

Three dysregulated microRNAs in serum as novel biomarkers for gastric cancer screening

Hui Wang · Lei Wang · Zheng Wu · Rong Sun · Haifeng Jin · Jifeng Ma · Lili Liu · Rui Ling · Jun Yi · Ling Wang · Jiefang Bian · Jianghao Chen · Nanlin Li · Shifang Yuan · Jun Yun

Received: 23 September 2014 / Accepted: 20 October 2014 / Published online: 4 November 2014
© Springer Science+Business Media New York 2014

Abstract Gastric cancer (GC) is one of the most threatening diseases. The symptoms of GC are complex and hard to detect, which also contribute to the poor prognosis of GC. Besides, the current diagnosis for GC is expensive and invasive. Thus, a fast, noninvasive biomarker is urgently needed for GC screening. MicroRNAs (miRNAs) are small noncoding RNAs, which are involved in a great variety of pathological processes, particularly carcinogenesis. MiRNAs are stable in gastric juice, plasma as well as serum, which facilitate it to be a promising biomarker for cancer. In this study, we selected three novel miRNAs, i.e., miR-233, miR-16, and miR-100, to investigate

their potential diagnostic value in GC screening. A total of 50 GC patients and 47 healthy controls were involved in this study. Blood serum samples were collected; RNAs were extracted and normalized with U6 snRNA as the internal control; qRT-PCR was performed for relative expression of target miRNAs. Levels of miRNAs expression were compared by Student's *t* test for the comparison between two groups, and one-way ANOVA was used for multiple comparisons. The expression of miR-223, miR-16, and miR-100 was all significantly higher in GC patients than controls (all $P < 0.001$). All the tested miRNAs were manifested to be valuable biomarkers for GC. Relative expression of these miRNAs was significantly correlated with clinical characteristics of GC patients, such as TNM stage ($P = 0.036$ for miR-223; $P < 0.001$ for miR-100), metastatic status ($P = 0.045$ for miR-223; $P = 0.031$ for miR-16; $P = 0.006$ for miR-100), tumor size ($P = 0.042$ for miR-223; $P = 0.031$ for miR-16; $P < 0.001$ for miR-100), and differentiation grade ($P = 0.036$ for miR-223; $P = 0.030$ for miR-16; $P = 0.034$ for miR-100). However, in T classification, which considered both tumor size and direct extent of primary tumor, the difference in target miRNAs expression was not significant. In summary, we confirmed the diagnostic value of serum miR-223, miR-16, and miR-100 in GC. Significantly elevated expression of the three miRNAs was also observed in advanced GC patients, which suggested their availability in cancer staging.

H. Wang, L. Wang and Z. Wu are the co-first authors.

H. Wang · L. Wang · R. Ling · J. Yi · L. Wang · J. Bian · J. Chen · N. Li · S. Yuan (✉) · J. Yun (✉)
Department of Vascular and Endocrine Surgery, Xijing Hospital, The Fourth Military Medical University, Xi'an 710032, Shanxi Province, China
e-mail: yuanshifang_xj@yeah.net

J. Yun
e-mail: yunjun_xj@163.com

Z. Wu
Department of Biotherapy, Fourth Hospital of Hebei Medical University, Shijiazhuang 050011, Hebei Province, China

R. Sun · H. Jin
Department of Gastroenterology, Bethune International Peace Hospital, Shijiazhuang 050082, Hebei Province, China

J. Ma
Department of Information, Bethune International Peace Hospital, 398 Zhongshan Western Road, Shijiazhuang 050082, Hebei Province, China

L. Liu
Department of Oncology, Tangdu Hospital, the Fourth Military Medical University, Xi'an, China

Keywords microRNA · miR-223 · miR-16 · miR-100 · Serum · Gastric cancer · Screening

Introduction

Gastric cancer (GC) has been one of the most threatening cancers around the world. Although the mortality rate has

declined greatly, as a result of the improvement of hygiene and advanced food preservation methods, it still causes 10,990 deaths in the United States only in the year of 2014 [1]. Moreover, the incidence rate and mortality rate are even higher in Eastern Asia, especially in China [2]. Chronic infection of *Helicobacter pylori* is one of the primary causes of GC. As is known to all, early detection of cancer can dramatically improve the prognostic condition of patients. But GC usually occurs with complex symptoms. For example, a GC patient may have the feeling of nausea, lack of appetite, difficulty in digestion and pain in abdomen, which can be confused with other digestive diseases [3]. Most patients with GC were diagnosed at advanced stages. The current diagnosis for GC is primarily via endoscopic examination, which is expensive and invasive. Nonetheless, the target protein for various cancer types, such as carcinoembryonic antigen (CEA), E-cadherin, CA-125, and alpha-fetoprotein, do not illustrate an acceptable specificity and sensitivity in GC diagnosis [4]. Thus, a sensitive, specific, noninvasive, and simple method for GC diagnosis is urgently needed.

