Serum MicroRNA Expression Profile as a Biomarker in the Diagnosis and Prognosis of Pancreatic Cancer

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BACKGROUND: Detection of pancreatic cancer (PaC), particularly at early stages, remains a great challenge owing to lack of specific biomarkers. We sought to identify a PaC-specific serum microRNA (miRNA) expression profile and test its specificity and sensitivity as a biomarker in the diagnosis and prognosis of PaC.

METHODS: We obtained serum samples from 197 PaC cases and 158 age- and sex-matched cancer-free controls. We screened the differentially expressed serum miRNAs with Illumina sequencing by synthesis technology using pooled serum samples followed by RT-qPCR validation of a large number of samples arranged in multiple stages. We used risk score analysis to evaluate the diagnostic value of the serum miRNA profiling system. To assess the serum miRNA–based biomarker accuracy in predicting PaC, we performed additional double-blind testing in 77 PaC cases and 52 controls and diagnostic classification in 55 cases with clinically suspected PaC.

RESULTS: After the selection and validation process, 7 miRNAs displayed significantly different expression levels in PaC compared with controls. This serum 7-miRNA–based biomarker had high sensitivity and specificity for distinguishing various stages of PaC from cancer-free controls and also accurately discriminated PaC patients from chronic pancreatitis (CP) patients. Among the 7 miRNAs, miR-21 levels in serum were significantly associated with overall PaC survival. The diagnostic accuracy rate of the serum 7-miRNA profile was 83.6% in correctly classifying 55 cases with clinically suspected PaC.

CONCLUSIONS: These data demonstrate that the serum 7-miRNA–based biomarker can serve as a novel noninvasive approach for PaC diagnosis and prognosis.

Pancreatic cancer (PaC)7 is the fourth leading cause of cancer-related deaths in Western countries and has the poorest survival rate (<5%) among the common malignancies (1, 2). Although surgical resection shows promise as an effective treatment for PaC, a lack of effective tools for diagnosis in the earliest stages results in low 5-year survival rates, which drop rapidly from >50% in patients with stage I to <5% in patients with more advanced stages (3–5). Both imaging techniques and serological markers, such as carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA), have relatively poor diagnostic sensitivity and specificity (6, 7). In addition, it is still difficult to distinguish chronic pancreatitis (CP) patients, a high-risk population of PaC, from PaC patients (8–11). Therefore, to improve the prognosis of PaC, it is urgent to develop specific and noninvasive biomarkers for PaC diagnosis, especially for early-stage tumors.

A new class of small noncoding RNAs known as microRNAs (miRNAs) has been reported to act as endogenous regulators of protein-coding genes at the posttranscriptional level (12). Recent studies by our group and others have shown that human serum contains miRNAs and that the expression patterns of these serum miRNAs have the potential to identify various types of diseases, including prostate cancer, large B-cell lymphoma, ovarian cancer, and non–small cell lung cancer (13–20). These findings suggest that there may...
be a unique serum miRNA expression pattern in PaC that could serve as a new noninvasive biomarker for PaC diagnosis.

In the present study, using both Illumina sequencing by synthesis (SBS) technology and reverse-transcription quantitative PCR (RT-qPCR) assays to characterize the genome-wide miRNA expression profile in serum from PaC patients and controls, we sought to identify a panel of serum miRNAs that could serve as a novel biomarker for diagnosis of PaC.

