Altered MiRNA Expression in Gastric Cancer: a Systematic Review and Meta-Analysis

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Key Words
Gastric cancer • MiRNA • Microarray • Biomarker • Diagnosis

Abstract

Background/Aims: Aberrant microRNA expression has the potential to be used for early diagnosis of gastric cancer or to predict survival and treatment response. This study performed a systematic review and meta-analysis of altered miRNAs in gastric cancer in order to assess the use of miRNAs as novel biomarkers for early detection and prognosis prediction of gastric cancer. Methods: We retrieved published articles from the PubMed online database and obtained different sets of data on miRNAs expression profiling in gastric cancer and highlighted the most frequently dysregulated miRNAs in gastric cancer. We then extracted studies that used quantitative RT-PCR and then pooled them together by using meta-disc software (version 1.4). Results: We found that there were 47 aberrantly expressed miRNAs in gastric cancer (29 up-regulated and 18 down-regulated) that were most frequently reported in the literature. In publications that provided information on specific miRNA expression vs. diagnostic value, the pooled data showed good sensitivity and specificity as well as high levels of overall accuracy. However, specimen types could be a factor that introduces substantial heterogeneity. Published studies also showed association of altered miRNA expression with clinicopathological data from gastric cancer patients. Conclusion: Thus, various miRNAs are differentially expressed in gastric cancer and some of them could be further evaluated as biomarkers for early diagnosis of gastric cancer and prediction of prognosis or treatment response.

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Introduction

Gastric cancer is the fourth most common cancer in the world, with an estimated 989,600 new cases and 738,000 deaths globally in 2008 [1]. Approximately 72% of gastric cancer occurs in developing countries and geographically, the highest incidence rate is in Asia (e.g., Korea, Japan, and China) and parts of South America, whereas the lowest rate is in North America [1]. The overall 5-year survival rate of patients with early stage gastric cancer can be above 90% [2, 3]. However, approximately two thirds of gastric cancer patients show tumor metastasis or local invasion at diagnosis. Surgery is the treatment of choice for early stages of the disease. However, the median survival time is only 6 to 9 months for later stage of disease after surgical treatment [4, 5]. Thus, early detection of gastric cancer is the key to prolong survival of patients and therefore health-care policy for gastric cancer should be focused on early detection and as well as novel treatment strategies. To date, the commonly used diagnostic tools for detection of gastric cancer are endoscopy, CT, MRI, and EUS and tumor markers (CA199, CEA, and CA724). However, these tools are valuable only in diagnosing late stage cancers [6]. Thus, identification and evaluation of novel molecular markers could therefore improve diagnosis and management of gastric cancer. For this reason, we sought to explore the application of microRNAs as a potential diagnostic and prognostic marker for human patients with gastric cancers.

MicroRNAs (miRNA) are endogenous, small, single-stranded non-coding RNAs that regulate gene expression via either translational repression or mRNA degradation [7, 8]. miRNAs are estimated to regulate up to 30% of genes in the human genome [9]. Over the past few years, studies of miRNA revealed that miRNA participate in regulation of various biological processes, such as cell proliferation, apoptosis, and differentiation, as well as tumor development, metastasis, angiogenesis, and immune reaction; thus, miRNA play important roles in homeostasis and tumorigenesis [10, 11]. In this study, we have reviewed the most recent literature on miRNA and performed a meta analysis for potential diagnostic and prognostic value for miRNA in gastric cancer. On the basis of recent data, we divided our studies into three parts, i.e., the data on miRNA chip analysis, qRT-PCR, and the relationship to clinicopathological factors.

Materials and Methods

Search strategy

We thoroughly searched PubMed, Web of Science, and EMBASE online databases for the relevant human studies of miRNAs published in English language up to March of 2014. The following keywords and phrases were used: (stomach neoplasms OR (stomach AND neoplasms) OR (gastric AND "cancer) OR (gastric cancer) OR GC) AND (microRNA OR miRNA). We also reviewed the references cited in these retrieved studies to identify additional studies. Two investigators (Wang and Xiao) independently reviewed the titles, abstracts, and full texts of manuscripts to identify eligible studies. Another investigator (Zhang) joined in data extraction together with the two former investigators. Any disagreements were resolved by a third investigator (Zhu). The data were then extracted from the selected studies and input into tables that contain different characteristics of interest.

