

Differential MicroRNA Expression Between Gastric Cancer Tissue and Non-cancerous Gastric Mucosa According to *Helicobacter pylori* Status

ORIGINAL
ARTICLE

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Background: MicroRNAs (miRNAs) are key post-translational mechanisms which can regulate gene expression in gastric carcinogenesis. To identify miRNAs responsible for gastric carcinogenesis, we compared expression levels of miRNAs between gastric cancer tissue and non-cancerous gastric mucosa according to *Helicobacter pylori* status.

Methods: Total RNA was extracted from the cancerous regions of formalin-fixed, paraffin-embedded tissues of *H. pylori*-positive (n = 8) or *H. pylori*-negative (n = 8) patients with an intestinal type of gastric cancer. RNA expression was analyzed using a 3,523 miRNA profiling microarray based on the Sanger miRBase. Validation analysis was performed using TaqMan miRNA assays for biopsy samples from 107 patients consisted of control and gastric cancer with or without *H. pylori*. And then, expression levels of miRNAs were compared according to subgroups.

Results: A total of 156 miRNAs in the aberrant miRNA profiles across the miRNA microarray showed differential expression (at least a 2-fold change, $P < 0.05$) in cancer tissue, compared to noncancerous mucosa in both of *H. pylori*-negative and -positive samples. After 10 promising miRNAs were selected, validations by TaqMan miRNA assays confirmed that two miRNAs (hsa-miR-135b-5p and hsa-miR-196a-5p) were significantly increased and one miRNA (hsa-miR-145-5p) decreased in cancer tissue compared to non-cancerous gastric mucosa at *H. pylori*-negative group. For *H. pylori*-positive group, three miRNAs (hsa-miR-18a-5p, hsa-miR-135b-5p, and hsa-miR-196a-5p) were increased in cancer tissue. hsa-miR-135b-5p and hsa-miR-196a-5p were increased in gastric cancer in both of *H. pylori*-negative and -positive.

Conclusions: miRNA expression of the gastric cancer implies that different but partially common gastric cancer carcinogenic mechanisms might exist according to *H. pylori* status.

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Key Words: Gastric cancer, microRNAs, *Helicobacter pylori*, Microarray

INTRODUCTION

Gastric cancer is one of the most common cancers and the first leading cause of cancer death in eastern Asian countries.¹ Although numerous efforts were undertaken to reduce the development and progression to cancer death. The rate of gastric cancer death is still high. Many studies have focused on gastric

cancer pathogenesis to overcome this poor situation. However, molecular pathogenesis of the gastric cancer remains insufficient. Meanwhile, *Helicobacter pylori* is known to be the major pathologic organism in the development of upper gastrointestinal peptic ulcer disease and is considered the causative agent of chronic gastritis, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer.² Continuous *H.*

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pylori infection could induce gastritis, intestinal metaplasia (IM), and eventually gastric neoplasia, including adenoma, dysplasia, and cancer. *H. pylori* eradication might reduce gastric cancer risk. However, 5.3% of gastric cancer patients were not infected with *H. pylori*. For this *H. pylori*-negative gastric cancer, another mechanism is expected to exist.^{3,6}

MicroRNAs (miRNAs) are about 20 length nucleotides of non-coding RNA. miRNA can regulate gene expression by hybridizing to the 3'-untranslated region of specific messenger RNA targets. miRNA is one of the most important mechanism functioning as a post-transcriptional regulator, such as DNA methylation or acetylation. miRNA can regulate many biological processes, including development of chronic inflammatory diseases or various neoplastic diseases. Moreover, even single miRNA can downregulate or upregulate multiple targets which related to same metabolic and signaling pathway.⁷ More than 1,000 miRNAs have been founded in humans. Recent studies regarding miRNA profiling indicated that many miRNAs were significantly dysregulated in gastric cancer mucosa.⁸ Additionally, another researcher suggested that *H. pylori* eradication might reduce the risk of cancer development by reducing the dysregulation of cancer-related miRNAs.⁹ However, the studies about effect of miRNA on gastric cancer usually have not been performed on the basis of clear discrimination of *H. pylori*-negative or -positive status.