Materials and Methods

STUDY DESIGN

The present study enrolled 197 patients who had been clinically classified as having PaC at the time of participation, between 2005 and 2009. A multiphase case-control study was designed to identify serum miRNAs as a surrogate marker for PaC (Fig. 1). In the initial biomarker screening stage, we subjected pooled serum samples from 25 PaC cases (Tianjin Medical University Cancer Institute and Hospital) and 25 matched controls (Jinling Hospital) to Illumina SBS technology to select miRNAs whose expression was altered in PaC cases compared to controls. Subsequently, we refined the number of serum miRNAs included as the PaC signature by a 2-phase experimental procedure using hydrolysis probe-based RT-qPCR assays. The training phase used serum samples from the 25 PaC cases and 25 controls that had been assessed by SBS technology, whereas the validation phase used serum samples from an additional 95 PaC cases (Changhai Hospital, Ruijin Hospital, and Beijing Cancer Hospital and Institute) and 81 controls (Jinling Hospital). The panel of serum miRNAs selected as the PaC biomarker was further examined in the second control group comprising 82 CP cases (Changhai Hospital and Ruijin Hospital). We analyzed an additional 77 PaC cases (First Affiliated Hospital of Nanjing Medical University and Jinling Hospital) and 52 controls (Jinling Hospital) in a blinded fashion (the investigators performing the molecular analysis on the blood samples were blinded to the patients’ clinical diagnosis). Finally, we analyzed an additional 55 cases suspected of having PaC (Jinling Hospital) based on preliminary diagnosis for the 7 miRNA levels by the same methods. All the protocols, including the diagnosis procedure and serum collection methods, were identical in these hospitals. Written informed consent was obtained from all patients and volunteers before the study, and the study was approved by the ethics committee of each participating institution.

PATIENTS AND CONTROL SUBJECTS

All blood samples were collected before any therapeutic procedures, including surgery, chemotherapy, and radiotherapy. PaC diagnosis was confirmed by histological examination or fine-needle aspiration cytology. Histological typing of the tumors was performed according to WHO criteria. All the enrolled PaC patients showed the tumor histotype of pancreatic ductal adenocarcinoma. Tumors were staged according to the sixth edition of the American Joint Committee on Cancer tumor-node-metastasis (TNM) system. For those patients who underwent surgery, definitive tumor stage was established on the basis of operative findings. For those patients unsuitable for surgical treatment, tumors were staged by means of ultrasonography, dynamic computed tomography, magnetic resonance imaging, angiography, and/or endoscopic ultrasonography. Among the 197 patients, 26 (13.2%), 48 (24.37%), 45 (22.84%), and 66 (33.5%) were classified as stage I, II, III, and IV carcinomas, respectively. Tumor stage could not be assessed in the remaining patients owing to lack of accurate information. Clinical and demographic characteristics of the PaC patients are summarized in Table 1.

In addition, as the second control, we analyzed 48 CP samples collected at Changhai Hospital and 34 at Ruijin Hospital between 2004 and 2009. CP was diagnosed by the presence of at least 1 of the following
criteria: (a) substantial changes in the pancreatogram shown by endoscopic retrograde cholangiopancreatography (ERCP); (b) pancreatic calcification shown by computed tomography or endoscopic ultrasonography (EUS); and (c) clinical presentation of abdominal pain with increases in serum pancreatic enzymes for at least 6 months. The absence of coexisting PaC was confirmed by EUS or ERCP. One of the CP patients underwent surgical treatment. Detailed clinical and demographic characteristics of the CP patients are also listed in Table 1.

We recruited the 158 healthy controls at the Healthy Physical Examination Center of Jinling Hospital. The health condition checkup included a detailed history; physical, radiological, and endoscopic examinations; blood tests; tumor marker tests (CA19-9, CEA); and abdominal sonography. Subjects with no evidence of pancreatic disease or other abnormalities were enrolled as cancer-free controls.

Serum samples from 55 patients suspected of having PaC were collected from Jinling Hospital. All of these cases showed a broad spectrum of PaC-like symptoms and were clinically classified by pathologic diagnosis 1–7 months after serum miRNA analysis. Results from the final pathologic diagnosis are listed in Supplementary Table 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol58/issue3.