MicroRNAs (miRNAs) are small noncoding RNAs. They target at a majority of protein-coding transcripts and act as the guide molecules in RNA silencing. MiRNAs are involved in a great variety of pathological processes, particularly carcinogenesis; the dysregulation of miRNAs biogenesis has also been proved to be associated with many diseases [5]. For example, miR-182 and miR-200a have been found to be able to control G-protein Subunit α -13 (GNA13) expression and further contribute to the progress of prostate cancer [6]; miR-135b can act as a downstream effector of oncogenic pathway and promotes cancer progression in colon cancer [7]. Besides, miRNAs are stable in gastric juice, plasma as well as serum, which facilitate it to be a promising biomarker for cancer. Considering the fact that miRNAs play an important part in cellular pathways, they are also suspected to have practical predictive value in cancer diagnosis, which has also been proved by several studies. It was found by Yang et al. [8] that elevated expression of miR-155 represents a novel predictor for rectal cancer detection. However, not many convincing studies have been performed regarding miRNA and GC. Thus, in this study, we selected three novel miRNAs, i.e., miR-233, miR-16, and miR-100, to investigate their potential diagnostic value in GC screening. Hopefully, we can provide some insight for the role of miRNAs in GC development.

Materials and methods

Subjects and samples

A total of 97 subjects, including 50 GC patients and 47 healthy controls, were recruited from 2010 to 2013 at

Xijing Hospital of the Fourth Military Medical University in Xi'an, China. The research was performed according to the protocol approved by the Ethics Committee of Xijing Hospital. A consent form was given by all subjects enrolled in the study. This research was conducted in compliance with the Declaration of Helsinki.

All participants have to fulfill the following criteria: (a) confirmed pathological diagnosis of GC; (b) no previous history of immune disease; (c) no previous history of chemotherapy or radiotherapy. TNM stages were further identified with tumors and regional lymph nodes collected during surgery according to TNM atlas [9]. The patients are classified as stages I, II, III, and IV based on the evaluation of metastatic status (yes or no), tumor size (≤ 5 or > 5 cm), differentiation grade (low, middle, or high), and T classification (T1, T2, T3, or T4) for direct extent of primary tumor.

Five milliliter peripheral blood was collected with EDTA tubes from subjects according to the consent. Blood serum was isolated from blood samples by centrifuging at 1,500 rpm for 10 min, followed by centrifuging at 12,000 rpm for 2 min. All samples were stored at -80°C in aliquot for further requirement.

RNA extraction

RNA extraction was performed with mirVana PARIS Kit (Ambion) according to the manufacturer's protocol. 400 μl of blood serum was used for total RNA extraction. Extracted RNA was dissolved with 10 μl RNase-free water. The absorbances at 260/280 (RNA/DNA) and 260/230 (RNA/Protein) were assessed with NanoDropTM 1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). Retrieved RNA concentration was further calculated and normalized with RNase-free water during cDNA synthesis. All purified RNA samples were either processed for qRT-PCR or kept at -80°C for future requirement.

Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR)

The reverse transcription reaction was conducted with TaqMan[®] microRNA Reverse Transcription Kit (Applied Biosystems). cDNA was synthesized in a 5- μl reaction, which contained 0.5 μl of different primer, 0.05 μl of 100 mM dNTPs, 0.5 μl of 10X reverse transcription buffer, 0.063 μl of 20 U/ μl RNase Inhibitor, and 0.33 μl of 50U/ μl Multiscribe Reverse Transcriptase. The volume of RNA sample was depended on its concentration retrieved from last step. RNase-free water was also added to control the total volume of the reaction. The reaction solutions were incubated at 16°C for 30 min, followed by 42°C for

30 min, 85 °C for 5 min, and held at 4 °C. All synthesized cDNA samples were diluted twofold with RNase-free water for quantitative PCR.

The mixture for qPCR contained 2 µl of cDNA solution, 5 µl of TaqMan® 2X Pfect Master Mix, 0.25 µl of specific primers for different miRNAs, and 2.75 µl of RNase-free water for a total volume of 10 µl. Bio-Rad IQ5 (Bio-Rad Laboratories, Inc) thermocycler was used for qPCR. Primers specific for U6 snRNA (Ambion, AM30303) were also added to the mixture as an internal control. The integrity of this reaction was also verified by U6 snRNA. The PCR amplification was performed as follows: an initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s.