Recently, the present investigators reported dysregulated miRNA status at both *H. pylori*-negative and -positive gastric cancer patients in Korea, based on the result from the formalin-fixed paraffin-embedded gastric cancer tissues.¹⁰ We concluded that different pathogenic mechanisms might be present between *H. pylori*-negative and -positive gastric cancer patients. In this study, miRNA was selected mainly based on the expression ratio of *H. pylori*-negative gastric cancer over *H. pylori*-positive gastric cancer tissue. Thus, direct comparisons between gastric cancer tissue and non-cancerous gastric mucosa according to *H. pylori*-positive and -negative groups had not been

performed. The present study was performed to clarify the potential miRNA responsible for gastric cancer development in clear discrimination of *H. pylori*-negative and -positive status.

MATERIALS AND METHODS

1. Study subject

Thirty-two gastric cancer patients matched for age, sex, and *H. pylori* status. Eight *H. pylori*-negative and 8 *H. pylori*-positive patients were enrolled. All of these patients were received curative gastric resection at Seoul National University Bundang Hospital and were included for microarray study. All subjects of this study received gastroscopy for gastric cancer screening and conformation of histological gastric adenocarcinoma diagnosis. For TaqMan miRNA assays, gastric cancer tissue was retrieved from gastric cancer patients who received endoscopic sub-mucosal dissection. We also collected *H. pylori*-negative and -positive control tissues. Table 1 demonstrates baseline characteristics of the study subjects. The subjects who underwent gastroscopy and *H. pylori* gastric cancer screening but did not show any significant gastroduodenal diseases, such as gastric cancer, dysplasia, mucosa-associated lymphoid tissue lymphoma, esophageal cancer, or peptic ulcer disease, were enrolled into the control group. The study protocol was approved by the Ethics Committee of Seoul National University Bundang Hospital (IRB No. B-1301-186-111). All participants provided their written informed consent to participate in this study.

2. *H. pylori* testing

Three types of *H. pylori* testing (histology, rapid urease test, and culture) were conducted in both the antrum and the body. Even if only one test result is positive, the patient was regarded *H. pylori*-positive. If all three tests were negative, *H. pylori* serology test was performed using anti-*H. pylori* immunoglobulin G in an ELISA (Green Cross Medical Science, Eumseong, Korea). IM was graded according to the modified sydney system in hematoxylin

Table 1. Characteristics of the subjects used in the analyses

Characteristic	miRNA microarray			TaqMan miRNA RT-PCR		
	Hp- GC (n = 8)	Hp+ GC (n = 8)	Hp- cont (n = 27)	Hp+ cont (n = 26)	Hp- cancer (n = 27)	Hp+ cancer (n = 27)
Age (yr)	69.3 ± 1.7	67.8 ± 3.4	54.2 ± 12.4	61.1 ± 8.0	64.7 ± 9.6	66.2 ± 8.5
Sex (male/female)	6/2	5/3	16/11	15/11	21/6	17/10
Intestinal type histology	8	8			27	27

Values are presented as mean ± SD or number only. miRNA, microRNA; Hp-, *Helicobacter pylori*-negative; Hp+, *Helicobacter pylori*-positive; GC, gastric cancer group; cont, non-cancer control group.

and eosin stained tissue.¹¹ All of the 54 *H. pylori*-negative cases (27 controls and 27 patients with gastric cancer) were sero-negative and IM was absent.

3. RNA isolation and microRNA microarray

After manual dissection under microscopic guidance avoiding the contamination of inflammatory cells and stromal cells, Hematoxylin & eosin-stained sections, 50 μm in thickness from cancerous and noncancerous regions of intestinal type of gastric cancer formalin-fixed paraffin-embedded (FFPE) samples, were reviewed by one pathologist. Each section was incubated in xylene and total RNA was extracted using a RecoverAll™ Total Nucleic Acid Isolation Kit (Life Technologies, Carlsbad, CA, USA). Each 400 ng RNA was dephosphorylated with 15 units of calf intestine alkaline phosphatase, followed by RNA denaturation with 40% dimethylsulfoxide. Dephosphorylated RNA was ligated with pCp-Cy3 mononucleotide and resuspended in Gene Expression Blocking Reagent and Hi-RPM Hybridization buffer. The denatured and labeled samples were pipetted onto assembled Agilent Human miRNA microarray Release 16.0 platform and hybridized at 55°C for 20 hours at 20 rpm. The hybridization images were analyzed using a DNA microarray scanner (Agilent Technologies, Palo Alto, CA, USA). The average fluorescence intensity for each spot was calculated and local background was subtracted. Data visualization and analysis were

performed with GeneSpring GX 7.3 software (Agilent Technologies). Signal cutoff measurements were $P < 0.01$.