### RNA ISOLATION AND RT-qPCR ASSAY

Venous blood samples (approximately 5 mL) were collected from each donor and placed in a serum separator

| Table 1. Demographic and clinical features of the PaC patients, CP patients, and cancer-free controls. |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Variable                | Control | PaC | CP | PaC vs control | PaC vs CP |
| n                       | 158     | 197 | 82 | 0.356        | 0.0004     |
| Mean age, years (SE)    | 58.39 (12.24) | 62.57 (13.23) | 44.75 (15.46) | 0.356^b     | 0.0004^b   |
| Sex                     | Male    | 103 | 62 | 0.860^c      | 0.0005^c   |
|                         | Female  | 73  | 94 | 82 (100)     | 82 (100)   |
| Diagnosis method        | Pathology | 197 | 82 | 0.356^b     | 0.0004^b   |
|                         | ERCP or EUS | 82 | 82 | 0.356^b     | 0.0004^b   |
| Stage                   | I       | 26  | 10 | 0.356^b     | 0.0004^b   |
|                         | II      | 48  | 24 | 0.356^b     | 0.0004^b   |
|                         | III     | 45  | 24 | 0.356^b     | 0.0004^b   |
|                         | IV      | 66  | 24 | 0.356^b     | 0.0004^b   |
| Smoking                 | Yes     | 24  | 10 | 0.356^b     | 0.0004^b   |
|                         | No      | 162 | 70 | 0.356^b     | 0.0004^b   |
|                         | Unknown | 12  | 9  | 0.356^b     | 0.0004^b   |
| Alcohol consumption     | Yes     | 20  | 10 | 0.356^b     | 0.0004^b   |
|                         | No      | 159 | 73 | 0.356^b     | 0.0004^b   |
|                         | Unknown | 18  | 7  | 0.356^b     | 0.0004^b   |
| Positive ratio          | CA19–9  | 2  | 2 | 0.356^b     | 0.0004^b   |
|                         | CEA     | 1  | 1 | 0.356^b     | 0.0004^b   |

^a Data are n (%), unless noted otherwise.
^b Student t-test.
^c Two-sided χ² test.
^d Fisher’s exact test.
tube. Samples were processed within 1 h. Serum was separated by centrifugation at 800g for 10 min at room temperature, followed by a 15-min high-speed centrifugation at 10 000g at room temperature to completely remove cell debris. The supernatant serum was recovered and stored at −80 °C until analysis.

We extracted total RNA from 100 μL serum by phenol/chloroform purification and centrifugation in isopropyl alcohol as described (13). For detailed methodology, see online Supplementary Methods.

We carried out TaqMan probe–based RT-qPCR assay with a commercial kit (Applied Biosystems) as described (21). For detailed methodology, see online Supplementary Methods.

SBS TECHNOLOGY
The sequencing procedure was conducted as described (13). For detailed methodology, see online Supplementary Methods.

STATISTICAL ANALYSIS
We performed risk score analysis to evaluate the association between PaC and the miRNA expression levels. The risk score of each miRNA in the training set, denoted as \( s_i \), was set as 1 if the expression level was higher than the upper 95% reference interval limit for the corresponding level in controls and 0 otherwise. Taking into account the association of each miRNA with PaC risk, each patient was assigned a risk score function (RSF) according to a linear combination of the miRNA expression levels. The RSF for sample \( i \) with the information from the 7 miRNAs was

\[
rsf_i = \sum_{j=1}^{7} W_j \cdot s_{ij}
\]

In the equation above, \( s_{ij} \) is the risk score for miRNA \( j \) on sample \( i \), and \( W_j \) is the weighting given to the risk score of miRNA \( j \). To determine the \( W_j \) for 7 univariate logistic regression models were fitted with the disease status with each of the risk scores. The regression coefficient of each risk score was used as the weight to indicate the contribution of each miRNA to the RSF. We then used frequency tables and ROC curves to evaluate the diagnostic effectiveness of the profile and find an appropriate cutoff. We then validated the procedure and cutoffs in the validation sample set.

The same procedure was repeated to construct an RSF to discriminate between CP and PaC patients, with the CP patients as controls. All statistical analysis was performed with the Statistical Analysis System software (v.9.1.3, SAS Institute). Data are presented as mean (SE). We used Student t-tests to compare the differences in serum miRNA concentrations between the cancer group and control group. \( P < 0.01 \) was considered statistically significant.

