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Research Article

Cancer Prevention Research

Combined Serum CA19-9 and miR-27a-3p in Peripheral Blood Mononuclear Cells to Diagnose Pancreatic Cancer

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Abstract

MicroRNAs are potentially very useful biomarkers in the diagnosis of cancer. We sought to identify specific microRNAs in peripheral blood mononuclear cells (PBMCs) whose levels might facilitate diagnosis of pancreatic cancer. We investigated PBMC microRNA expression in three independent cohorts [healthy, benign pancreatic/peripancreatic diseases (BPD), and pancreatic cancer], comprising a total of 352 participants. First, we used sequencing technology to identify differentially expressed microRNAs in PBMC of pancreatic cancer, BPD, and healthy controls ($n = 20$ in each group). Then the selected microRNAs were analyzed using the quantitative reverse transcriptase PCR assays in the remaining 292 samples. The predictive value of the microRNAs was evaluated by logistic regression models and the receiver operating characteristic curve (AUC). We found that miR-27a-3p level in PBMCs could discriminate pancreatic cancer from BPD with a sensitivity of 82.2% and specificity of 76.7% (AUC = 0.840; 95% CI, 0.787–0.885%). Combination of PBMC miR-27a-3p and serum CA19-9 levels provided a higher diagnostic accuracy with a sensitivity of 85.3% and specificity of 81.6% (AUC = 0.886; 95% CI, 0.837–0.923%). The satisfactory diagnostic performance of the panel persisted regardless of disease status (AUCs for tumor-node-metastasis stages I–III were 0.881, 0.884, and 0.893, respectively). PBMC miR-27a-3p level represents a potential marker for pancreatic cancer screening. A panel combining serum CA19-9 and PBMC miR-27a-3p level could have considerable clinical value in diagnosing pancreatic cancer. *Cancer Prev Res; 6(4); 331–8. ©2013 AACR.*

Introduction

Pancreatic cancer is a lethal malignancy with an overall 5-year survival rate of only approximately 5% (1). In 2008, pancreatic cancer was responsible for an estimated 268,800 deaths worldwide (2). The poor prognosis of this disease is partly because of late clinical presentation and the lack of effective early detection measures. As a result, only 15% to 20% of patients with pancreatic cancer are candidates for potentially curative treatments at the time of diagnosis (3, 4). Therefore, it is important to identify new and more effective biomarkers for early detection of pancreatic cancer.

MicroRNAs are endogenous single-stranded RNA molecules that are 18 to 24 nucleotides in length (5). Mature microRNAs repress translation of mRNA into protein, and many microRNAs are highly conserved among species (6). Many studies have showed that microRNAs play roles in the regulation of crucial biological processes, including cellular proliferation, development, differentiation, metabolism, apoptosis, and immunity (7–13).

Tumors are equipped with multiple mechanisms to evade early events in immunological surveillance by regulating their susceptibility to lysis (14). These mechanisms might involve modulation of microRNAs, which have significant impacts on the function of antitumor T cells (15). Based on this idea, it would be useful to study the role of microRNAs in peripheral blood mononuclear cells (PBMCs) in the context of diagnosis and prognosis of malignant tumors. Several studies have identified transcripts expressed differentially between PBMCs from cancer patients and normal subjects, and some of these expression changes seem to reflect specific immune responses of circulating cells (16, 17). However, few studies have been done on PBMC microRNAs, which could be of use as diagnostic biomarkers for pancreatic cancer.

Our study investigated PBMC microRNA expression profiles with independent validation in a large cohort of 352 participants, with the purpose of identifying microRNA markers for the diagnosis of pancreatic cancer. Healthy subjects, patients with pancreatic cancer, and patients with

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benign pancreatic/peripancreatic diseases (BPDs) were included in the cohort. The diagnostic performance of PBMC microRNA levels was assessed and compared with the widely used marker serum CA19-9.