To measure the expression of different miRNAs, the cycle threshold (Ct) values were acquired from Bio-Rad iQ5 2.1 Standard Edition Optical System Software 2.1.94.0617. Relative target miRNA expression was further calculated with Ct values. The relative expression level was defined as $2^{-\Delta\Delta C_t}$, with U6 expression being the standard.

Statistical analysis

Student's *t* test and one-way ANOVA test were applied to assess the difference in relative target miRNA expression between GC patients and healthy controls, in addition to the association of miR-223, miR-16, and miR-100 expression and clinical characteristics (gender, age, TNM stage, metastatic status, tumor size, T classification, and differentiation grade). The diagnostic value of target miRNAs for GC was identified with receiver operating characteristic (ROC) curves and the area under the curve (AUC). The optimal cutoff values were selected with the Youden index. Two-side *P* values were calculated, and a *P* value less than 0.05 was regarded as statistically significant. All statistical analyses were conducted with STATA version 12.0 software (Stata Corp, College Station, TX), and GraphPad Prism 5.0 (GraphPad Software Inc., California) was applied to generate graphs.

Results

Characteristics of subjects

A total of 97 subjects were involved in our study, with 50 GC patients and 47 healthy controls. The gender and age of patients and controls were normalized; no significant difference was observed ($P > 0.05$). Among the 50 enrolled GC patients, 27 were males and 23 were females. 21 patients were under the age of 60 and 29 were elder than that. Earlier TNM stages (I and II) were observed in 31

patients; advanced stages (III and IV) were identified in 19 patients. And metastasis was developed in 22 of 50 patients. In terms of tumor size, 33 patients had a smaller tumor with diameter less than 5 cm. In further T classification considering tumor size and direct extent of primary tumor, 11 of them were diagnosed as T1 or T2 and 23 patients were identified as T3. 17 GC patients with primary tumor larger than 5 cm in diameter were classified as T4. Besides, patients were also categorized by differentiation grade. Low differentiation grade was observed in 21 patients; 29 patients had a moderate or high differentiation grade.

Preliminary analyses on target miRNAs expression and GC susceptibility

To investigate whether the expression of miR-223, miR-16, and miR-100 was aberrant in blood serum of GC patients, unpaired Student's *t* test was conducted on relative expression level of target miRNAs in 50 GC patients compared to 47 healthy controls. The expression of miR-223, miR-16, and miR-100 was all significantly higher in GC patients than controls ($P < 0.0001$, $t = 7.265$; $P < 0.0001$, $t = 8.643$; $P < 0.0001$, $t = 4.144$, respectively). The results were in consistence with our expectation and confirmed the results of previous studies. Scatter dot plots of the serum level of miR-223, miR-16, and miR-100 were plotted in Figs. 1a, 2a, and 3a.

Diagnostic value of miR-223, miR-16, and miR-100 for GC

The diagnostic value of three selected miRNAs was further evaluated with ROC curves and AUC value. All the tested miRNAs, namely miR-223, miR-16, and miR-100, manifested to be valuable biomarkers for GC. AUCs of 0.85 (95 % CI 0.78–0.93) for miR-223, 0.90 (95 % CI 0.84–0.96) for miR-16, and 0.71 (95 % CI 0.61–0.82) for miR-100 were observed. At the optimal cutoff value of 6.23, a sensitivity of 0.81 and a specificity of 0.78 were considered to be the maximal for miR-223. Similarly, at the cutoff value of 16.66, sensitivity and specificity of miR-16 in GC diagnosis were 0.79 and 0.78, respectively. For miR-100, the cutoff value was 3.33 and sensitivity and specificity as maximal were 0.71 and 0.58. ROC curves of miR-223, miR-16, and miR-100 were illustrated in Figs. 1b, 2b, and 3b.