Results

PATIENT DESCRIPTION
All 197 patients enrolled in this study were clinically and pathologically diagnosed with pancreatic ductal adenocarcinoma. As shown in Table 1, there were no significant differences in the distribution of smoking \( (P = 0.967) \), alcohol consumption \( (P = 0.953) \), age \( (P = 0.356) \), and sex \( (P = 0.860) \) between the cancer patients and the cancer-free controls.

BIOMARKER SELECTION AND VALIDATION PHASE
In the initial screening phase by SBS technology, serum samples were pooled from 25 PaC patients or 25 healthy donors (approximately 1.0 mL each). As shown in online Supplementary Table 2, a genome-wide expression profiling of serum miRNAs obtained by SBS technology showed that serum from PaC patients had 44 upregulated miRNAs and 19 downregulated miRNAs compared with serum from controls.

We further examined these differentially expressed serum miRNAs by hydrolysis probe–based RT-qPCR in a training sample set including 25 PaC cases and 25 controls (the same samples used in SBS technology). The reliability and reproducibility of RT-qPCR assay for measuring serum miRNAs was tested first (online Supplementary Fig. 1, A–C). The results suggested that miRNA can be efficiently extracted and amplified from serum and the RT-qPCR results of serum miRNAs can be reliably compared across multiple samples. In this phase, we retained only those miRNAs with a mean fold-change ≥2 and a \( P \) value <0.01. As shown in online Supplementary Table 3, this phase generated a panel of 7 miRNAs (miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185, and miR-191) that were significantly altered in PaC samples.

To verify the accuracy and specificity of these 7 miRNAs as a PaC signature, we assessed their expression levels using another independent sample set consisting of 95 PaC cases and 81 cancer-free controls (validation set). As shown in Table 2, the trend of the expression alteration of the 7 miRNAs was generally concordant between the training set and the validation set, and all 7 miRNAs were upregulated ≥2-fold. As a result of this multiphase testing and analysis, we selected a profile of 7 serum miRNAs to be the potential signature for PaC. The differential expression of these 7 miRNAs between the PaC cases and controls is shown in online Supplementary Fig. 2, A–G.
To evaluate the diagnostic value of this 7-miRNA profiling system, we performed a risk score analysis on the data set and used it to predict PaC case and control status. First, we calculated the risk scores of samples in the training set. According to their risk scores and at a set cutoff, samples could be divided into a high-risk group, representing the predicted PaC cases, or a low-risk group, representing the predicted controls. At the optimal cutoff value (5.95), the diagnostic sensitivity and specificity of the serum 7-miRNA–based marker for PaC detection were 89% and 100%, respectively. None of the controls had a risk score \( \geq 5.95 \), whereas 22 of the 25 PaC samples exhibited a risk score \( \geq 5.95 \) (Table 3). Second, we used the same risk score formula with cutoff 5.95 to calculate the risk score of samples from the validation set. The diagnostic sensitivity and specificity obtained for the validation set were 94% and 93%, respectively, and of 95 PaC cases and 81 controls from the validation set, only 5 PaC cases and 6 controls were incorrectly predicted (Table 3). We constructed ROC curves for continuous predictors using these RSFs to estimate the diagnostic sensitivity and specificity of the serum 7-miRNA–based biomarker. The areas under the curve (AUCs) were 0.992 (0.027) and 0.985 (0.021) for the training set and validation set, respectively (Fig. 2, A and B).

We analyzed the differential expression of miRNAs between the PaC and control serum samples by performing unsupervised clustering that was blinded to the clinical annotations. The dendrogram generated by the cluster analysis showed a clear separation of the PaC samples from the control samples based on the serum 7-miRNA profile. In the training set, none of the 25 control samples and 2 of 25 PaC samples were misclassified (online Supplementary Fig. 3A). In the validation set, 95 PaC cases and 81 controls were also clearly separated into 2 main classes, with only 1 PaC case and 2 control samples misclassified (online Supplementary Fig. 3B).