Materials and Methods

Study design and patients

Blood samples from 352 patients who met the eligibility criteria (Supplementary Table S1) were collected at Zhongshan Hospital between January 2010 and January 2012. The samples were allocated to 2 sequential phases (Fig. 1).

Discovery phase. Preoperative PBMCs were collected from 20 patients with pancreatic cancer and 20 patients with BPD. As an additional control, PBMCs were also collected from 20 healthy subjects. Patient characteristics are presented in Supplementary Table S2. MicroRNA profiles were generated by sequencing small RNAs from the 3 groups of samples. By comparing microRNA profiles between the pancreatic cancer and healthy groups, and between the pancreatic cancer and BPD groups, we established 2 differential microRNA expression patterns and subsequently compared them to each other. MicroRNAs that were significantly upregulated (fold change $\geq$2, normalized expression level of microRNA $\geq$100 in each sample) in both pairwise comparisons were chosen for further testing by quantitative reverse transcriptase PCR (qRT-PCR). Subsequently, 5 of the microRNAs identified via sequencing were selected as candidates for further testing by qRT-PCR.

Validation phase. Five differentially expressed microRNAs identified via sequencing were first tested by qRT-PCR in an independent cohort of PBMC samples from 100 participants. Three microRNAs that were differentially expressed between the pancreatic cancer group and both control groups (healthy subjects and BPD patients) were further tested in an additional 192 participants.

