EUROPEAN JOURNAL OF CANCER 47 (2011) 784–791

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A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis

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ARTICLE INFO

Article history:
Received 19 August 2010
Received in revised form 20 October 2010
Accepted 27 October 2010
Available online 26 November 2010

Keywords:
Gastric cancer
Serum microRNA
Biomarker
Diagnosis
Solexa
qRT-PCR

ABSTRACT

Background: Prognosis of patients with gastric cancer (GC) is generally poor due to the lack of non-invasive tools for GC detection. The purpose of present study was to identify a serum microRNA (miRNA) expression profile that can serve as a novel diagnostic biomarker for GC detection and to assess its clinical applications in monitoring disease progression.
Methods: Serum samples were taken from 164 GC patients and 127 age- and gender-matched tumour-free controls. An initial screening of miRNA expression by Solexa sequencing was performed using serum samples pooled from 20 patients and 20 controls, respectively. Differential expression was validated using hydrolysis probe-based stem-loop quantitative reverse transcription polymerase chain reaction (qRT-PCR) in individuals samples, the samples were arranged in two phases.
Results: The Solexa sequencing results demonstrated that 19 serum miRNAs were markedly upregulated in the GC patients compared to the controls. The qRT-PCR analysis further identified a profile of five serum miRNAs (miR-1, miR-20a, miR-27a, miR-34 and miR-423-5p) as a biomarker for GC detection. The analysis results showed that the expression level of five serum miRNAs was correlated to tumour stage. The areas under the receiver operating characteristic (ROC) curve of this five-serum miRNA signature were 0.879 (95% confidence interval (CI) 0.822–0.936) and 0.831 (95% CI 0.767–0.898) for the two sets of serum samples, respectively, markedly higher than those of the biomarkers carcinoembryonic antigen (CEA) (0.503) and carbohydrate antigen 19-9 (CA19-9) (0.600).
Conclusions: We identified five-miRNA signature for GC diagnosis by genome-wide serum miRNA expression profiling. Expression levels of this serum miRNA-based biomarker also indicate tumour progression stages.

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doi:10.1016/j.ejca.2010.10.025

1. Introduction

Gastric cancer (GC) is the fourth most common human malignant disease and the second most frequent cause of cancer-related death worldwide.[1] Currently, surgical resection is the most effective treatment and prolongs the survival of patients with early gastric cancer; however, for advanced gastric cancer, it frequently recurs as nodal and haematogenous metastases and peritoneal dissemination, and the prognosis for individuals with advanced disease remains poor.[2] Therefore, improvement in diagnosis and treatment could increase the long-term survival of patients with resectable-stage GCs. Unfortunately, most early-stage GCs are asymptomatic and difficult to detect.[3]

Chromoendoscopy and random biopsies can diagnose some early-stage GC patients, but the invasiveness of these diagnostic procedures and potential sampling errors with random endoscopic biopsy limit their efficacy. Meanwhile, for the diagnosis of GC, few highly sensitive or highly specific tumour markers are available, and current diagnostic tools for GC, including the serological markers carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA), have low specificity and sensitivity. Several groups have undertaken high-throughput analyses of GC expression profiles by DNA microarrays and microdissection.[4] Good markers for diagnosis and progression of GC, however, have not yet been identified. These facts partially account for the extremely poor prognosis and high mortality rate of GC. Therefore, novel biomarkers and diagnostic methods for early detection of GC are urgently needed to reduce the disease morbidity and mortality.

MicroRNAs (miRNAs) are a subset of non-coding RNA molecules (21–23 nucleotides in length) that are believed to regulate gene expression.[5] Altered expression of miRNAs has been associated with several diseases, particularly cancer.[6] Using tissue miRNA expression profiles as prognostic biomarkers in cancer has been demonstrated by several studies.[7–10] Because serum and plasma are relatively easy to access, circulating biomarkers are one of the most promising means of diagnosis. Previous studies from our group and others have shown that human serum contains miRNAs, and that the expression pattern of these serum miRNAs can potentially be used to identify various types of cancer, including prostate cancer, large B-cell lymphoma, ovarian cancer, liver cancer and non-small cell lung cancer.[11–16] Therefore, identifying a unique serum miRNA expression profile in GC can potentially assist tumour diagnosis and cancer treatment.