Inclusion and exclusion criteria

The inclusion and exclusion criteria of this meta-analysis were as follows: i). Studies on miRNA in tissue or blood in gastric cancer patients using miRNA microarray and reported on dysregulated miRNAs were included; ii). For studies on miRNA in tissue or specimens including serum, plasma, and peripheral blood using qRT-PCR, if diagnosis value such as sensitivity and specificity were available and (or) data on dysregulated miRNA was available, the article was included; iii). If the sample size in both experimental and control groups was larger than 10 in clinic validation using qRT-PCR, then the study was included; and iv). Only original research papers that were full-text available and published in English were included; otherwise, they were excluded.
**Data extraction**

We extracted the data from each included study according to the types of study, i.e., i). In miRNA microarray studies, we extracted data including platform of miRNA microarray chip, specimen type, sample size, the cut-off value for up- and down-regulated miRNA expression, and the list of dysregulated miRNAs; ii). In the validation studies using miRNAs as biomarkers for tumor diagnosis by method of qRT-PCR, we focused on the sensitivity and specificity of the dysregulated miRNA, and additional information including specimen type, sample size, the cutoff value, control gene for normalization, AUC (the area under the Receiver operating characteristic curves (ROC) curve) and so on were collected; iii). The previously screened miRNAs were associated with clinicopathological data, e.g., gender, age, tumor size, location, cell proliferation, and TMN stage, lymphatic metastases, pathological type, the target gene of miRNA, prediction of response to radiotherapy and chemotherapy and prognosis of gastric cancer patients.

**Statistical analysis**

The analytical software meta-disc version 1.4 (version 1.4; Ramony Cajal Hospital, Madrid, Spain) was used to analyze the diagnostic value and to calculate the 95% confidence interval. A two-sided p < 0.05 was considered statistically significant. Inconsistency (I²) was calculated to test heterogeneity with an I² value greater than 50% indicating substantial heterogeneity. Based on the heterogeneity values, we used the random-effect model throughout the statistical tests to compute the data. A regression analysis was performed to explore the possible sources of heterogeneity. A subgroup analysis of the specimen type was performed for sensitivity, specificity, LR+ (positive likelihood ratio), LR- (negative likelihood ratio), and DOR (diagnostic odds ratio). Summary receiver operating characteristic (sROC) curve and the area under the curve (AUC) were also calculated to predict sensitivity and specificity. Other data on miRNA chip and associated clinicopathological data in the validation studies that were not appropriate to be pooled are listed in tables.

**Results**

**Data on altered miRNA expression using miRNA microarray analysis**

In this part of data, we aim to summarize data generated from miRNA microarray analysis on differentially expressed miRNA in gastric cancer in order to highlight the value of using the most commonly altered miRNAs as potential biomarkers in diagnosis of gastric cancer. We found 19 studies reporting altered miRNA expression using various high-throughput miRNA chips as shown in Table 1.

In terms of samples, plasma from gastric cancer and healthy people was used in two studies [10, 11], plasma from pre- and post-operative gastric cancer patients were used in one study [21], whereas all other studies utilized tissues from tumors and adjacent normal or real normal mucosa, except for formalin-fixed paraffin-embedded tissue being used in one study [25]. The sample numbers within each study range from 3 pairs to 353 individuals. Except for one study [24] that used qRT-PCR and could only detect a total of 72 miRNAs, all other studies used miRNA chips and detected at least 326 miRNAs. Three studies [10, 12, 27] did not provide data on miRNA fold changes while in all other studies, a 1.5 fold change was used to pick up-regulated or down-regulated miRNAs. Histopathological grading information was provided in seven [12, 13, 16, 18, 19, 24, 26] studies.

Analysis of data from the 19 included studies that used miRNA microarray chips revealed a number of miRNAs with altered expression. Although we found hundreds of altered miRNAs, only members from the miR-17-92 cluster and its two paralogues were consistently upregulated [12-19, 22, 23, 25-27]. We have also summarized miRNAs that were up-regulated in at least 4 of the 19 studies and down-regulated in at least 3 studies in Table 2. Specifically, a total of 25 miRNAs, including miR-21 and miR-23a, were most frequently up-regulated and 14 miRNA (including miR-29c and miR-30a-5p) were down-regulated.