4. Selection of microRNA candidates and Taqman microRNA validation assay

The microarray showed several miRNAs which were at least 2-fold change with statistical significance ($P < 0.05$). We selected manually 10 miRNAs from each *H. pylori*-positive and -negative groups, based on high signal intensity above 100. Remarkable intergroup difference between cancer versus non-cancer. miRNA was extracted from the frozen gastric cancer tissue in gastric cancer patients and gastric body in control cases, which had been obtained during gastroscopy and had been kept at -80°C , with a mirVana™ miRNA Isolation Kit (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed using 5 μL of miRNA and TaqMan MicroRNA Reverse Transcription Kit and miRNA-specific stem-loop primers (Applied Biosystems, Foster City, CA, USA). The assays were carried in duplicate. The 20 μL reaction mixture contained reverse transcription reaction product, TaqMan Universal PCR Master Mix without uracil-N-glycosylase, TaqMan miRNA assay mix, and nuclease-free water. Amplification was performed using the StepOnePlus real-time PCR system (Applied Biosystems) at 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Relative miRNA expression levels are presented as $2^{-\Delta\Delta\text{Ct}}$ method.

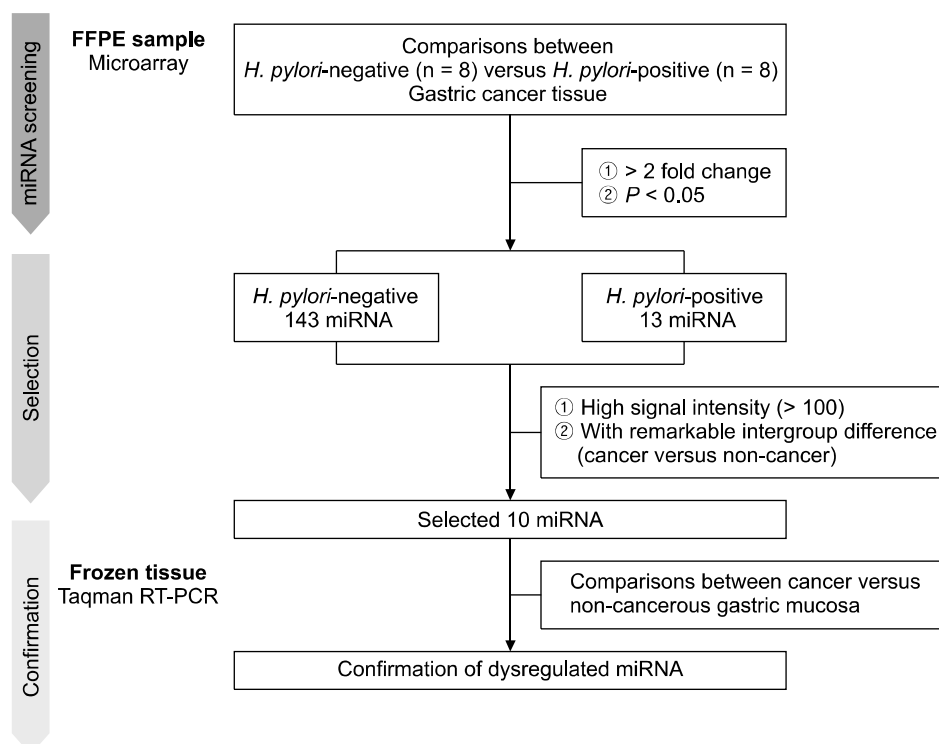


Figure 1. Schematic flow of the study. miRNA, microRNA; FFPE, formalin-fixed paraffin-embedded.

