRT)-PCR with the PrimeScript RT-PCR kits (Takara, Shiga, Japan). The sequences of primers used are shown in Table 1. The results are expressed as means $\pm$ SD from at least 3 separate experiments performed in triplicate, and differences between groups were analyzed using the 2-tailed Student's *t*-test; statistical analysis was performed using SPSS software (SPSS, Armonk, NY, USA). *P* values of <0.05 were considered significant.

The RiboArray lncRNA DETECT Human Array with a coverage of 8264 unique human lncRNAs was synthesized by Ribobio (Guangzhou, China) by applying the Combimatrix platform. The procedures were performed according to the manufacturer's protocol. Briefly, 3 *H. pylori*-infected GES-1 samples and 3 noninfected samples were used to extract total RNA. One microgram of total RNA was reverse transcribed into aRNA, and then, 6 μ g of aRNA was labeled with Cy3 fluorophore using the Amino Allyl MessageAmpTM IIaRNA Kit (Invitrogen). Microarrays with labeled samples were hybridized overnight at 40°C and washed using buffers recommended in the manufacturer's protocol. Slides were scanned using the Genepix 4000B laser scanner (Axon Instruments, Union City, CA, USA), and images were analyzed using Genepix Pro 7.0 software (Axon Instruments). Signals that were <1.5 times stronger than the background were removed. lncRNA microarray analysis, including labeling, hybridization, scanning, normalization, and data analysis, was performed by Ribobio. We selected lncRNAs with *P*-values <0.01 for further analysis.

GES-1 cells were infected with *H. pylori* (MOI, 100:1). Twenty-four hours later, successful infection

Table 1. Primers used in quantitative PCR

Primer	Sequence
β -Actin sense primer	ACAATGTGGCCGAGGCTTT
β -Actin antisense primer	GCACGAAGGCTCATCATCA
XLOC_004562 sense primer	ATAACACCATCTCGGCAGGAA
XLOC_004562 antisense primer	AGGGTCTAAGTGCTCTAAGATCAAG
XLOC_005912 sense primer	AGCCTCCTGACAGTGCGTG
XLOC_005912 antisense primer	CTGACAGATCCTATGCGGC
XLOC_000620 sense primer	AGACCACGGGAGGGAC
XLOC_000620 antisense primer	GAGTTTCTTCTACTGCTGGG
XLOC_004122 sense primer	GCAGGAACACCGGTACTT
XLOC_004122 antisense primer	CTTTTACTTAGGAGCCACTTCTTG
XLOC_014388 sense primer	CTGCTGAGCGATGAAGTGGAT
XLOC_014388 antisense primer	CTCCTGACTTCACGCCTGTTAC

Fig. 1. *Helicobacter pylori* infection results abnormal lncRNAs expression. (A) Differential lncRNAs in GES-1 cells. (B) Differential lncRNAs in gastric mucosal tissues. HP, *Helicobacter pylori*. (C) Associations between long noncoding RNAs and intestinal metaplasia. IM, intestinal metaplasia.

IncRNA Profile in Response of *Helicobacter pylori*

Table 2. Differentially expressed lncRNA genes under infection with *H. pylori*

Gene position	Gene name	Variation trend	Fold change (Rv/control)	P
chr1:170567994-170616480: +	XLOC_000461	up	2.1	0.008306
chr1:233860044-233866505: +	XLOC_000620	up	1.7	0.008343
chr1:38765780-38777050: -	XLOC_000789	up	1.7	0.008369
chr1:79490857-79493876: -	XLOC_000877	up	1.9	0.008482
chr1:81000662-81050048: -	XLOC_000883	up	1.5	0.008505
chr1:192486768-192536347: -	XLOC_001133	up	1.5	0.008595
chr2:105719097-105720244: +	XLOC_001607	up	1.6	0.008684
chr2:73383765-73385965: -	XLOC_002160	up	1.9	0.008823
chr2:75049906-75059445: -	XLOC_002169	up	1.8	0.008827
chr3:40684764-40685532: +	XLOC_002621	up	1.7	0.008836
chr3:86954826-86959897: -	XLOC_003177	up	1.5	0.009106
chr5:134601126-134603585: +	XLOC_004562	up	1.9	0.009174
chr6:119671081-119738843: +	XLOC_005448	up	1.8	0.009189
chr6:164085402-164092730: -	XLOC_005912	up	1.6	0.009327
chr9:35013511-35015067: +	XLOC_007336	up	1.5	0.009451
chrX:134530353-134531689: +	XLOC_008074	up	1.6	0.009588
chr10:7115610-7118468: +	XLOC_008375	up	1.9	0.009611
chr11:10315851-10324233: +	XLOC_009060	up	2.1	0.009699
chr12:115334602-115336010: +	XLOC_009891	up	2.0	0.009724
chr14:26851402-26866526: -	XLOC_010979	up	1.5	0.00977
chr15:78439742-78441489: -	XLOC_011537	up	1.7	0.009881
chr16:13925216-13929301: +	XLOC_011649	up	1.8	0.009944
chr17:56154493-56160565: -	XLOC_012535	up	1.8	0.009955
chr22:42760405-42765214: -	XLOC_014388	down	0.47	0.000471
chr9:134222637-134224656: -	XLOC_007875	down	0.52	0.001071
chr11:112352897-112356504: +	XLOC_009280	down	0.67	0.001616
chr4:152326613-152330005: -	XLOC_004122	down	0.37	0.001761
chr2:45240352-45240807: -	XLOC_002077	down	0.67	0.001837
chr6:147480060-147502128: +	XLOC_005496	down	0.64	0.001906
chr15:48080271-48083451: -	XLOC_011469	down	0.60	0.001933
chr6:86354448-86356991: -	XLOC_005778	down	0.64	0.003352
chr8:31133100-31179420: +	XLOC_006764	down	0.61	0.004006
chr13:109962482-109964121: +	XLOC_010492	down	0.55	0.004222
chr3:150618940-150621181: -	XLOC_003285	down	0.52	0.005124
chr22:20186251-20192441: -	XLOC_014299	down	0.54	0.005424
chr7:26638502-26657903: -	XLOC_006389	down	0.62	0.005529
chr2:214032965-214039035: +	XLOC_001835	down	0.60	0.005581
chr10:3793258-3805418: +	XLOC_008352	down	0.48	0.005943
chr9:7925577-7961234: +	XLOC_007279	down	0.57	0.006514
chr4:38278023-38281503: -	XLOC_003926	down	0.50	0.006633
chr7:56184205-56186864: +	XLOC_006107	down	0.50	0.00777
chr15:97972958-97975172: +	XLOC_011373	down	0.66	0.008168
chr18:12739783-12749484: -	XLOC_012776	down	0.48	0.008477
chr20:25730058-25732440: -	XLOC_013708	down	0.66	0.009693