MiR-223, miR-16, and miR-100 expressions in GC patients with different clinical status

To further evaluate the diagnostic value of miR-223, miR-16, and miR-100 as biomarkers in different clinical status

Fig. 1 Diagnostic performance of serum miR-223 in differentiating gastric cancer patients from healthy controls. **a** Relative expression levels of miR-223 in gastric cancer patients and healthy controls. **b** Receiver operating characteristic (ROC) curve analysis of miR-223 in differentiating gastric cancer patients from healthy controls

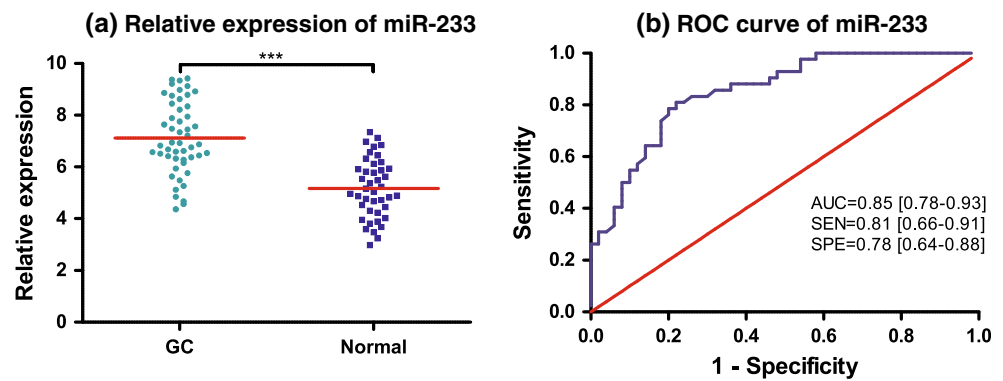


Fig. 2 Diagnostic performance of serum miR-16 in differentiating gastric cancer patients from healthy controls. **a** Relative expression levels of miR-16 in gastric cancer patients and healthy controls. **b** Receiver operating characteristic (ROC) curve analysis of miR-16 in differentiating gastric cancer patients from healthy controls

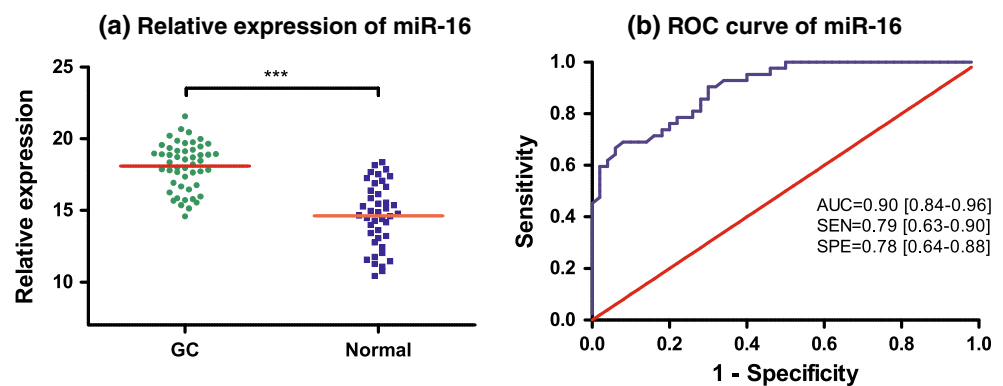
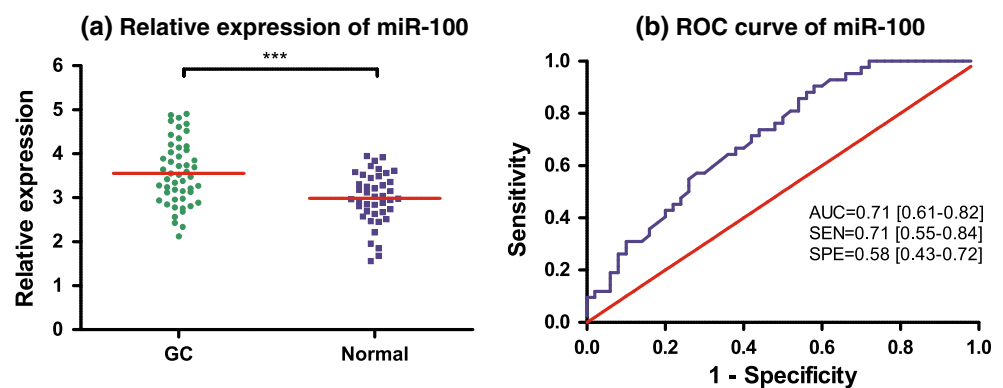


Fig. 3 Diagnostic performance of serum miR-100 in differentiating gastric cancer patients from healthy controls. **a** Relative expression levels of miR-100 in gastric cancer patients and healthy controls. **b** Receiver operating characteristic (ROC) curve analysis of miR-100 in differentiating gastric cancer patients from healthy controls



of GC patients, enrolled subjects were stratified according to different clinical status; difference in miRNAs expression in various groups was assessed. The results were illustrated in Table 1. Expression of miR-223, miR-16, and miR-100 was increased as GC progressing.