To ascertain whether a serum miRNA expression signature can distinguish GC from cancer-free controls, we conducted genome-wide serum miRNA expression profiling by Solexa sequencing followed by a stem-loop quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay using hydrolysis probes to systematically and extensively evaluate serum miRNA expression. By statistical analysis, we obtained a profile of five serum miRNAs, which can serve as a biomarker for GC detection. The correlation between serum miRNAs and GC progression was further assessed.

2. Materials and methods

2.1. Study design, patients and control subjects

A multi-stage, case–control study was designed to identify a serum miRNA profile as a surrogate marker for GC (Fig. 1). All samples were collected from consenting individuals according to the protocols approved by the ethics committee of each participating institution. In total, 164 patients with primary GCs and 127 control subjects were enrolled in our study. In the initial biomarker screening stage, GC serum samples pooled from 20 non-metastatic GC and 20 metastatic GC patients and control donors pooled from 20 normal samples who were treated at Jinling Hospital of Jiangsu Province were subjected to Solexa sequencing (miR Base 12.0, 692 miRNAs in total) to identify the miRNAs that were significantly differentially expressed. Subsequently, sequential validation was performed using a hydrolysis probe-based qRT-PCR assay to refine the number of serum miRNAs as a GC signature. In the biomarker selection stage, 22 GC serum samples and 22 controls (Jinling Hospital and Tianjin Medical University Cancer Institute and Hospital) formed a training set, whereas an additional 142 GC serum samples and 105 normal subjects (Jinling Hospital and Tianjin Medical University Cancer Institute and Hospital) formed a validation set. All patients were diagnosed with GCs between 2008 and 2009, and blood samples were collected prior to any therapeutic procedures, such as surgery, chemotherapy and radiotherapy. Histopathology of the patients was confirmed by surgical resection of the tumours and tumour stage was determined based on the surgery findings. For patients who were unsuitable for surgical treatment, histopathology and tumour stage were confirmed by histobiopsy and imaging technology. Tumours were staged according to the tumour-node-metastasis staging system of the International Union against Cancer.[17] Histological grade was assessed according to the World Health Organization (WHO) criteria.[18]

The demographics and clinical features of the patients are listed in Table 1. Control subjects were recruited from a large pool of individuals seeking a routine health check-up at the Healthy Physical Examination Centre of Jinling Hospital. People who showed no evidence of disease were selected as tu-

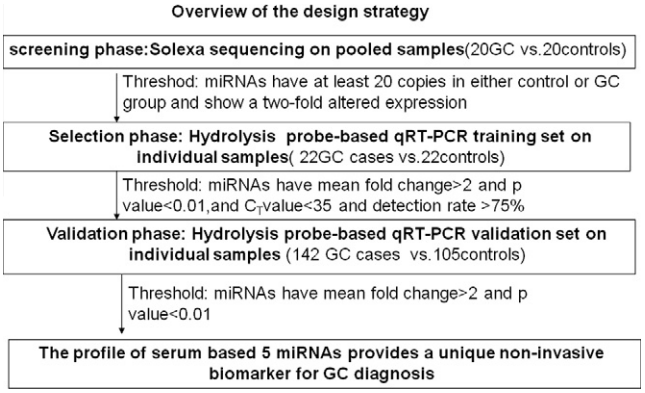

Fig. 1 – A flow chart of the experimental design.

Table 1 – Demographic and clinical features of the gastric cancer (GC) patients and healthy controls in the training set and the validation set.