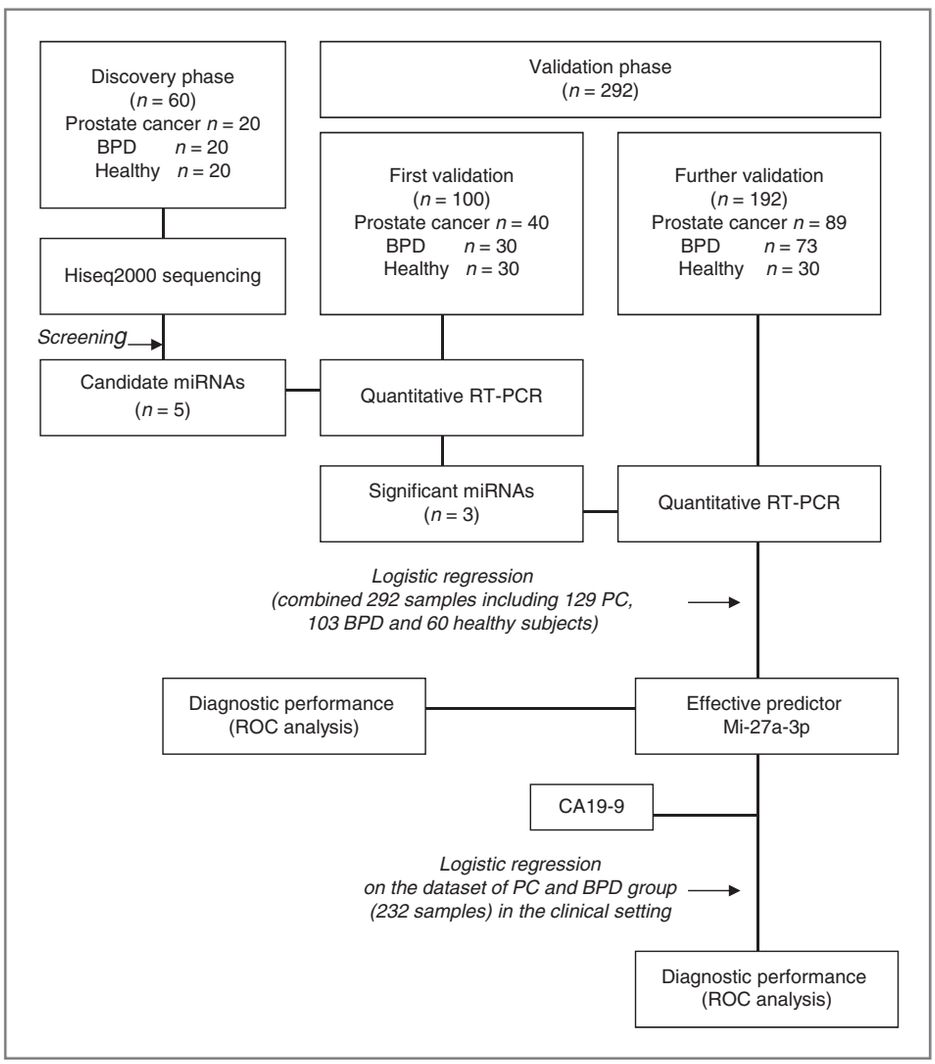

Figure 1. Study design.

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In each study phase, blood samples were obtained from 3 categories of participants: healthy subjects, patients with BPD, and patients with pancreatic cancer. The investigation protocol was approved by local Institutional Review Boards, and informed consent was obtained from all study participants. No patient received chemotherapy or radiotherapy before blood sampling.

PBMC preparation and RNA isolation

For preparation of PBMCs, peripheral blood (5 mL) was drawn into EDTA tubes and transferred to the laboratory within 30 minutes for blood processing. PBMCs were isolated using lymphocyte separation medium (Sigma-Aldrich), following the manufacturer's instructions.

For the PBMC samples, total RNA (covering all the small noncoding RNAs) was extracted using Trizol Reagent (Invitrogen) according to the manufacturer's instructions. The concentration was quantified using a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies). Each total RNA pellet was resuspended in 30 μL nuclease-free water and stored at −80°C.

Sequencing and qRT-PCR

First, to identify candidate microRNAs for use in diagnosing pancreatic cancer, we applied HiSeq 2000 technology (Illumina) to sequence small RNAs from 60 PBMC samples (see Supplementary Text S1 for details). For this sequencing procedure, we used pools of equal amounts (1 μg) of total RNA from 20 pancreatic cancer patients, 20 BPD patients, and 20 healthy subjects.

To validate the candidate microRNAs identified by sequencing, we conducted qRT-PCR using Taqman microRNA assays (Applied Biosystems). The assays were first done on 100 samples for 5 candidates (miR-27a-3p, miR-16-5p, miR-15b-5p, miR-26a-5p, and miR-342-3p) that met the aforementioned criteria for significant upregulation. The expression level of RNU6B snRNA was used as a stable endogenous control for purposes of normalization. All assays were carried out in triplicate. ΔC_T was calculated by subtracting the average C_T value of RNU6B snRNA from the average C_T value of the microRNAs of interest. Relative expression levels were expressed as $2^{-\Delta CT}$. Subsequently, the assays were done on an additional 192 samples for 3 candidates (miR-27a-3p, miR-16-5p, and miR-15b-5p) that were significantly differentially expressed in the first validation. The sequencing data has been deposited and assigned GEP accession numbers: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE37710.

Statistical analysis

Differential expression of PBMC microRNAs, and differences in serum CA19-9 between pancreatic cancer and control groups (healthy and BPD) was analyzed using the Mann–Whitney test. The correlation between PBMC microRNA expression and serum CA19-9 in patients with pancreatic cancer and clinicopathological features was evaluated using the Mann–Whitney or Kruskal–Wallis test. A multivariate logistic regression model was used to select diagnostic microRNA markers based on the validation dataset of PBMC microRNA expression. PBMC miR-27a-3p and serum CA19-9 were analyzed using a logistic regression model to select significant predictors for pancreatic cancer based on the validation dataset of pancreatic cancer and BPD. Factors that are associated with the level of miR-27a-3p and CA19-9 (expressed as the natural logarithm of their relative expression values) were evaluated using a multiple linear regression model including pancreatic cancer, jaundice (cases with serum total bilirubin level >17.1 μmol/L) in the validating dataset (pancreatic cancer, healthy, and BPD groups). The area under the receiver operating characteristics (ROC) curve (AUC) was used as an accuracy index to evaluate the diagnostic performances of the individual markers and the panel. Methods used for sample-size estimation are presented in Supplementary Text S2. All *P*-values are 2-sided, and $P < 0.05$ was considered statistically significant. MedCalc (version 10.4.7.0; MedCalc, Mariakerke, Belgium) software was used to perform the ROC and regression analysis. The predictive panel was validated using the open source package "pROC" for R and S-PLUS (18).