**Validation of some dysregulated miRNAs in clinical samples**

We collected all studies that evaluated the diagnostic value of miRNAs as a novel biomarker in gastric cancer patients. The flowchart explaining our search procedure can be

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This text appears to be a excerpt from a scientific article discussing the altered miRNA expression in gastric cancer. It includes a detailed methodology for data extraction, statistical analysis, and results focusing on the alteration of miRNAs in gastric cancer tissues and plasma. The text also highlights the validation of these miRNAs as biomarkers for tumor diagnosis.
Wang et al.: Altered MiRNA Expression in Gastric Cancer

Table 1. Studies of miRNA microarray profiling in fresh gastric cancer tissue samples.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Platform of miRNA microarray</th>
<th>miRNA probes</th>
<th>Samples from cancer/normal tissue (N or pairs)</th>
<th>Cutoff (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tchernitsa [12]</td>
<td>NCode Multi-Species miRNA Microarray Probe Set V (IrvineGen, CA, USA)</td>
<td>857</td>
<td>6 pairs (3/4 without lymph node metastases), intestinal-type</td>
<td>median 2</td>
</tr>
<tr>
<td>Chen [14]</td>
<td>miRCURY LNA microRNA Array, v.11.0 (Exiqon)</td>
<td>NA</td>
<td>5 pairs</td>
<td>3</td>
</tr>
<tr>
<td>Yao [15]</td>
<td>Mercury LNA Array, v.11.0, qRT-PCR</td>
<td>847</td>
<td>3 pairs</td>
<td>2/1.5</td>
</tr>
<tr>
<td>Li [16]</td>
<td>miRCURY LNA Array microRNA chip (Exiqon) v.14.0</td>
<td>984</td>
<td>6 pairs , intestinal-type GC</td>
<td>2</td>
</tr>
<tr>
<td>Wang [17]</td>
<td>miRCURY LNA Arrays Exiqon, Vedbaek, Denmark v.16.0</td>
<td>NA</td>
<td>17 pairs</td>
<td>2</td>
</tr>
<tr>
<td>Zhang [18]</td>
<td>miRNA chip, v12.0 (Agilent Technologies, Foster City, CA)</td>
<td>885</td>
<td>3 pairs, distal gastric adenocarcinoma</td>
<td>1.5</td>
</tr>
<tr>
<td>Tsukamoto [19]</td>
<td>miRNA microarray (Agilent Technologies, Santa Clara, CA, USA)</td>
<td>470</td>
<td>14 (intestinal type) and 8 (diffuse type) / 5 pairs</td>
<td>2</td>
</tr>
<tr>
<td>Su [20]</td>
<td>AFX miRNA expression chips (Affymetrix, CA, USA)</td>
<td>NA</td>
<td>16 pairs</td>
<td>1.5</td>
</tr>
<tr>
<td>Konishi [21]</td>
<td>3D-Gene miRNA microarray platform (TORAY, Kamakura, Japan)</td>
<td>NA, miRBase 15.0</td>
<td>3 Pre-operative plasma/post-operative plasma</td>
<td>2</td>
</tr>
<tr>
<td>Gorur [10]</td>
<td>The BioMark 96,96 Dynamic Array (Fluidigm, South San Francisco, CA), qRT-PCR</td>
<td>994,704</td>
<td>20 / 190, blood samples</td>
<td>mean</td>
</tr>
<tr>
<td>Guo [22]</td>
<td>miParaflo™ microfluidic chip (LC Sciences, Houston, TX, USA)</td>
<td>NA, miRBase 10.0</td>
<td>3 pairs</td>
<td>1.5</td>
</tr>
<tr>
<td>Ding [23]</td>
<td>miParaflo microfluidic technology (LC Sciences, TX, USA)</td>
<td>NA</td>
<td>6 pairs</td>
<td>1.5</td>
</tr>
<tr>
<td>Katada [24]</td>
<td>qRT-PCR</td>
<td>72</td>
<td>42 pairs undifferentiated GC</td>
<td>2</td>
</tr>
<tr>
<td>Li [25]</td>
<td>The TagMan Human miRNA Array v1.0 (Early Access) , qRT-PCR</td>
<td>365</td>
<td>30 pairs, formalin-fixed paraffin-embedded specimens</td>
<td>2</td>
</tr>
<tr>
<td>Uechi [26]</td>
<td>Ohio State University custom microRNA microarray chip (OSU, CCC v3.0)</td>
<td>326</td>
<td>184 / 103 diffuse-type and 81 intestinal-type / 169</td>
<td>1.5</td>
</tr>
<tr>
<td>Carvalho [27]</td>
<td>miRNAChipHuman_V2(Portugal)</td>
<td>NA</td>
<td>37 / 10 (pooled as 4 samples) / 6 pairs</td>
<td>NA</td>
</tr>
<tr>
<td>Deng [28]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 2. Expression of the most frequently altered miRNAs in gastric cancer detected by the miRNA microarray