Variables	Cases (n = 164)		Controls (n = 127)		P-value
	No.	%	No.	%	
Average age (years)	60.2 ± 10.2		60.0 ± 10.4		$P = 0.858$[a] $P = 0.232$[b]
Sex	Male	138	84	99	60
	Female	26	16	28	40
					$P = 0.346$[b]
Smoking status	Ever/current	41	25	23	18
	Never	117	71	100	79
	Unknown	6	4	4	3
					$P = 0.214$[b]
Alcohol consumption	Ever/current	22	13	26	20
	Never	137	84	99	78
	Unknown	5	3	2	2
TNM stage	I	29	18		
	II	56	34		
	III	48	29		
	IV	23	14		
	Unknown	8	5		
Family history of GC	Yes	17	12		
	No	147	88		
					$P = 0.791$[c]
Significant cardiac dysfunction	Yes	20	12	13	10
	No	144	88	111	90
					$P = 0.088$[c]
Neurological disease or diabetes	Yes	1	1	5	4
	No	163	99	119	96
					$P = 0.045$[c]
Carcinoembryonic antigen (CEA)	Positive	11	7	2	2
	Negative	153	93	125	98
					$P = 0.006$[c]
Carbohydrate antigen 19-9 (CA19-9)	Positive	15	9	2	2
	Negative	149	91	125	98

[a] Student's-t test.
[b] Two-sided χ^2 test.
[c] Fisher's exact test.

mour-free control subjects. Controls were matched to the patients based on age, gender and ethnicity.

2.2. *Serum preparation, RNA isolation and qRT-PCR assay*

Venous blood samples (~5 ml) were collected from each patient and healthy volunteer. In GC patients, blood samples were collected before any therapeutic procedure and before surgical resections of the primary tumours. Hydrolysis probes (Applied Biosystems, Foster City, CA, USA) were used for the qRT-PCR analysis, according to the manufacturer's instructions.[10,11] A detailed description of the experimental protocols is deposited in the Supplementary data.

2.3. *Solexa sequencing and in silico analysis*

The sequencing procedure was conducted as previously described.[11] Briefly, the serum samples from 20 GC patients and 20 healthy donors were pooled, respectively, and the total RNA was extracted. Finally, the reads were processed for in silico analysis as previously described.[19] A detailed description of the experimental protocols is deposited in the Supplementary data.

2.4. *Serum CA19-9 and CEA determination*

The serum values of CA19-9 and CEA were measured using commercial kits (CA19-9 RIA, Abbott AxSYM System, Chicago, IL; CEA fluoroimmunometric assay, Beckman Coulter Inc., Fullerton, CA). The upper limits of normal values for CA19-9 and CEA were 37 U/ml and 5 U/ml, respectively.

2.5. *Statistical analysis*

Risk score analysis was performed to evaluate the association between GC and miRNA expression levels. The risk score of

each miRNA in the training set, denoted as s, was set as 1 if the expression level was greater than the upper 95% reference interval for the corresponding miRNA level in controls, whereas the score was set as 0 if otherwise. When taking into account the correlation of each miRNA with GC risk, each patient was assigned a risk score function (RSF) according to a linear combination of the expression level of the miRNA. The RSF for sample i using the information from the five miRNAs was as follows: $RSF_i = \sum_{j-1}^{5} W_j \cdot s_{ij}$.

In the equation above, s_{ij} is the risk score for miRNA j on sample i, and W_j is the weight of the risk score of miRNA j. To determine the W_s, five univariate logistic regression models were fitted with the disease status with each of the risk scores. Parameters of the univariate logistic regression analysis for miR-1, miR-20a, miR-27a, miR-34a, and miR-423-5p in sample set I are 2.8980, 2.7505, 2.8980, 2.3389, and 1.1309, respectively. Parameters of the univariate logistic regression analysis for miR-1, miR-20a, miR-27a, miR-34a, and miR-423-5p in sample set II are 2.0728, 1.9735, 2.8655, 2.2618, and 1.9185, respectively. The regression coefficient of each risk score was used as the weight to indicate the contribution of each miRNA to the RSF. The frequency table and receiver operating characteristic (ROC) curves were then used to evaluate the diagnostic effects of the profiling and to find an appropriate cutoff point. Validation of the procedure and the cutoffs were performed in the test sample.