Significantly higher expression of miR-223 and miR-100 was observed in patients of advanced TNM stages (III and IV. miR-223: $P = 0.036$; miR-100: $P < 0.001$), while the increase in miR-16 expression was not significant. Expression of all the three miRNAs was raised in patients with metastasis (miR-223: $P = 0.045$; miR-16: $P = 0.031$; miR-100: $P = 0.006$) and low differentiation grade (miR-223: $P = 0.036$; miR-16: $P = 0.030$; miR-100: $P = 0.034$). In

respect of tumor size, significant increased expression of miR-223, miR-16, and miR-100 was also detected in patients with larger tumors (miR-223: $P = 0.042$; miR-16: $P = 0.031$; miR-100: $P < 0.001$). Interestingly, if we further categorized the subjects by T classification, which considered both tumor size and direct extent of primary tumor, the difference in target miRNAs expression between different T types was not significant (miR-223: $P = 0.173$; miR-16: $P = 0.490$; miR-100: $P = 0.388$). Meanwhile, we observed that the expression of miR-100 was also significantly different regarding various age groups ($P = 0.001$) and gender groups ($P = 0.033$), which might be attributed to our limited sample size.

Table 1 Clinical data statistical analysis of gastric cancer patients

Characteristics	Sample size	Relative expression of miR-223	<i>P</i> value	Relative expression of miR-16	<i>P</i> value	Relative expression of miR-100	<i>P</i> value
<i>Gender</i>							
Female	23	5.36 ± 2.30	0.247 ^a	14.66 ± 2.15	0.351 ^a	2.68 ± 0.95	0.033 ^a
Male	27	6.23 ± 2.86		15.26 ± 2.34		3.21 ± 0.76	
<i>Age</i>							
<60	21	5.12 ± 2.65	0.106 ^a	14.71 ± 3.12	0.125 ^a	2.98 ± 0.86	0.001 ^a
≥60	29	6.53 ± 3.21		16.25 ± 3.65		4.05 ± 1.25	
<i>TNM stage</i>							
I + II	31	5.53 ± 3.54	0.036 ^a	15.35 ± 7.24	0.213 ^a	3.45 ± 1.68	<0.001 ^a
III + IV	19	8.21 ± 5.26		18.63 ± 9.56		5.46 ± 1.86	
<i>Metastatic status</i>							
Yes	22	8.56 ± 6.25	0.045 ^a	20.45 ± 11.35	0.031 ^a	6.21 ± 3.86	0.006 ^a
No	28	5.89 ± 2.56		14.65 ± 6.98		3.85 ± 1.85	
<i>Tumor size</i>							
≥5 cm	17	8.69 ± 4.23	0.042 ^a	20.56 ± 12.36	0.031 ^a	6.56 ± 3.67	<0.001 ^a
<5 cm	33	6.21 ± 3.85		14.75 ± 6.25		2.65 ± 1.32	
<i>T classification</i>							
T1 + T2	11	5.21 ± 2.54	0.173 ^b	15.06 ± 9.25	0.490 ^b	3.85 ± 2.56	0.388 ^b
T3	23	7.56 ± 4.65		17.36 ± 9.65		4.25 ± 2.12	
T4	16	9.25 ± 7.45		19.85 ± 11.75		5.23 ± 3.56	
<i>Differentiation grade</i>							
Low	21	5.68 ± 2.13	0.036 ^a	14.62 ± 7.85	0.030 ^a	3.56 ± 1.65	0.034 ^a
Moderate + high	29	9.40 ± 7.68		21.56 ± 12.56		5.23 ± 3.21	

^a Student's *t* test^b One-way ANOVA test

Discussion

In this study, we investigated the diagnostic value of miR-223, miR-16, and miR-100 in distinguishing GC patients from healthy controls and their potential role as a biomarker for different clinical status of GC patients. 50 GC patients and 47 healthy controls were involved in our study. Aberrant expression of all target miRNAs was observed in GC patients; a relatively high diagnostic value for GC was confirmed. The level of expression increased as GC progressing. Patients in advanced phases tended to have a higher level of serum miR-223, miR-16, and miR-100.