5. Statistical analysis

No interarray normalization was applied on the array, because the similarity between matched normal and cancer sample arrays was unknown. To identify distinct miRNAs hybridization signals, one-way analysis of variance (ANOVA) ($P < 0.05$) were employed for microarray clustering analysis. All statistical analyses were performed using the SPSS ver. 13.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

1. MicroRNA microarray and selection of promising candidate microRNAs

Schematic flow of the study is demonstrated in Figure 1. Microarray result shows that 156 miRNAs were up- or down-regulated by at least a 2-fold change with statistical significance ($P < 0.05$). Specifically, 143 miRNAs were obtained from the *H. pylori*-negative group and 13 miRNAs were obtained from the *H. pylori*-positive group. Additionally, we selected more promising 10 miRNAs which have high microarray signal intensity (> 100) and remarkable difference between cancer versus non-cancer (Fig. 1). Finally, hsa-miR-146b-5p, hsa-miR-142-3p, hsa-miR-31-5p, hsa-miR-145-5p, hsa-miR-135-5p, hsa-miR-18a-5p, hsa-miR-196a-5p, hsa-miR-21b-5p, hsa-miR-375, and ebv-miR BART19-3p were selected for confirmation analysis. Fold change graph of these 10 miRNAs were listed at Figure 2A (*H. pylori*-negative group) and 2B (*H. pylori*-positive group).

2. Validation study by TaqMan microRNA assay

Selected 10 miRNA candidates were tested for validation assay. At Taqman RT-PCR result, hsa-miR-135b-5p ($P = 0.002$) and hsa-miR-196a-5p ($P < 0.001$) were significantly increased and hsa-miR-145-5p ($P = 0.040$) was significantly decreased in cancer tissue compared to non-cancerous gastric mucosa at *H. pylori*-negative group (Fig. 3A). For *H. pylori*-positive group, hsa-miR-18a-5p ($P = 0.043$), hsa-miR-135b-5p ($P = 0.001$), and hsa-miR-196a-5p ($P < 0.001$) were increased in cancer tissue (Fig. 3B). Among them, hsa-miR-135b-5p and hsa-miR-196a-5p were increased irrespective of *H. pylori* status with statistical significance (Fig. 4).

DISCUSSION

Recent many studies have founded that epigenetic factors could intervene additional aspects of the gene-environment interaction in the development of cancer.¹² There have been many studies which have concluded that miRNAs are involved in the pathogenesis of cancer. Especially for gastric cancer, many favorable outcomes have been published.¹³ Meanwhile, *H. pylori* infection is most critical environmental factor for gastric carcinogenesis unlike other type of cancer. But, numerous gastric cancers were not developed from the *H. pylori* infectious status, regardless of current or past infection.² Such unique type of gastric cancer usually is called *H. pylori*-negative gastric cancer