All the statistical analyses were performed using the Statistical Analysis System software (v.9.1.3; SAS Institute, Cary, NC). Data are presented as the median ± SD. Non-parametric Mann-Whitney U test was used to compare the difference in serum miRNA concentration between the cancer group and healthy group. $P < 0.01$ was considered statistically significant.

3. Results

3.1. Description and clinical features of the patients

All 164 patients enrolled in the present study were clinically and pathologically diagnosed with GC. As shown in Table 1, there was no significant difference in the distribution of smoking ($P = 0.346$), alcohol consumption ($P = 0.214$), age ($P = 0.858$) and gender ($P = 0.232$) between the cancer patients and the normal subjects. Among the 164 patients, 29 (18%), 56 (34%), 48 (29%) and 23 (14%) were classified as stages I, II, III and IV, respectively. Elevated levels of CEA (>5 U/ml) and CA19-9 (>37 U/ml) were found in 11 (7%) and 15 (9%) patients, respectively.

3.2. Genome-wide expression profiling of serum miRNAs in GC patients and tumour-free controls

Solexa sequencing was used to identify the miRNAs with significantly altered expression. The Solexa data showed that miRNAs were the major components of small RNAs (<30 bp) in serum (Supplemental data Table 1 and Supplemental Fig. 1). Among the 692 serum miRNAs detected by Solexa sequencing, 332, 204 and 179 miRNAs were found in healthy controls, non-metastatic patients and metastatic patients, respectively (Supplemental Table 2). The expression of a miRNA was considered 'significantly altered' only if at least 20 copies were detected by Solexa sequencing, together with a larger than two-fold change in its expression level between the patients and control groups. Based on these criteria, 19 miRNAs were found differentially expressed in GC and were further analysed by qRT-PCR (Supplemental Table 3). Another two miRNAs, miR-20a and miR-34a, were also tested by qRT-PCR because they had been shown to be dysregulated in malignant GC tissues.[20–22]

3.3. Evaluation of miRNA expression by real-time qRT-PCR analysis

The standard curve of synthetic single-strand miR-16 was linear on a semi-logarithmic plot in a range from 10 to 10^7 fmol/L (Supplemental Fig. 2A). The pattern and working range of these standard curves performed by the five mature miRNAs are similar to that of miR-16. The concentration ranges of all five miRNAs in individual serum samples were within the working ranges of their corresponding standard curves. Based on this, miR-16 standard curve was able to serve as a tool in calculating the concentration of all five miRNAs and in rounding out the variation caused by individual performer and machines. Employing miR-16 standard curve can obtain the similar results with those calculated by using individual standard curve. Considering the potential application of this biomarker in clinic, we used a common miR-16 standard curve in order to simplify the process of detecting five miRNAs. The limit of detection and dynamic range for each miRNA were listed in Supplemental data Table 4.

The C_T values among the replicate assays were very similar ($R = 0.993$), indicating that the RNA extraction method was reproducible (Supplemental Fig. 2B). Analytical reproducibility of the qRT-PCR assay was also accurate ($R = 0.990$) (Supplemental Fig. 2C). Taken together, these results suggest that circulating miRNAs can be efficiently extracted from serum and quantitatively analysed by qRT-PCR.

We next used qRT-PCR assay to confirm the expression of 21 candidate miRNAs which were selected from the previous step. We identified five miRNAs that showed differential expression and these miRNAs were chosen for the next validation. In the training set, 22 GC and 22 controls were examined by qRT-PCR. This phase generated a list of five miRNAs that had a significant differential expression pattern (Table 2). These miRNAs were miR-1, miR-20a, miR-27a, miR-34a and mR-423-5p. Compared to their levels in the control samples, these five miRNAs in the GC samples were increased to 3.56, 2.37, 3.03, 2.19 and 2.00 folds, respectively (Table 2).