Several studies have been conducted on miRNAs as biomarkers for GC. Cui et al. [10] proposed that miR-21 and miR-106a in gastric juice can be utilized as potential biomarker for GC with a relatively high diagnostic value. MiR-199-3p has also been found to be upregulated in the cytoplasm of tumor cells, and elevated expression of miR-199-3p in plasma can be applied as a biomarker [11]. In addition, the predictive value of miRNAs in GC staging has also been investigated in many researches. Many miRNAs do not exhibit an predictive value in GC staging [12]. Nonetheless, in this study, all the three target miRNAs

were proved to be significantly upregulated in advanced GC stages. It was also proposed that miRNA might be related to the prognosis of cancer, since miRNAs play important role in metastasis and tumor genesis. Future studies on miR-223, miR-16, and miR-100 level and GC prognosis are recommended.

MiRNAs are involved in carcinogenesis in several ways. MiRNA can regulate cell cycle progression and proliferation, inhibit apoptosis by targeting at related gene, and participate in tumor cell invasion and metastasis [13]. Generally, miRNAs can be classified as oncogenic miRNAs (oncomiRs) and tumor suppressive miRNAs (tsmiRs). OncomiRs inhibit tumor suppressors, which further leads to cell proliferation and reduced apoptosis, while tsmiRs help to facilitate the proper function of tumor suppressors (Fig. 4). For example, miR-21 is an oncogenic miRNA; it targets at the coding region of p57Kip2 in prostate cancer and vitiates the function of p57Kip2-mediated responses [14]. Whereas miR-152 is a tumor suppressive miRNA, it inhibits cell proliferation and invasion; the apoptosis of glioblastoma stem cells was also promoted by miR-152 [15]. Aberrant expression of either oncomiRs or tsmiRs can result in carcinogenesis. It has been proved by previous

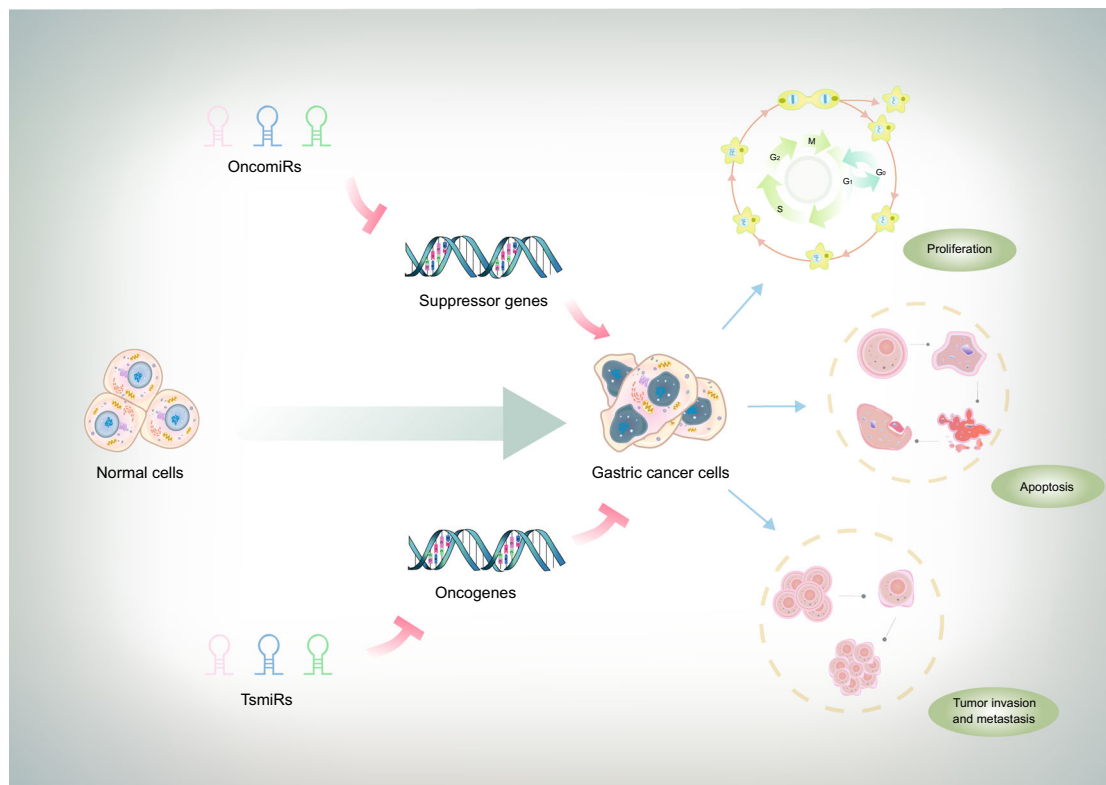


Fig. 4 Schematic diagram depicting microRNAs dysregulation in gastric cancer. MicroRNAs dysregulation may result in gastric cancer cell formation and progression through affecting the expression of

target genes and then the progress of cell cycle, apoptosis, and tumor invasion and metastasis

studies that the hypermethylation of promoter region of tumor suppressive miRNAs may lead to tumor formation and cancer progression [16]. Moreover, the overexpression of oncogenic miRNAs may reduce the expression of cytokines and growth factors, which have a known role in cell adhesion and cell proliferation. Thus, oncomiRs contribute to the process of carcinogenesis [17].