<table>
<thead>
<tr>
<th>Up-regulated miRNAs (± 4 folds; n=25)</th>
<th>Down-regulated miRNAs (± 3 folds; n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-17-92 family: miR-17, miR-18a, miR-18b, miR-19a, miR-20a, miR-20b, miR-25, miR-92, miR-93, miR-106a, miR-106b</td>
<td>miR-29c, miR-30a-5p, miR-30c, miR-30d, miR-31, miR-101, miR-146a, miR-375, miR-378, miR-451, miR-486-5p, miR-504-3p, miR-504-5p, miR-504-5p, miR-769-3p</td>
</tr>
<tr>
<td>miR-192, miR-194, miR-214, miR-215, miR-222, miR-223, miR-335</td>
<td></td>
</tr>
</tbody>
</table>

seen as Figure 1 briefly. A total of 17 studies evaluate 28 panel of miRNAs in total were included. The number of patients in each study ranged between 42 and 391, and the specimens used were plasma, serum, peripheral blood, or gastric juice. The adopted endogenous reference, quantitative method, and the cut-off values were not unified, e.g., for the plasma, U6 was used in three studies [27, 29, 32], while Cel-miR-39 used in one study [33]. As for the feasibility to obtain blood sample, almost all studies recruited healthy individual as a normal control except Zhang et al [34] who recruited normal mucosa or minimal gastritis in 99 patients as a control. In addition, Li et al [30] focused on early stages of gastric cancer and Zhang et al [18] focused on serum in distal gastric adenocarcinomas compared with 20 age-matched healthy individuals.
Meta-analysis

We utilized the meta-disc software version 1.4 to analyze the data. We generated forest plots as Figure 2 and demonstrated that the pooled sensitivity was 0.77 (95%CI: 0.75-0.79; Q=143.42, p=0.000, I²%=81.2%) and the specificity was 0.78 (95%CI: 0.76-0.80; Q=179.97, P=0.000, I²%=85.0%), indicating a significant heterogeneity among these studies. We then used random-effects models to re-analyze the data and analyzed the diagnostic threshold. The spearman correlation coefficient was 0.166 (p=0.398), indicating that the heterogeneity was not caused by diagnostic threshold. Next, we generated forest plots of DOR. The DOR of each study did not display a straight line distribution and the Cochran-Q value was 77.51 (P=0.000), indicating substantial heterogeneity. This may be caused by discrepancy of studied population, specimen type, endogenous reference, or total sample size. Meta-regression analysis (data not shown) on the probable factors suggested that specimen type was probably the cause for heterogeneity.

Subgroup analysis

Due to high heterogeneity, the data (such as sensitivity) could not be simply pooled, but was only suitable for subgroup analyses. Filtering studies based on specimen type reduced heterogeneity to acceptable levels; for example, on sROC curve, the maximum joint sensitivity and specificity (Q value) was 0.7832 and the AUC was 0.8521, indicating a high level of overall accuracy. In a group of five studies, which included seven panels of miRNAs from serum, the pooled sensitivity was 0.79 (95%CI: 0.75-0.83; I²%=44.6%), and the pooled specificity was 0.71 (95%CI: 0.67-0.76; I²%=86.2%). However, in the 2 studies that included 4 miRNAs from peripheral blood, the pooled sensitivity, specificity, LR+, LR-, and DOR were 0.58 (95%CI: 0.50-0.65; I²%=22.2%), 0.90 (95%CI: 0.82-0.95; I²%=42.1%), 4.52 (95%CI: 2.52-8.09; I²%=0%), 0.48 (95%CI: 0.39-0.59; I²%=21.0%), and 9.85 (95%CI: 4.70-20.66; I²%=0%), respectively. In the subgroup of studies using plasma samples, the heterogeneity was high; thus, a pooled study was not appropriate. The pooled sensitivity, specificity, LR+, LR- and DOR in plasma vs. gastric juice are 0.85 vs. 0.65, 0.78 vs. 0.81, 4.51 vs. 3.42, 0.20 vs. 0.41, and 28.8 vs. 18.80, respectively.
Wang et al.: Altered MiRNA Expression in Gastric Cancer

Clinical studies that supplied the ratio of dysregulated miRNAs in gastric cancer tissues

Since obtaining normal tissue specimens is an invasive procedure, we were unable to retrieve a large number of studies using normal tissues to validate the diagnostic value of altered miRNAs. However, tissues adjacent or distant to tumor lesions is easier to acquire and more reliable as it eliminates inter-individual discrepancies. We identified 34 multi-
center, cohort studies from 4 countries that provided data on altered expression of miRNAs. Specifically, two studies [21, 31] utilized plasma from pre- and post-operative gastric cancer patients, while one study [35] used peripheral blood, two studies [36, 37] used FFPE tissues, and two studies from the same laboratory [38, 39] assessed down-regulated miR-181b and miR-182 in 10 gastric adenocarcinoma tissues. Furthermore, only a few studies performed subgroup data analyses for association with clinicopathological features and the miRNAs studied were different in each of these studies. The altered miRNA expression and its frequency are shown in Figure 3. Respectively, there are 29 up-regulated miRNAs (including
miR-107, miR-222, and miR-106b and 18 down-regulated miRNAs (including miR-143, miR145, miR622 and miR148a) in gastric cancer tissues vs. controls. The mean ratio of these dysregulated miRNAs was 0.766.