In the validation set, the concentration of these five selected miRNAs was examined by qRT-PCR in a larger cohort comprised of 142 GC patients and 105 matched controls. The miRNA expression pattern alterations in the validation set were consistent with those of the training set. The levels of the five miRNAs were significantly higher in the GC cases compared to the control subjects. When compared to their concentrations in normal controls, the fold changes were 2.13, 2.12, 2.04, 2.02 and 2.20, respectively. The differential expression of the five miRNAs in the 164 GC samples compared to the 127 controls is shown in Fig. 2.

Table 2 – Differentially expressed serum microRNAs (miRNAs) in GC cases compared to the controls in both the training set and the validation set. The expression levels are presented as median ± SD (fmol/L).

miRNA	Controls (n = 22)	Training set GC (n = 22)	Fold change	P-value	Controls (n = 105)	Validation set GC (n = 142)	Fold change	P-value
miR-1	38.30 ± 68.80	248.01 ± 134.77	3.56	5.27×10^{-6}	97.59 ± 123.20	191.78 ± 214.19	2.13	8.86×10^{-17}
miR-20a	83.92 ± 50.58	183.23 ± 115.96	2.37	2.94×10^{-5}	94.16 ± 140.33	210.81 ± 176.03	2.12	9.45×10^{-18}
miR-27a	97.66 ± 75.83	181.73 ± 229.43	3.03	1.95×10^{-3}	78.17 ± 44.71	156.71 ± 112.49	2.04	5.14×10^{-14}
miR-34a	147.22 ± 109.09	318.58 ± 237.56	2.19	7.72×10^{-3}	130.67 ± 93.65	285.50 ± 164.39	2.02	1.66×10^{-16}
miR-423-5p	36.78 ± 119.51	195.93 ± 71.88	2.00	5.13×10^{-4}	181.84 ± 961.60	262.88 ± 2896.67	2.20	3.54×10^{-3}

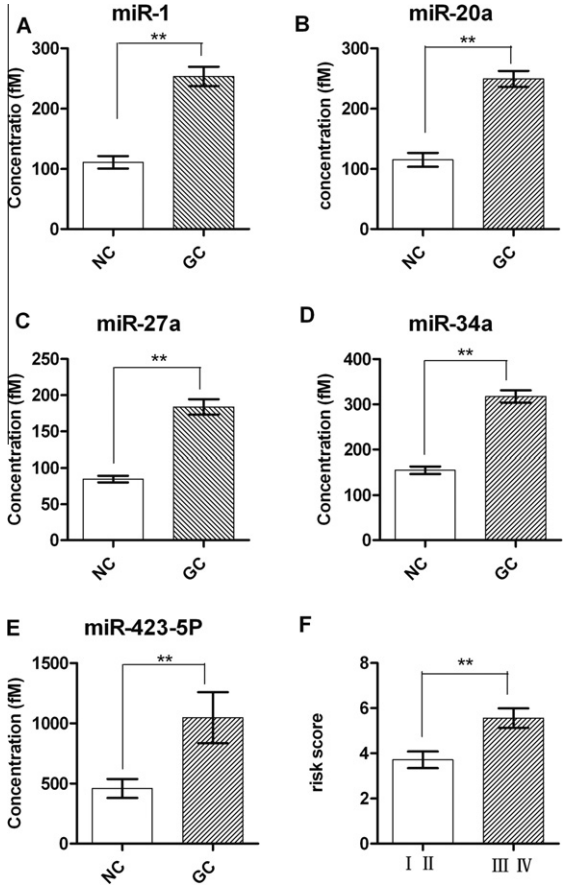

Fig. 2 – (A–E) Detection of gastric cancer (GC) using the five-serum microRNA (miRNA) profile as a biomarker. Serum levels of the five miRNAs were measured in 164 GC cases and 127 healthy control subjects (in both the training set and the validation set) using a hydrolysis probe-based qRT-PCR assay. C_T values were converted to an absolute value based on the standard curve. (F) Risk score values in GC patients between stages I, II and III, IV. **P < 0.01.