To determine whether the serum 7-miRNA–based biomarker was affected by clinical features of PaC patients, including TNM stage, age, sex, and smoking history, we analyzed the relationship of the 7 serum miRNA expression levels with demographic and clinical factors using Student t-test or 1-way ANOVA. No obvious differences were observed when PaC cases were stratified by sex, age, or smoking history, although the expression levels of the 7 serum miRNAs were correlated with the tumor stage of PaC patients. As shown in online Supplementary Fig. 2H, the risk score value was differentially distributed in PaC cases at different tumor stages. The mean risk score of PaC cases at later stages (III and IV) was significantly higher than that at earlier stages (I and II). In particular, the expression levels of miR-21 and miR-24 were positively correlated.

### Table 2. Serum miRNAs differentially expressed in PaC cases compared to cancer-free controls.

<table>
<thead>
<tr>
<th></th>
<th>Training set</th>
<th>Validation set</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PaC</td>
</tr>
<tr>
<td>miR-20a</td>
<td>68.44 (9.46)</td>
<td>214.68 (23.29)</td>
</tr>
<tr>
<td>miR-21</td>
<td>8.82 (1.66)</td>
<td>37.37 (5.87)</td>
</tr>
<tr>
<td>miR-24</td>
<td>24.95 (4.64)</td>
<td>78.78 (13.57)</td>
</tr>
<tr>
<td>miR-25</td>
<td>5.02 (0.91)</td>
<td>25.51 (2.54)</td>
</tr>
<tr>
<td>miR-99a</td>
<td>16.16 (3.64)</td>
<td>43.09 (4.64)</td>
</tr>
<tr>
<td>miR-185</td>
<td>22.08 (4.91)</td>
<td>46.48 (6.74)</td>
</tr>
<tr>
<td>miR-191</td>
<td>50.38 (11.85)</td>
<td>144.70 (21.82)</td>
</tr>
</tbody>
</table>

*Data are mean, fmol/L (SE).*
to advanced clinical stages of PaC (online Supplementary Fig. 2, I and J).

SEPARATION OF PaC CASES FROM CP CASES BY THE SERUM 7-miRNA–BASED BIOMARKER
To test whether the 7-miRNA signature could serve as a specific biomarker to discern PaC from CP, we measured the expression levels of these miRNAs in the serum from 82 CP patients by hydrolysis probe–based RT-qPCR and compared the results with those from PaC patients. The results clearly indicated that the 7-miRNA signature could be used to separate PaC from CP cases (online Supplementary Fig. 2, A–G). Interestingly, no significant differences were observed in the levels of these 7 miRNAs between CP patients and healthy controls (online Supplementary Fig. 2, A–G). The ROC curve also indicated that this 7-miRNA–based biomarker could accurately discern CP cases from PaC cases [AUC 0.993 (0.008)] (Fig. 2C) but not from healthy controls [AUC 0.644 (0.073)] (Fig. 2D). Likewise, cluster analysis with the 7-miRNA signature showed a clear separation of PaC from CP samples. Among the 120 PaC and 82 CP cases, only 3 CP cases were misclassified as PaC (online Supplementary Fig. 2, I and J).
In contrast, the 7-miRNA signature could not distinguish the CP cases from the healthy controls (online Supplementary Fig. 3D).

PREDICTION OF PaC PATIENT SURVIVAL RATE BY USE OF SERUM miRNA LEVEL

We next investigated the association between the serum miRNA expression level and the survival rate of PaC patients. As shown in Fig. 3, PaC patients with high levels of serum miR-21 exhibited a lower survival rate than those with low levels ($P < 0.05$, log-rank test). Subsequently, we performed a univariate and multivariate Cox proportional hazards regression model to determine the influence of serum miRNA level as well as clinicopathological characteristics (sex, age, TNM stage, etc.) on patient survival. The results showed that serum miR-21 level could serve as an important indicator for predicting the survival rate of PaC patients independent of other clinic/pathological factors (online Supplementary Table 4).