In this study, we selected three candidate miRNAs, i.e., miR-223, miR-16, and miR-100, as biomarkers for GC. The expression of miR-223 was elevated in response to *Helicobacter pylori* infection [13]. Overexpression of miR-223 stimulated cell proliferation as well as the colony formation of *H.pylori*, which is closely related to GC development [18]. It was suspected that miR-223 targeted at Stathmin1, an oncoprotein upregulated in GC cell line [19]. MiR-223 may also target at FBXW7/hCdc4 at post-transcriptional level, which regulated proliferation, apoptosis, and invasion in GC [20]. In addition, the expression of tumor suppressor EPB41L3 was also inhibited by miR-223 [21]. Upregulation of miR-16 stimulates GC by targeting at nuclear factor-kappa B (NF- κ B), the binding process was also enhanced by nicotine consumption [22]. MiR-16 was also manifested to be involved in multidrug resistance by increasing Bcl-2 protein level and the

luciferase activity of BCL2 in GC cells [23]. The overexpression of Bcl-2 also resulted in impaired cell apoptosis. The pathway could be modulated by dihydroartemisinin (DHA) [24]. Besides, reduced expression of miR-100 was closely related to metastasis by targeting Argonaute 2 expression and modulating EMT and cancer cells stemness in prostate cancer cells [25]. MiR-100 was also found to play a critical role in cellular proliferation by targeting RAP1B in colorectal cancer cells [26]. It was also indicated by previous study that expression of miR-100 could inhibit carcinogenesis in GC cells [27]. Interestingly, in the present study, we manifested that the elevated expression of miR-100 in blood serum was associated with GC progression, which is in contrast to previous findings from cancer cell lines. The contradictory might imply a dual role of miR-100 in carcinogenesis.

A critical step of the present study is the normalization of RNA samples and the selection of internal control, since no consensus internal control has been identified. In this study, we selected U6 snRNA as internal control, which plays a central role in RNA splicing. The transcription and maturation of U6 snRNA are essential for cellular homeostasis [28]. It also exhibits a consistent expression across all patients and controls. Meanwhile, a limitation of

this study is that the numbers of patients and controls enrolled in this study are relatively small. The scope of the present study was also limited in terms of generalizability. All eligible subjects were from Asian population; the result may not be applicable to Caucasian and African ethnicities.

In summary, we confirmed the diagnostic value of serum miR-223, miR-16, and miR-100 in GC. All of them can be applied as novel, noninvasive biomarkers for GC. Significantly elevated expression of the three miRNAs was also observed in advanced GC patients, which suggested their availability in cancer staging. It was also suspected that the target miRNAs might play an important part in GC development. Future study on the cutoff value for serum miR-223, miR-16, and miR-100 in cancer diagnosis and staging was recommended.

Acknowledgments This work was supported by a grant from National Natural Science Foundation of China (Grant No. 81101504).

Conflict of interest The authors declare that they have no conflict of interest.