3.4. *Separation of GC cases and control donors by risk score analysis*

Risk score analysis based on the five-miRNA expression profile was used to distinguish serum samples of the GC cases from those of the control donors. First, the risk score of each serum sample was calculated, and based on their scores, serum samples were then divided into a high-risk group, representing the possible GC cases, and a low-risk group, representing the control subjects. At the optimal cutoff value of 2.25 with the value of sensitivity + specificity considered to be maximal, the positive predictive value and negative predictive value of the five-serum miRNA signature in set I samples (82 GCs and 63 controls) was 0.88 and 0.77, respectively. Similarly, when the same cutoff point was used to analyse the rest 146 samples in set II (82 GCs and 64 controls), the positive predictive value and negative predictive value of the five-serum miRNA signature was 0.81 and 0.75, respectively (Table 3).

Receiver operating characteristic (ROC) curves were then constructed to estimate the sensitivity and specificity of the five-serum miRNA signature. The areas under the curve (AUC) were 0.879 (95% CI 0.822–0.936) and 0.831 (95% CI 0.767–0.898) for the serum samples in sets I and II, respectively (Fig. 3A and B). Using the same serum samples, we compared the AUC of five miRNAs with that of CEA and CA19-9, two currently available blood-based biomarkers for GC detection.[23] The AUC values of the five-serum miRNA signature were markedly higher than those of CEA (0.503 95% CI 0.411–0.595) and CA19-9 (0.600 95% CI 0.507–0.684) (Fig. 3C and D). The results indicate that the five-serum miRNA signature is a more accurate biomarker than CEA and CA19-9 for GC diagnosis.

3.5. *Expression levels of the selected serum miRNAs in GC at different stages*

Furthermore, except the miR-423-5p, the expression levels of another 11 miRNAs (miR-25 miR-92a, miR-320a, miR-324-3p, miR-339-3p, miR-451, miR-629, miR-652, miR-320b, miR-320c, miR-1246) that showed a more than two-fold change in expression between metastatic and non-metastatic GC in the Solexa analysis (See Supplemental data Table 3) were also examined by qRT-PCR in the individual samples of GC patients. None of the 11 miRNAs had significantly altered expression between non-metastatic and metastatic cancer as assessed by the qRT-PCR assay.

Table 3 – Risk score analysis of GC cases and control donors.

	Score	1–2.55	2.55–10.89	PPV[a]	NPV[b]
Set I	Control	54	9	0.88	0.77
	GC	16	66		
Set II	Control	49	15	0.81	0.75
	GC	17	65		

[a] Positive predictive value.
[b] Negative predictive value.