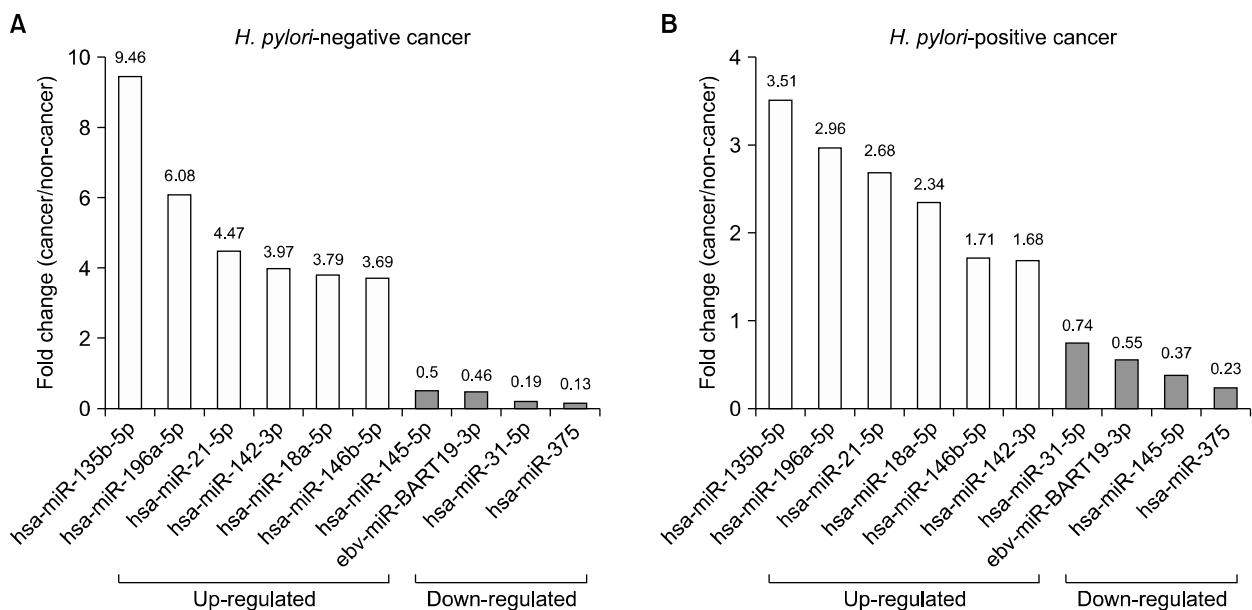


Figure 2. Selection of promising candidate microRNAs in *Helicobacter pylori*-negative and -positive gastric cancer. (A) A fold change graph of 10 microRNA (miRNA) in *H. pylori* negative cancer. (B) A fold change graph of 10 miRNA in *H. pylori*-positive cancer.