Association of differentially expressed miRNAs with clinicopathological factors

Association of differentially expressed miRNAs with clinicopathological factors from gastric cancer patients was shown in Table 3. Differential expression of miRNAs was associated with age, gender, tumor size, TNM stage, tumor differentiation, lymphatic metastasis, tumor invasion, pathological type, response to radiotherapy and chemotherapy, and prognosis. These differentially expressed miRNAs were also associated with altered expression of some of their target genes.

Discussion

In this study, we retrieved and analyzed published studies on altered miRNA expression in gastric cancer. Our data showed aberrantly expressed miRNAs in gastric cancer, with 25 up-regulated and 14 down-regulated miRNAs being the most frequently altered. Among them, the miR-17-92 cluster was generally up-regulated in gastric cancer tissues. Our data analysis also showed that specific miRNAs could be used (with moderate sensitivity and specificity, and a high level of the overall accuracy) in the diagnosis of gastric cancer. However, it is to be noted that there was substantial heterogeneity in the data most likely attributable to the specimen types. Furthermore, we found that the mean ratio of dysregulated miRNAs in a large number of clinical studies was 0.766. The published studies also showed association of altered miRNA expression with clinicopathological data from gastric cancer patients. Thus, future studies are required to further validate these differentially expressed miRNAs in gastric cancer as biomarkers for early diagnosis of gastric cancer, prognosis and prediction of treatment responses.

To date, there are a number of available biomarkers, such as CEA, CA199, and CA724 for diagnosis of gastric cancer, but their sensitivity and specificity are low [6]. So we necessarily need a novel effective biomarker for diagnosing gastric cancer at early stages. Detection of altered expression of miRNAs could have several advantages [59-61], i). miRNAs are usually stable in fresh tissues, FFPE tissue, cells and peripheral blood [31, 62-66] because they are short and resistant to degradation; ii). Each miRNA is specifically expressed in tissue specimens [67]; iii). miRNAs have been shown to be differentially expressed in gastric cancer vs. normal tissues; and iv). Altered miRNA expression has been shown to contribute to tumorigenesis and cancer progression; v). Association of miRNA expression with tumor stages has been demonstrated [68, 69]. Thus, research on miRNA could help us to develop novel biomarkers and to better understand the molecular mechanism of gastric cancer development.

Indeed, our current study showed 25 most frequently upregulated and 14 downregulated miRNAs in gastric cancer. Future studies with a larger sample size are needed to confirm these data. Furthermore, different miRNAs have different target genes and thus, future studies should also analyze expression of their target genes for generating combined biomarkers to be used for the early detection of gastric cancer or prediction of survival and treatment responses. Finally, the specificity of these miRNA biomarkers should be further confirmed by comparing samples from the patients with gastric cancers and the patients with other type of cancers.

Furthermore, the functional analysis of miRNAs might be also important for the choice of diagnostic miRNAs. For example, miR-451 has been linked to controlling expression of p-glycoprotein, a critical protein controls multidrug resistance in cancer cells [70]. And miR-106 family (miR-106b, miR-93 and miR-25) overexpresses in gastric cancer stem cells [71]. Thus, the physicians should take the functions of these miRNAs into consideration when they look for good candidates for gastric cancer biomarkers.
However, our current study does have some limitations; for example, there are always unique data from published studies, but not unified set of data for meta analysis. The biological functions of blood miRNAs are not clear, making data interpretation difficult. Moreover, the drawback of these retrieved studies is that consideration was not given for gastric cancer risk factors, such as *Helicobacter pylori* infection, genetics, diet and other risk factors that may contribute to altered expression of miRNAs. Infection of *Helicobacter pylori* is an important factor in gastric cancer development and the expression of different miRNAs were shown to be significantly associated with infection of Helicobacter pylori [72]. In addition, each study has its own study design, use of miRNA chips, endogenous reference, pathology type, and localization of gastric cancer lesions, which makes the analysis difficult.

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**Disclosure Statement**

The authors have declared that no competing interests exist.

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Wang et al.: Altered MiRNA Expression in Gastric Cancer


Wang et al.: Altered MiRNA Expression in Gastric Cancer


Wang et al.: Altered MiRNA Expression in Gastric Cancer


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