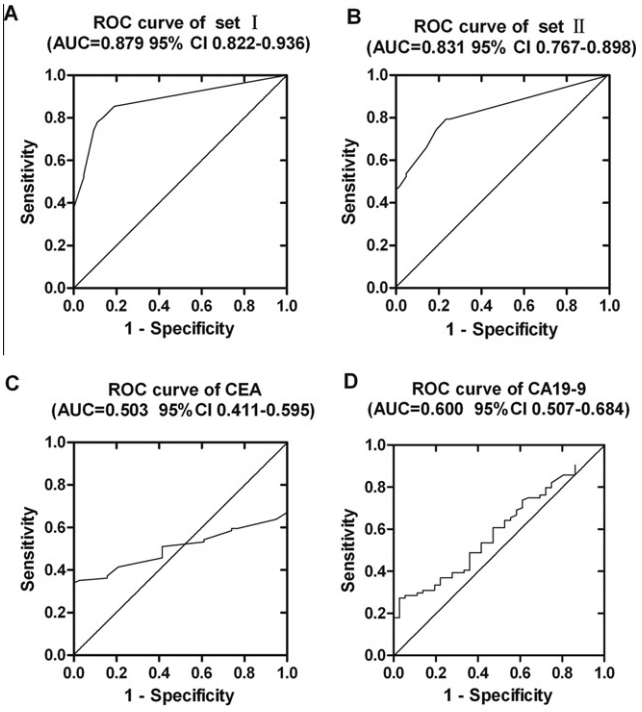

Fig. 3 – (A and B) Receiver operating characteristic (ROC) curves for the five-serum miRNA profile to distinguish GC serum samples from control serum samples in the training set (A) and the validation set (B). (C and D) ROC curves for carcinoembryonic antigen (CEA) (C) and carbohydrate antigen 19-9 (CA19-9) (D) to distinguish GC serum samples from control serum samples.

Expression levels of the five serum miRNAs in the GC patients at different stages were analysed by risk score. As shown in Fig. 2F, the expression levels of five serum miRNAs were significantly different in the GC patients at different tumour stages ($P < 0.01$). In particular, risk score based on the five-serum miRNA signature was progressively higher from earlier stage (I and II) to later stage (III and IV) GC cases.

3.6. *Unsupervised cluster analysis*

The unsupervised cluster method was also used to analyse the differential expression of miRNAs between the GC and control serum samples. The dendrogram generated by the cluster analysis showed a clear separation of the GC samples from the control samples based on the five-serum miRNA profile (Supplemental Fig. 3). In the training set, only one out of 22 GC samples and five out of 22 control samples were incorrectly classified (Supplemental Fig. 3A). In the validation set, 142 GC cases and 105 controls were classified into two main categories and 16 GC cases and 25 control samples were misclassified (Supplemental Fig. 3B).

4. Discussion

Dysregulation of miRNA expression in cancer has been widely reported previously.[24] However, the studies mainly focused on miRNAs expressed in tumour tissues and cells, Katada and colleagues[25] demonstrated that the survival rate was significantly lower in undifferentiated GC patients with high expression levels of miR-20b or miR-150 and that miR-27a expression was correlated with lymph node metastasis. Chan and colleagues[26] found that miR-21 was overexpressed in 92% of the GC patients. Although tissue miRNAs can provide an accurate diagnosis for various types of cancer including GC, collecting tissue samples is an invasive procedure and depends on surgical sections after the initial clinical classification. Recently, serum and plasma miRNAs have emerged as potential new blood-based markers for detecting cancers and other diseases.[11–14,16,27] In this study, we systematically determined the expression levels of serum miRNAs in patients with GC and identified five serum miRNAs (miR-1, miR-20a, miR-27a, miR-34a, and miR-423-5p) significantly up-regulated in the GC patients compared to the control subjects. To our best knowledge, this is the first study identifying a serum miRNA-based GC signature by genome-wide serum miRNA expression profiling.

The early studies on searching for serum miRNA-based cancer biomarkers generally focused on individual cancer-specific miRNAs.[13] However, the specificity of biomarkers based on a single tumour-specific miRNA is generally poor. For example, elevated plasma or serum level of miR-122, which is liver-specific, could result from not only liver cancer but also HBV infection, cirrhosis and general liver injury.[15] The diverse, complex molecular events involved in the initiation and development of a severe malignancy require the functional alteration of not only tumour cell growth-related genes and/or tissue-specific genes, but also the genes associated with the body's immune responses. Accordingly, there should be multiple miRNAs targeting those genes involved in tumourigenesis. By Solexa sequencing, we screened the whole miRNA profile in both GC and control serum samples and identified five GC-associated miRNAs, including miR-1, miR-20a, miR-27a, miR-34 and miR-423-5p. Functionally, these miRNAs can be classified into three groups: (i) immune response-related (miR-20a and miR-423-5p), (ii) tissue-specific (miR-1) and (iii) tumour cell growth/cycle-related (miR-27a and miR-34). Our results clearly demonstrate that a combination of multiple serum miRNAs is a more comprehensive indicator for tumour detection than the conventional single protein-based or carbohydrate molecule-based biomarkers. The sensitivity and specificity of GC detection by this five-miRNA biomarker are 80% and 81%, respectively, significantly higher than those of any single-factor index, such as CA19-9 and CEA. For the same serum samples, the specificity of GC detection by CA19-9 and CEA is 17% and 18%, respectively. These results demonstrate that the expression profile of the five serum miRNAs can serve as an accurate biomarker for GC diagnosis. Previous study by Tsujiura and colleagues[28] showed that serum miR-106 and miR-let7a can be used to distinguish GC patients from tumour-free controls. However, these miRNAs were not present in our five-serum profile.