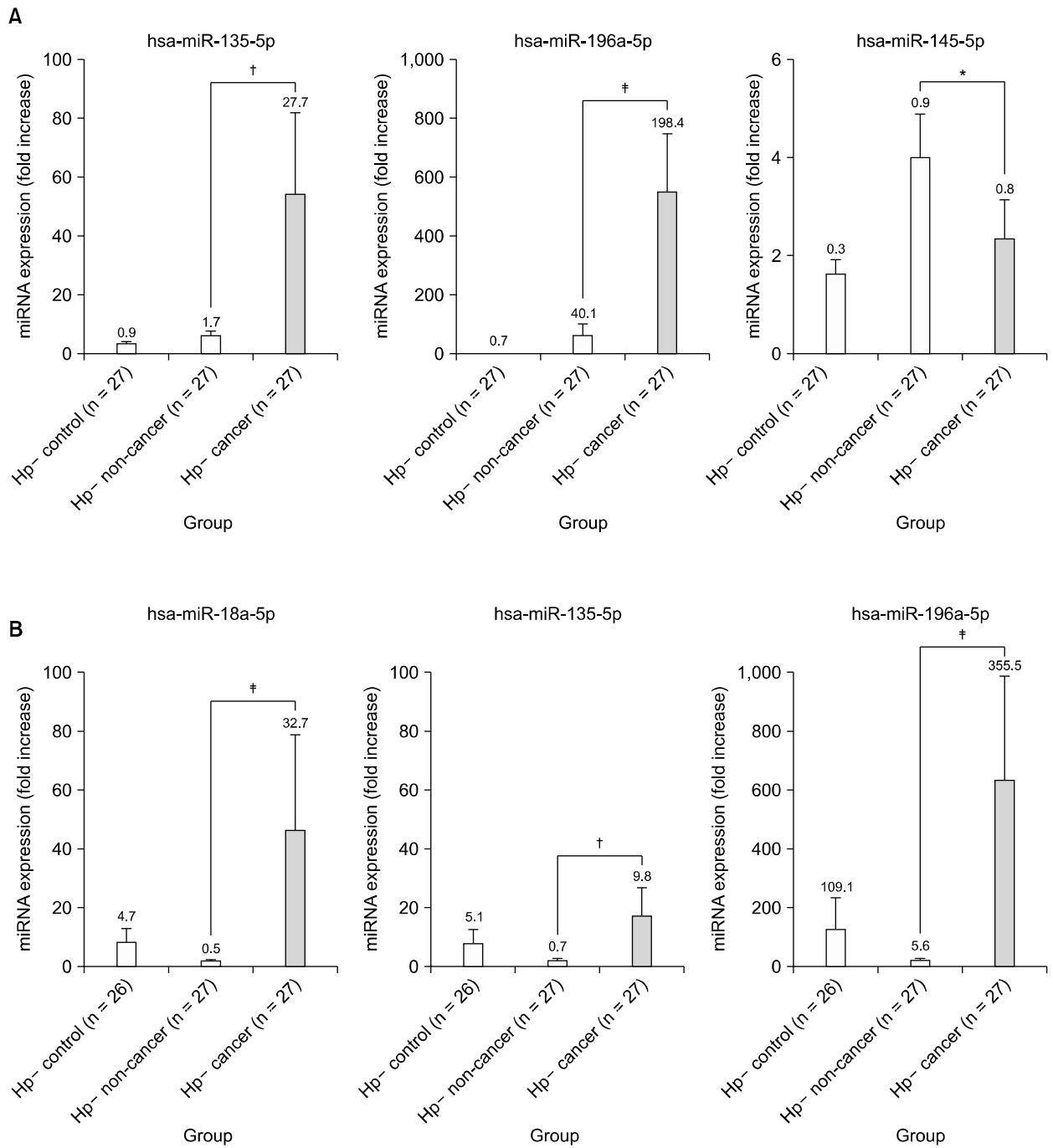


Figure 3. Validation by TaqMan microRNA assay. MicroRNA (miRNA) expression levels in (A) the *Helicobacter pylori*-negative (Hp-) and (B) *H. pylori*-positive (Hp+) groups. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

and their proportion is estimated about 5% to 10% of total gastric cancer.^{5,6,10}

Up to recently, the presumptive roles of miRNAs have been studied in gastric cancer. miRNAs in gastric cancer patients is usually dysregulated.⁷ Some kind of down-regulated miRNA was regarded to have tumor suppressive activity. And another kind of

up-regulated miRNA was regarded to have carcinogenic property. miRNA was also founded in patient's serum. Some researchers proposed that some kind of miRNA could be used as tumor marker to improve the diagnostic certainty and evaluation of prognosis. However, such results have been usually derived from the comparisons between gastric cancer versus non-cancer

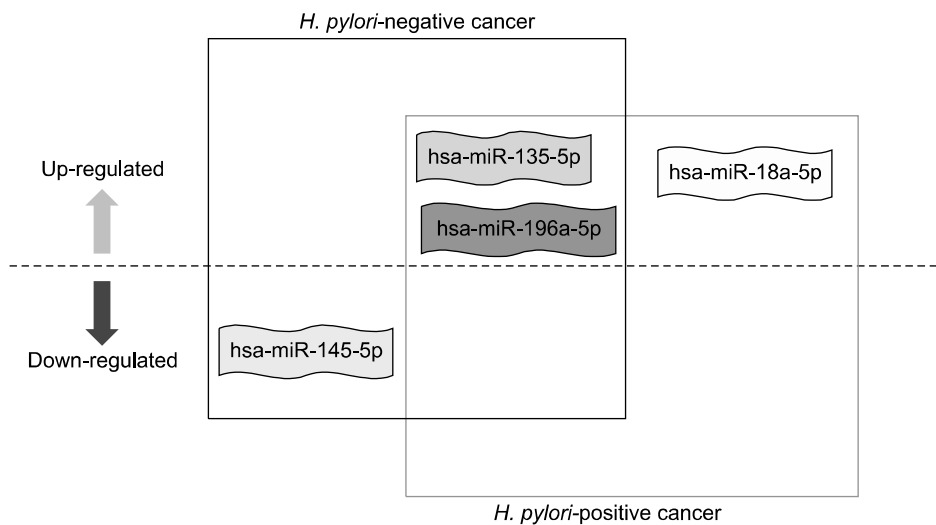


Figure 4. Confirmed 4 dysregulated microRNAs (miRNAs) according to *Helicobacter pylori* status.

mucosal tissue without clear discrimination of *H. pylori*-negative or -positive status. Moreover, comparisons between cancer versus non-cancerous mucosa in same patients compared with non-cancer control had not been tested before. The present investigators assumed that comparisons between cancer versus non-cancerous mucosa in same patients could more easily draw cancer-related miRNAs according to *H. pylori* status. Moreover, it could separate miRNAs responsible for *H. pylori*-induced carcinogenesis from *H. pylori*-independent carcinogenic mechanism.