VALIDATION OF THE PREDICTIVE UTILITY OF THE SERUM 7-miRNA BIOMARKER BY A DOUBLE-BLIND TEST

We tested another 129 serum samples (77 PaC cases and 52 controls) in a double-blind fashion to validate the accuracy of the serum miRNA–based biomarker for PaC diagnosis. After analyzing the expression levels of the 7 miRNAs in these serum samples and classifying them based on the previously built diagnostic model, a clear separation of PaC cases from controls was observed, with the accuracy rate of the serum 7-miRNA profile as a PaC biomarker being 86.8%, a rate higher than that for CA19-9 (76.0%) and CEA (55.8%) for the same sample set (online Supplementary Table 5).

APPLICATION OF THE 7-miRNA BIOMARKER IN DISCRIMINATIVE DIAGNOSIS OF CASES CLINICALLY SUSPECTED OF HAVING PaC

Using 55 clinically suspected cases of PaC, we further tested the accuracy of the serum 7-miRNA biomarker for PaC prediction in a prospective fashion. First, we analyzed the expression levels of the 7 miRNAs in these serum samples and classified them based on the profile of the serum 7-miRNA biomarker. All 55 cases were then followed and determined by pathologic diagnosis after 1–7 months. Results from the final pathologic diagnosis served as a standard (100% accuracy) to test the accuracy of the 7-miRNA biomarker for PaC diagnosis. As shown in online Supplementary Table 6, the forecast accuracy rate of the serum 7-miRNA profile as a PaC biomarker among those suspicious cases was 83.6%, which was significantly higher than those of CA19-9 (56.4%) and CEA (36.4%) for the same sample set.

Discussion

Previous studies have demonstrated an association between the aberrant miRNA expression and the development of PaC (23–26); however, these studies mainly focused on miRNAs expressed in tumor tissues and cells. The limitation of tissue miRNAs as a cancer biomarker is that the collection of tissue sample is an invasive procedure. The reliance on surgical sections after the initial clinical classification strongly limits the application of tissue miRNAs in cancer diagnosis.

The discovery of serum miRNAs as potential biomarkers overcomes the problem of collecting tissue samples by an invasive process. Recent studies by our group (13, 20) and others (14–19) have demonstrated that miRNAs are stably expressed in serum and plasma and can be readily detected by various assays such as SBS technology, miRNA microarray, and RT-qPCR. More importantly, the unique serum miRNA expression profiles for various diseases including cancers may serve as fingerprints for their detection (13–20). A serum miRNA–based biomarker would make it possible to comprehensively analyze tumors without the need for biopsy, surgery, and other invasive procedures. Consistent with this concept, our present study provides a proof-of-principle approach for the identification of a serum miRNA–based biomarker from a genome-wide serum miRNA expression profile. This approach involves an SBS screen of a pooled serum sample followed by RT-qPCR validation of individual
samples. Our results show that a combination of multiple serum miRNAs can serve as a more comprehensive indicator for tumor detection than the conventional single protein–based or single carbohydrate molecule–based biomarkers, such as CA19-9 and CEA. In particular, using the serum samples from patients with only stage I/II PaC, we also observed significant differential expression of the 7 miRNAs between PaC cases and controls (online Supplementary Table 7). For 26 PaC cases at stage I, the positive rate of PaC detection by the serum 7-miRNA biomarker was 96.2%, significantly higher than that of CA19-9 (46.2%) or CEA (30.8%) in the same sample set. For 48 PaC cases at stage II, the positive rate was 91.7%, also higher than that of CA19-9 (62.5%) or CEA (31.3%). Because patients with early-stage PaC generally can undergo complete resection of tumors, our data suggest that the serum 7-miRNA as a biomarker for defining early PaC may be a novel effective tool in improving the outcomes and prognosis of PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients. Furthermore, although CP shares a broad spectrum of PaC-like symptoms and difficult to clearly distinguish from PaC patients.

In conclusion, we have identified a serum 7-miRNA–based biomarker for more accurately discerning PaC cases from cancer-free controls and CP cases. The serum 7-miRNA–based biomarker may serve as a novel noninvasive approach for PaC diagnosis and prognosis.

References

Croce CM, Cohn DE. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol 2009;112:55–9.