Results

Patient characteristics

The characteristics of the study participants in the validation phase are presented in Table 1. Serum total bilirubin and CA19-9 of the study participants are also presented in Supplementary Table S3. A total of 352 participants were recruited, including 149 patients with pancreatic cancer, 80 healthy subjects, and 123 patients with BPD. The BPD group used for sequencing ($n = 20$) included patients with chronic pancreatitis ($n = 11$), serous cystoadenoma ($n = 4$), and pseudocyst ($n = 5$). The BPD group used in the validation datasets ($n = 103$) included patients with chronic pancreatitis ($n = 33$), pseudocyst ($n = 15$), autoimmune pancreatitis ($n = 4$), serous cystoadenoma ($n = 15$), benign cyst ($n = 2$), lymphoepithelial cyst ($n = 1$), and biliary calculus disease ($n = 33$). The pancreatic cancer group used for sequencing ($n = 20$) comprised 20 patients with pancreatic ductal adenocarcinoma (PDAC). The pancreatic cancer group used in the validation datasets ($n = 129$) included patients with PDAC ($n = 106$), neuroendocrine carcinoma ($n = 10$), intraductal papillary mucinous carcinoma ($n = 6$), solid pseudopapillary carcinoma ($n = 5$), and acinar cell carcinoma ($n = 2$).

MicroRNA screening

First, HiSeq 2000 sequencing was done to identify microRNAs that were significantly differentially expressed among the pancreatic cancer, healthy, and BPD groups. Supplementary Fig. S1 illustrates the hierarchical clustering of the differentially expressed microRNAs in the 3 possible pairwise comparisons: pancreatic cancer versus healthy, pancreatic cancer versus BPD, and BPD versus healthy. By comparing microRNA profiles between the pancreatic cancer and healthy groups and between the pancreatic cancer and BPD groups, we established 2 differential microRNA

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Table 1. Characteristics of study participants in the validation datasets

Characteristic	Pancreatic cancer ($n = 129$)	BPD ($n = 103$)	Healthy ($n = 60$)
Age			
<60 years	63 (48.8%)	59 (57.3%)	29 (48.3%)
≥60 years	66 (51.2%)	44 (42.7%)	31 (51.7%)
Gender			
Male	78 (60.5%)	48 (46.6%)	31 (51.7%)
Female	51 (39.5%)	55 (53.4%)	29 (48.3%)
TNM stage			
IA	10 (7.8%)		
IB	23 (17.8%)		
IIA	23 (17.8%)		
IIB	31 (24.0%)		
III	32 (24.8%)		
IV	10 (7.8%)		
CA19-9			
<37 U/mL	32 (24.8%)	73 (70.9%)	60 (100%)
≥37 U/mL	97 (75.2%)	30 (29.1%)	0 (0%)
Total bilirubin			
<17.1 μmol/L	92 (71.3%)	58 (56.3%)	60 (100%)
≥17.1 μmol/L	37 (28.7%)	45 (43.7%)	0 (0%)
Fasting blood glucose			
<6.1 mmol/L	76 (58.9%)	83 (80.6%)	60 (100%)
≥6.1 mmol/L	53 (41.1%)	20 (19.4%)	0 (0%)

expression patterns that we then compared to each other. Five microRNAs, miR-27a-3p, miR-16-5p, miR-15b-5p, miR-26a-5p, and miR-342-3p, were significantly upregulated in the pancreatic cancer group relative to both the healthy and BPD groups (fold change ≥2, normalized expression level of microRNA ≥100 in each sample; Supplementary Table S4). We selected these 5 differentially expressed microRNAs as candidates for further testing via qRT-PCR.