Although miRNAs in serum or other body fluids are quite stable and readily detected by various assays[11], the source of circulating miRNAs remains unclear. Our previous study has shown that serum miRNAs are derived from not only circulating blood cells, but also from other tissues affected by the disease.[11] In circulation, tumour cell-derived miRNAs

might be stored in microvesicles secreted by various cell types.[11,12,29] The finding of tumour cell/tissue source of serum miRNAs supports our observation that the serum miRNA profile serves as a tumour fingerprint.

Functional study of miRNAs in tumour tissue is also helpful for evaluating serum miRNAs as indicators for various types of cancer. Among the five serum miRNAs identified in the GC patients, many are involved in general tumourigenesis. For instance, miR-27a has been shown to be up-regulated in the tissue samples of digestive system neoplasms including gastric, colon, etc.[30,31] Moreover, increased expression of miR-20a and miR-34a has been observed in colon cancer, hepatocellular carcinoma (HCC), pancreatic cancer and other cancers.[21,22,32–34] Future studies are required to identify the target genes of these five serum miRNAs and the mechanism that regulates the biogenesis of these miRNAs.

We further evaluated the potential of using these biomarkers as diagnostic markers for early GC. This biomarker showed differentially-expressed miRNAs in stages I and II GC serum samples compared to control serum samples (See Supplemental data results, Supplemental Table 5). The result implicated a potential application of this biomarker in diagnosing GC at an early stage. Since patients with early stage GC can undergo complete resection of tumours, our data suggest that the five-serum miRNA as the biomarker for defining the early events of GC is an effective way to change the outcomes and improve the prognosis.

From a clinical point of view, the serum miRNA signature for GC may be also useful for indicating the progression stage of GC. Although no significant difference was observed when the GC cases were stratified by demographic and clinical factors including gender, age, smoking history and alcohol consumption of the patients, we identified a clear correlation between serum miRNA expression and GC tumour stage. These results indicate that the high risk score or high expression level of these serum miRNAs in GC patients is associated with advanced clinical stages of this disease. Given that the TNM system is currently the most important tool used by clinical oncologists to estimate tumour burden, predict prognosis and survival and determine the best combination of treatment, prediction of GC at different TNM stages by this five-serum miRNA signature would have a significant value in treatment selection.

According to pathology diagnosis, the GC patients can be divided into adenocarcinoma, signet-ring cell carcinoma, mucinous carcinoma and special type of carcinoma in our study, and each number of them is 145, 3, 11 and 5, respectively. This data show a similar trend with the epidemiology statistics (adenocarcinoma generally possesses an overwhelming majority of the GC). Since most of GC cases in the present study are adenocarcinoma, it maybe not suitable for discriminating different types of GC. Therefore, it remains unknown whether the five miRNAs can discriminate different types of GC. To test this, more cases of the signet-ring cell carcinoma, mucinous carcinoma and other special type of carcinoma are required.

In sum, we identified a unique five-serum miRNA signature for GC detection. This work will serve as the basis for further studies about the clinical value of serum miRNAs in predicting therapeutic efficacy, maintaining surveillance and forecasting prognosis.

Role of the funding source

Test materials, equipment, testing, etc. are supported by the fund.

Conflict of interest statement

None declared.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (30890044, 30772484, 30971344 and 30570731), Key Science Project supported by the Government of TianJin (10JCZDJC18400).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.10.025.

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