In the present study, we identified that 4 miRNAs (hsa-miR-145-5p, hsa-miR-135b-5p, hsa-miR-18a-5p, and hsa-miR-196a-5p) were significantly changed. hsa-miR-145-5p was down-regulated in *H. pylori*-negative cancer and hsa-miR-18a-5p was up-regulated in *H. pylori*-positive cancer. hsa-miR-196a-5p and hsa-miR-135b-5p were increased regardless of *H. pylori* status (Fig. 4). hsa-miR-196a-5p is well known to regulate various critical cellular process, such as proliferation, apoptosis, and differentiation. Moreover, hsa-miR-196a-5p has been found to be overexpressed in several cancers, including gastric cancer.¹⁴ miRNA-196a promotes cell proliferation by downregulating p27(ki p1).⁸ And it also promotes cellular proliferation by targeting various transcription factors, such as HOXB8, HMGA2, and annexin A1.¹⁵ From these results, we concluded that miRNA-196a might be have pivotal role in gastric cancer as in other types of cancers. The result of our study could support previous outcomes.

Interestingly, hsa-miR-145-5p was decreased significantly in *H. pylori*-negative cancer. It is suggested that depressed hsa-miR-145-5p activity was related with increase of epithelial-mesenchymal transition (EMT).¹⁶ EMT is a process that epithelial cells become

mesenchymal stem cells. Although it had been recognized as physiologic cellular plasticity,¹⁷ however, recent reports have suggested that EMT might have important role in carcinogenesis and progression. Cytotoxin-associated gene A (CagA) is an oncoprotein and a major virulence factor of *H. pylori*. Recent article regarding EMT on gastric cancer suggests that CagA of *H. pylori* plays a decisive role that triggers a EMT through several transcription factors activation.¹⁸ Up to recently, it was speculated that miRNA-related EMT might be regulated by hsa-miR-335, hsa-miR-21, hsa-miR-31, hsa-miR-205, and hsa-miR-10b.¹⁹ However, we assumed that hsa-miR-145-5p might have additional role in the development of *H. pylori*-negative gastric cancer through EMT. Further studies are needed to clarify the association between hsa-miR-145-5p and EMT. In case of miRNA-18a, its role in gastric cancer was not well investigated. But recent study suggests that hsa-miR-18a can modulate P53 expression in gastric cancer.²⁰ hsa-miR-18a was increased only in *H. pylori*-positive tissues in the present study. So, it is inferred that hsa-miR-18a might regulate some genetic processes related with *H. pylori*-positive gastric carcinogenesis. Additional studies are needed to clarify the exact role of hsa-miR-18a.

There are many concerns about our FFPE samples used in present study. At result of microarray test, above one hundred miRNAs have statistical significance,¹⁰ which indicates the usefulness of FFPE sample for screening miRNA enough.²¹ We selected final 10 candidates for Taqman RT-PCR by the signal intensity and difference between cancer versus non-cancer. Compared with previous study, systematic approach using miRNA database was not performed at the present study. So, we could not get enough statistically meaningful miRNA result. Moreover, mean age of the patients in *H. pylori*-negative non cancer control was younger than that of cancer group at Taqman

PCR. Further studies in more subjects are needed to overcome the bias.

In conclusion, 4 miRNAs (hsa-miR-145-5p, hsa-miR-135b-5p, hsa-miR-18a-5p, and hsa-miR-196a-5p) were significantly changed. miRNA expression of the gastric cancer in the present study implied that different but partially common gastric carcinogenic mechanisms according to *H. pylori* status might be present. Moreover, the present study confirmed that miRNAs could be molecular basis for the study of gastric carcinogenesis, especially on clear discrimination of *H. pylori* status. Functional study is needed to clarify how these miRNAs (especially hsa-miR-18a-5p and hsa-miR-145) could lead to gastric carcinogenesis and what target genes might be regulated.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