References

- Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*. 2014;64(1):9–29.
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61(2):69–90.
- D'Angelo G, Di Rienzo T, Ojetti V. Microarray analysis in gastric cancer: a review. *World J Gastroenterol*. 2014;20(34):11972–6.
- Ebert MP, Rocken C. Molecular screening of gastric cancer by proteome analysis. *Eur J Gastroenterol Hepatol*. 2006;18(8):847–53.
- Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol*. 2014;15(8):509–24.
- Rasheed SAK, Teo CR, Beillard EJ, Voorhoeve PM, Casey PJ. microRNA-182 and microRNA-200a control G-protein subunit α -13 (GNA13) expression and cell invasion synergistically in prostate cancer cells. *J Biol Chem*. 2013;288(11):7986–95.
- Valeri N, Braconi C, Gasparini P, Murgia C, Lampis A, Paulus-Hock V, et al. microRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. *Cancer Cell*. 2014;25(4):469–83.
- Yang Y, Peng W, Tang T, Xia L, Wang XD, Duan BF, et al. MicroRNAs as promising biomarkers for tumor-staging: evaluation of miR21 miR155 miR29a and miR92a in predicting tumor stage of rectal cancer. *Asian Pac J Cancer Prev*. 2014;15(13):5175.
- Wittekind C, Asamura H, Sobin LH. *TNM Atlas*. London: John Wiley & Sons; 2014.
- Cui L, Zhang X, Ye G, Zheng T, Song H, Deng H, et al. Gastric juice microRNAs as potential biomarkers for the screening of gastric cancer. *Cancer*. 2013;119(9):1618–26.
- Li C, Li JF, Cai Q, Qiu QQ, Yan M, Liu BY, et al. MiRNA-199a-3p in plasma as a potential diagnostic biomarker for gastric cancer. *Ann Surg Oncol*. 2013;20(3):397–405.
- Cai H, Yuan Y, Hao Y-F, Guo T-K, Wei X, Zhang Y-M. Plasma microRNAs serve as novel potential biomarkers for early detection of gastric cancer. *Med Oncol*. 2013;30(1):1–7.
- Nishizawa T, Suzuki H. The role of microRNA in gastric malignancy. *Int J Mol Sci*. 2013;14(5):9487–96.
- Mishra S, Lin CL, Huang TH, Bouamar H, Sun LZ. microRNA-21 inhibits p57Kip2 expression in prostate cancer. *Mol Cancer*. 2014;13(1):212.
- Ma J, Yao Y, Wang P, Liu Y, Zhao L, Li Z et al. MiR-152 functions as a tumor suppressor in glioblastoma stem cells by targeting Kruppel-like factor 4. *Cancer Lett*. 2014;355(1):85–95.
- Leung CM, Tsai KW, Pan HW. DNA methylation in aggressive gastric carcinoma. 2013;223–242. doi:10.5772/52135.
- Crone SG, Jacobsen A, Federspiel B, Bardram L, Krogh A, Lund AH, et al. microRNA-146a inhibits G protein-coupled receptor-mediated activation of NF-kappaB by targeting CARD10 and COPS8 in gastric cancer. *Mol Cancer*. 2012;11:71.
- Ma L, Chen Y, Zhang B, Liu G. Increased microRNA-223 in *Helicobacter pylori*-associated gastric cancer contributed to cancer cell proliferation and migration. *Biosci Biotechnol Biochem*. 2014;78(4):602–8.
- Kang W, Tong JH, Chan AW, Lung RW, Chau SL, Wong QW, et al. Stathmin1 plays oncogenic role and is a target of microRNA-223 in gastric cancer. *PLoS One*. 2012;7(3):e33919.
- Li J, Guo Y, Liang X, Sun M, Wang G, De W, et al. microRNA-223 functions as an oncogene in human gastric cancer by targeting FBXW7/hCdc4. *J Cancer Res Clin Oncol*. 2012;138(5):763–74.
- Li X, Zhang Y, Zhang H, Liu X, Gong T, Li M, et al. MiRNA-223 promotes gastric cancer invasion and metastasis by targeting tumor suppressor EPB41L3. *Mol Cancer Res*. 2011;9(7):824–33.
- Shin VY, Jin H, Ng EK, Cheng AS, Chong WW, Wong CY, et al. NF-kappaB targets miR-16 and miR-21 in gastric cancer: involvement of prostaglandin E receptors. *Carcinogenesis*. 2011;32(2):240–5.
- Xia L, Zhang D, Du R, Pan Y, Zhao L, Sun S, et al. MiR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. *Int J Cancer J Int Du Cancer*. 2008;123(2):372–9.
- Sun H, Meng X, Han J, Zhang Z, Wang B, Bai X, et al. Anti-cancer activity of DHA on gastric cancer—an in vitro and in vivo study. *Tumour Biol*. 2013;34(6):3791–800.
- Wang M, Ren D, Guo W, Wang Z, Huang S, Du H, et al. Loss of miR-100 enhances migration, invasion, epithelial-mesenchymal transition and stemness properties in prostate cancer cells through targeting Argonaute 2. *Int J Oncol*. 2014;45(1):362–72.
- Peng H, Luo J, Hao H, Hu J, Xie SK, Ren D, et al. microRNA-100 regulates SW620 colorectal cancer cell proliferation and invasion by targeting RAP1B. *Oncol Rep*. 2014;31(5):2055–62.
- Shi DB, Xing AY, Gao C, Gao P. Expression of microRNA-100 in human gastric cancer. *Zhonghua Bing Li Xue Za Zhi Chin J Pathol*. 2013;42(1):15–9.
- Mroczek S, Dziembowski A. U6 RNA biogenesis and disease association. *Wiley Interdiscip Rev RNA*. 2013;4(5):581–92.