Independent validation on PBMCs samples

The 5 microRNAs identified via sequencing were first tested by qRT-PCR in an independent cohort of PBMC samples from 100 participants (Supplementary Table S5). Three of 5 microRNAs were significantly upregulated in the pancreatic cancer group ($n = 40$) relative to both the healthy group ($n = 30$) and the BPD group ($n = 30$). Those 3 microRNAs were further tested in an additional 192 participants. The combined set of 292 PBMC samples (including 129 pancreatic cancer patients, 103 BPD patients, and 60 healthy subjects) was used to evaluate the differential expression of the 3 microRNAs (Supplementary Tables S3). Significantly upregulated expression of miR-27a-3p, miR-16-5p, and miR-15b-5p was observed in patients with pancreatic cancer compared with subjects in both the healthy and BPD groups: average fold-change = 3.16, 2.30, and 2.40 for miR-27a-3p, miR-16-5p, and miR-15b-5p, respectively (Table 2; Fig. 2A–C). Thus, we analyzed these 3 microRNAs using a multivariate logistic regression model. In this model, as shown in Table 2, only miR-27a-3p ($P < 0.001$) effectively discriminated the pancreatic cancer group from the healthy and BPD groups. The corresponding AUCs were 0.857 (95% CI, 0.812–0.895%; sensitivity = 82.2%; specificity = 79.1%).

The expression level of miR-27a-3p in PBMCs was comparable between strata by sex, age (≤60 or >60 years of age), levels of fasting blood glucose (≤17.1 or >17.1 μmol), and CA19-9 (≤37 or >37 U/mL), or tumor-node-metastsis staging system [the International Union Against Cancer (UICC) tumor-node-metastasis classification in 2002; TNM] stage. However, the level of miR-27a-3p expression in pancreatic cancer and BPD patients seem to be associated with elevated levels of total bilirubin (>17.1 μmol/L) with the median (range) level of 85.48 (5.39–873.10) in patients with elevated serum total bilirubin versus 73.77 (3.05–337.79) in their counterparts ($P = 0.013$, Mann–Whitney test). Furthermore, we also noticed a higher level of total bilirubin [13.1(3.5–385.5)] in patients with elevated level of CA19-9 versus those with normal level [11.1(3.5–298.9); $P = 0.005$, Mann-Whitney

Table 2. MicroRNA profile and diagnostic performance in the validating dataset of pancreatic cancer, healthy group, and BPD

MicroRNA	Pancreatic cancer vs. healthy group and BPD			
	Univariate			
	P^a	Average fold change	AUC	Multivariate P^b
miR-27a-3p	<0.001	3.16	0.857	<0.001
miR-16-5p	<0.001	2.30	0.773	0.154
miR-15b-5p	<0.001	2.40	0.782	0.463

[a]Mann–Whitney test.

[b]$P < 0.05$, significant predictor for pancreatic cancer.