REFERENCES

- Jun JK, Choi KS, Lee HY, Suh M, Park B, Song SH, et al. Effectiveness of the Korean National Cancer Screening Program in reducing gastric cancer mortality [published online ahead of print January 29, 2017]. *Gastroenterology* 2017. doi:10.1053/j.gastro.2017.01.029.
- Belair C, Darfeuille F, Staedel C. Helicobacter pylori and gastric cancer: possible role of microRNAs in this intimate relationship. *Clin Microbiol Infect* 2009;15:806-12.
- Shiota S, Thrift AP, Green L, Shah R, Verstovsek G, Rugge M, et al. Clinical manifestations of Helicobacter pylori-negative gastritis [published online ahead of print January 18, 2017]. *Clin Gastroenterol Hepatol* 2017. doi: 10.1016/j.cgh.2017.01.006.
- Nordenstedt H, Graham DY, Kramer JR, Rugge M, Verstovsek G, Fitzgerald S, et al. Helicobacter pylori-negative gastritis: prevalence and risk factors. *Am J Gastroenterol* 2013;108:65-71.
- Matsushima K, Isomoto H, Inoue N, Nakayama T, Hayashi T, Nakayama M, et al. MicroRNA signatures in Helicobacter pylori-infected gastric mucosa. *Int J Cancer*. 2011;128:361-70.
- Yoon H, Kim N, Lee HS, Shin CM, Park YS, Lee DH, et al. Helicobacter pylori-negative gastric cancer in South Korea: incidence and clinicopathologic characteristics. *Helicobacter* 2011;16:382-8.
- Wu WK, Lee CW, Cho CH, Fan D, Wu K, Yu J, et al. MicroRNA dysregulation in gastric cancer: a new player enters the game. *Oncogene* 2010;29:5761-71.
- Zhang Z, Li Z, Li Y, Zang A. MicroRNA and signaling pathways in gastric cancer. *Cancer Gene Ther* 2014;21:305-16.
- Rossi AF, Cadamuro AC, Biselli-Périco JM, Leite KR, Severino FE, Reis PP, et al. Interaction between inflammatory mediators and miRNAs in Helicobacter pylori infection. *Cell Microbiol* 2016;18:1444-58.
- Chang H, Kim N, Park JH, Nam RH, Choi YJ, Lee HS, et al. Different microRNA expression levels in gastric cancer depending on Helicobacter pylori infection. *Gut Liver* 2015;9:188-96.
- Kim N, Park RY, Cho SI, Lim SH, Lee KH, Lee W, et al. Helicobacter pylori infection and development of gastric cancer in Korea: long-term follow-up. *J Clin Gastroenterol* 2008;42:448-54.
- Shin CM, Kim N, Jung Y, Park JH, Kang GH, Kim JS, et al. Role of Helicobacter pylori infection in aberrant DNA methylation along multistep gastric carcinogenesis. *Cancer Sci* 2010;101:1337-46.
- Noto JM, Peek RM. The role of microRNAs in Helicobacter pylori pathogenesis and gastric carcinogenesis. *Front Cell Infect Microbiol* 2012;1:21.
- Chen C, Zhang Y, Zhang L, Weakley SM, Yao Q. MicroRNA-196: critical roles and clinical applications in development and cancer. *J Cell Mol Med* 2011;15:14-23.
- Haykal S, Waddell TK, Hofer SO. Re: moving towards in situ tracheal regeneration: the bionic tissue engineered transplantation approach. *J. Cell. Mol. Med. Vol. 14, No. 7, 2010, pp. 1877-1889. J Cell Mol Med* 2011;15:24-5.
- Jiang SB, He XJ, Xia YJ, Hu WJ, Luo JG, Zhang J, et al. MicroRNA-145-5p inhibits gastric cancer invasiveness through targeting N-cadherin and ZEB2 to suppress epithelial-mesenchymal transition. *Oncotargets Ther* 2016;9:2305-15.
- Baum B, Settleman J, Quinlan MP. Transitions between epithelial and mesenchymal states in development and disease. *Semin Cell Dev Biol* 2008;19:294-308.
- Lee DG, Kim HS, Lee YS, Kim S, Cha SY, Ota I, et al. Helicobacter pylori CagA promotes Snail-mediated epithelial-mesenchymal transition by reducing GSK-3 activity. *Nat Commun* 2014;5:4423.
- Willis BC, Borok Z. TGF-beta-induced EMT: mechanisms and implications for fibrotic lung disease. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L525-34.
- Chen YJ, Wu H, Zhu JM, Li XD, Luo SW, Dong L, et al. MicroRNA-18a modulates P53 expression by targeting IRF2 in gastric cancer patients. *J Gastroenterol Hepatol* 2016;31:155-63.
- Glud M, Klausen M, Gniadecki R, Rossing M, Hastrup N, Nielsen FC, et al. MicroRNA expression in melanocytic nevi: the usefulness of formalin-fixed, paraffin-embedded material for miRNA microarray profiling. *J Invest Dermatol* 2009;129:1219-24.