was confirmed by the release of the proinflammatory cytokines IL-8 and GRO- α . Quantitative alterations in lncRNAs were then identified using lncRNA microarray analysis. Among lncRNAs in which alterations were identified, 23 lncRNAs were upregulated and 21 lncRNAs were downregulated compared with the control (Table 2). To validate the microarray analysis findings, we selected 5 lncRNAs that were highly expressed in GES-1 cells and could be found in our coexpression network database among the differential lncRNAs and analyzed their expression using qRT-PCR. The results shown in Fig. 1A confirmed that XLOC_004562, XLOC_005912, and XLOC_000620 were highly expressed during *H. pylori* infection, whereas the expression of XLOC_004122 and XLOC_014388 was decreased ($P < 0.05$).

Then, we analyzed the associations between lncRNAs and their clinical values in human gastric mucosal tissues. Of 57 *H. pylori*-positive chronic gastritis patients, 34 were males and 23 were females. Of 22 *H. pylori*-negative healthy control subjects, 15 were males and 7 were females, and there were no significant differences in gender, height, weight, and body mass index between the 2 groups. Histological data revealed that 49 (86.0%) *H. pylori*-infected patients showed evidence of inflammatory infiltrates with lymphocytes and mononuclear cells, and 15 (26.3%) showed intestinal metaplasia, whereas *H. pylori*-negative subjects mostly had normal mucosa. XLOC_004122 and XLOC_014388 were down-regulated in *H. pylori*-positive patients (Fig. 1B, $P < 0.05$), whereas no significant changes were observed in

XLOC_004562, XLOC_005912, and XLOC_000620. Furthermore, among *H. pylori*-positive patients, we found that the expression of XLOC_004122 and XLOC_014388 was decreased in those with intestinal metaplasia (Fig. 1C, $P < 0.05$). It is known that *H. pylori* may promote the development of inflammation of the gastric mucosa, which then induces gastric atrophy and intestinal metaplasia, resulting in gastric cancer. Our study indicated that XLOC_004122 and XLOC_014388 may be closely related to this process.

We next followed-up 13 patients before and after treatment for *H. pylori* infection. Two patients showed no significant release of *H. pylori* infection and were excluded. Of the remaining 11 patients, 7 (63.6%) and 5 (45.5%) exhibited increased expression of XLOC_004122 and XLOC_014388, respectively, indicating that the 2 lncRNAs could be used to monitor the status of *H. pylori* infection.

Genetic studies have focused on noncoding RNAs for years. These abundant transcriptomes were previously regarded as “transcriptional noise”; however, over the past decade, many studies have reported that these non-coding RNAs have a series of important roles in both transcriptional and posttranscriptional regulation (6–8). Recent publications have found that lncRNAs were involved in many regulation actions in various diseases, including breast cancer (9), colon cancer (10), prostate cancer (11), hepatocellular carcinoma (12), and leukemia (13). Studies have revealed the contribution of lncRNAs as protooncogenes, tumor suppressor genes, and drivers of metastatic transformation; for example, GAGE6, p15, and HOTAIR (14–16). In particular, the lncRNA expression profile in gastric cancer has been widely studied. Lin et al. used microarray technology to characterize the role of lncRNAs in gastric cancer pathogenesis and identified 10 differentially expressed lncRNAs potentially regulating the p53 signaling pathway (17). Liu et al. found that ncRuPAR, a newly discovered lncRNA, inhibited gastric cancer progression by downregulating protease-activated receptor-1 (18). Li et al. reported that the overexpression of lncRNA H19 can enhance carcinogenesis and metastasis of gastric cancer (19). However, there have been no reports on the expression profiles of lncRNAs in *H. pylori*-infected surroundings. In the current study, for the first time, we used a genome-wide microarray system to assess differences in responses of *H. pylori* infection in GES-1 cells and identified a small number of lncRNAs that were aberrantly expressed. Although their functions were not determined clearly, coexpression network analysis indicated that these lncRNAs were enriched for many protein-coding genes related to the immune response. For example, a total of 327 mRNAs were coexpressed with XLOC_014388, and gene ontology analysis showed that most of them participated in the regulation of cell junction and kinase activity. Among 218 mRNAs coexpressed with XLOC_004122, many were involved in the calcium signaling and Toll-like receptor pathways (data

not shown). Our findings suggest that novel identified lncRNAs may play a partial or key role in the immune response to *H. pylori*, and this may provide potential targets for the future treatment of *H. pylori*-related diseases.

Conflict of interest None to declare.

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