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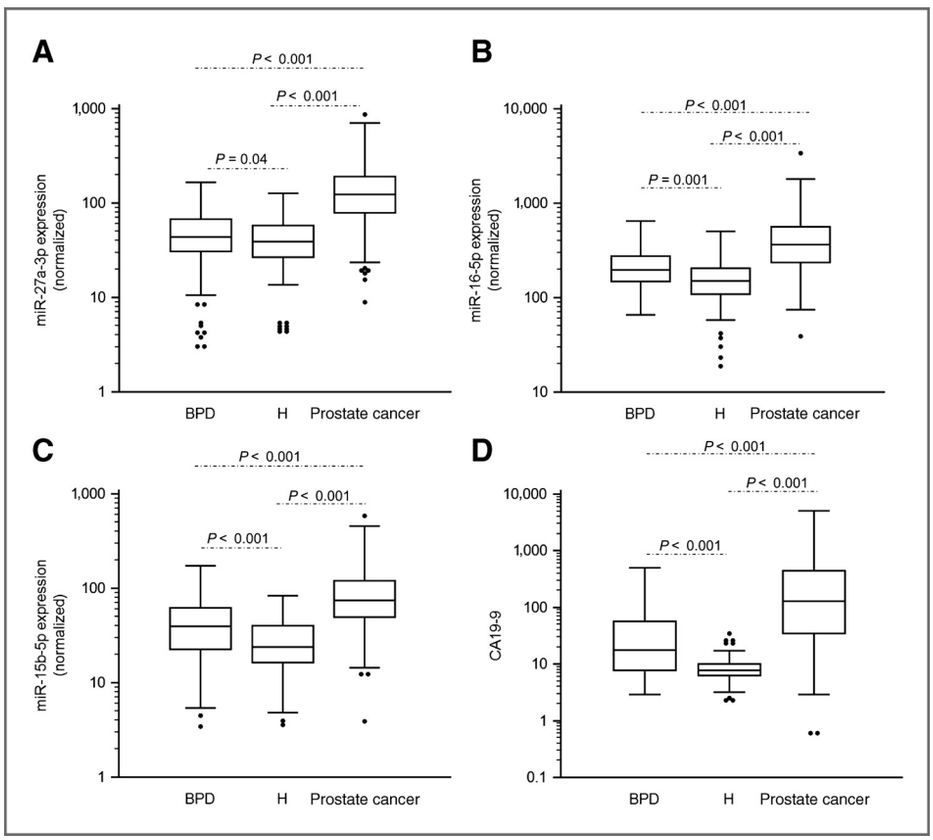

Figure 2. MicroRNA validation by qRT-PCR analysis. Box plots of expression levels of (A) miR-27a-3p, (B) miR-16-5p, (C) miR-15b-5p, and (D) CA19-9 in healthy subjects (H; $n = 60$) and patients with pancreatic cancer ($n = 129$) and BPD ($n = 103$). Expression levels of the miRNAs ($\log_{10}$ scale at y-axis) are normalized to RNU6B. The lines inside the boxes denote the medians. The boxes mark the interval between the 25th and 75th percentiles. The whiskers denote the interval between the 10th and 90th percentiles. Filled circles indicate data points outside the 10th and 90th percentiles. Statistically significant differences were determined using Mann–Whitney tests.

test]. Multiple linear regression analysis revealed that both pancreatic cancer (partial regression coefficient = 1.15, $P < 0.001$) and jaundice (partial regression coefficient = 0.59, $P < 0.001$) were predictors for PBMC miR-27a-3p expression in the validating dataset (comprising pancreatic cancer, healthy, and BPD groups).

We next assessed the performance of miR-27a-3p in differentiating the pancreatic cancer group from the BPD and healthy groups. This analysis showed that miR-27a-3p was moderately accurate at discriminating pancreatic cancer from the BPD group (AUC = 0.840; 95% CI, 0.787–0.885%; sensitivity = 82.2%; specificity = 76.7%) and the healthy group (AUC = 0.886; 95% CI, 0.831–0.927%; sensitivity = 82.9%; specificity = 83.3%).

Evaluating the diagnostic performance of serum CA19-9

Discrimination between pancreatic cancer patients and healthy subjects does not reflect the putative performance of the diagnostic test in a clinical setting. In this context, BPD patients represent a more suitable control. Therefore, we evaluated the diagnostic accuracy of serum CA19-9 in differentiating between the pancreatic cancer and BPD groups. For the purposes of this evaluation, we used the dataset from 232 patients from the validation phase (129 pancreatic cancer and 103 BPD; Fig. 2D). The resulting AUC was 0.788 (95% CI, 0.730–0.839%; sensitivity = 72.9%; specificity = 75.7%).

Multiple linear regression analysis revealed that both pancreatic cancer (partial regression coefficient = 2.10; $P < 0.001$) and jaundice (partial regression coefficient = 1.11; $P < 0.001$) were predictors for serum CA19-9 in the validating dataset (pancreatic cancer, healthy, and BPD groups).

Establishing the predictive panel in a clinical setting

There were significant differences in the distributions of gender, total bilirubin, and fasting blood glucose between pancreatic cancer and BPD groups (Pearson χ^2 test, $P < 0.05$) in a clinical setting. Thus, the 5 variables (including gender, total bilirubin, fasting blood glucose, miR-27a-3p, and CA19-9) were analyzed in the validation phase by a multivariate logistic regression model. Only miR-27a-3p and CA19-9 were sufficiently effective in the model to discriminate pancreatic cancer from BPD group:

$$\text{logit}(P = \text{pancreatic cancer}) = -2.4597 + 0.0239 \times \text{miR-27a-3p} + 0.0064 \times \text{CA19-9}$$

The diagnostic performance of the established panel was then evaluated using ROC analysis. The corresponding AUC was 0.886 (95% CI, 0.837–0.923%; sensitivity = 85.3%; specificity = 81.6%). The panel consisting of PBMC miR-27a-3p level and serum CA19-9 was significantly more effective than either individual marker alone at discriminating pancreatic cancer from BPD (vs. PBMC miR-27a-3p level: $P = 0.005$; vs. serum CA19-9 level: $P < 0.001$; Table 3). We validated the final predictive panel using 2000 stratified

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Table 3. Diagnostic performance of PBMCs miR-27a-3p and serum CA 9-9 in the validating dataset of pancreatic cancer and BPD group

	Pancreatic cancer vs. BPD		
Variable	**Sensitivity**	**Specificity**	**AUC (95% CI)**
miR-27a-3p	82.2%	76.7%	0.840 (0.787–0.885)
CA 19-9	72.9%	75.7%	0.788 (0.730–0.839)
combination[a]	85.3%	81.6%	0.886 (0.837–0.923)

[a]Combination of PBMC miR-27a-3p and serum CA19-9.

bootstrap replicates (18), and the resulting 95% CI of resampling AUCs was 0.840 to 0.928%.

Next, we evaluated the diagnostic performance of the predictive panel at different TNM stages. The corresponding AUCs for patients of TNM stages I to III were 0.881, 0.884, and 0.893, respectively.

Discussion

Lack of effective early detection measures is one of the most important factors contributing to the poor prognoses of pancreatic cancer patients. Serum CA19-9 has served for many years as a serum marker for pancreatic cancer diagnosis and screening. However, the major limitation of CA19-9 as a diagnosis marker for pancreatic cancer is lack of specificity because it is also elevated in BPD (3, 4). Extensive efforts to identify a better serum or plasma marker have met with limited success.

Immune system evasion in cancer patients is partially because of functional repression of immune cells (14, 15) and is initiated as early as the premalignant disease stage of pancreatic cancer (19). Several studies have shown that microRNAs play a crucial role in modulating adaptive or innate immune responses (12, 20–22). Sasaki and colleagues (15) showed that in the tumor microenvironment, which is skewed toward type-2 T cells, miR-17-92 expression in T cells is downregulated; consequently, the persistence of tumor-specific T cells and the efficacy of tumor control are decreased. Furthermore, CD4+ T cells derived from miR-17-92 transgenic mice exhibit an enhanced type-1 phenotype associated with increased interferon-γ production and very late antigen (VLA)-4 expression. Baine and colleagues (17) reported the first in-depth comparison between global gene expression profiles of PBMCs from pancreatic cancer patients and healthy controls. Those authors identified a gene predictor set that could potentially be further developed for use in diagnostic algorithms for pancreatic cancer. These discoveries regarding aberrant expression of both microRNAs in immune cells and mRNAs in PBMCs of cancer patients led us to evaluate the utility of PBMC microRNA levels in the diagnosis of pancreatic cancer.

For diagnosis of pancreatic cancer, the median sensitivity of serum CA19-9 is 79% (70–90%), and the median specificity is 82% (68–91%; ref. 23), consistent with the diagnostic performance in our study. By contrast, a recent retrospective study showed that CA19-9 has poor clinical utility as a tumor marker for pancreatic cancer, with an AUC of only approximately 0.7 (24). This inconsistency might be related to case selection. Furthermore, elevation in serum CA19-9 is associated with hyperbilirubinemia irrespective of the presence of benign or malignant disease, compromising its diagnostic specificity (25). Our study also confirmed that hyperbilirubinemia is a predictor for elevation of serum CA19-9. Therefore, caution is warranted when interpreting the results in jaundiced patients. Marrelli and colleagues (26) showed that serum CA19-9 decreases to normal levels when patients with benign pancreaticobiliary diseases are stented, but remains elevated in cases of malignancy. As with serum CA19-9, elevation of PBMC miR-27a-3p level was also related to hyperbilirubinemia in our study, potentially mitigating its diagnostic accuracy. However, owing to the complementary effect between the 2 biomarkers, the combination of serum CA 19-9 and miR-27a-3p levels yielded higher accuracy in differentiating pancreatic cancer from BPD in a clinical setting, especially for patients at early stages (TNM stage I). In addition, whereas serum CA19-9 has been reported (23) to correlate with tumor stage, we did not observe such a correlation in this study. This discrepancy could be attributed to the diversity of histological types of pancreatic cancer included in our cohorts.

Our study revealed that PBMC miR-27a-3p level was moderately accurate at differentiating pancreatic cancer from the control groups. In addition, its diagnostic performance was independent of disease staging; hence, it could serve as a potential diagnostic marker for pancreatic cancer at early stages. Previous reports of differential expression of miR-27a, both identified and verified, have primarily pertained to cancer cells (27–29). Liu and colleagues (27) showed that miR-27a is upregulated in human gastric adenocarcinoma, and that suppression of miR-27a inhibits gastric cancer cell growth. Their results supported the idea that miR-27a functions as an oncogene. Mertens-Talcott and colleagues (28) found that miR-27a exerts oncogenic activity by regulating ZBTB10, which results in overexpression of Sp proteins and Sp-dependent genes that are important for cell survival and angiogenesis. In the MCF-7

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breast-cancer cell line, miR-27a indirectly regulates expression of estrogen receptor α, thereby influencing hormone responsiveness (29). Our study, however, is to our knowledge the first to report the importance of the miR-27a-3p expression profile in PBMCs from pancreatic cancer patients. Further functional study is needed to confirm the role of miR-27a-3p in PBMCs. In this study, although the expression levels of miR-16-5p and miR-15b-5p in the pancreatic cancer group were significantly higher than those in the control groups, multivariate logistic regression analysis showed that these 2 microRNAs were not significant predictors for pancreatic cancer.

Our study has 2 unique advantages: First, we screened PBMC microRNAs using HiSeq 2000 sequencing technology, which enabled us to identify potential diagnostic markers with high accuracy. Moreover, our control group included not only healthy subjects, but also patients with BPD; as noted earlier, in a clinical setting, BPD patients represents a more suitable control group than healthy subjects. Our results indicate that our diagnostic panel could effectively differentiate pancreatic cancer from BPD.

Although our results are promising, there are several limitations in this study. First, we used pools of equal amounts of total RNA from 20 pancreatic cancer patients, 20 BPD patients, and 20 healthy subjects in the sequencing procedure, compromising the ability to identify differentially expressed microRNAs in PBMC. Moreover, pancreatic cancer group in this study included different types of pancreatic cancer. The potential marker miR-27a-3p we identified needs to be validated in a homogenous patient population with PDAC, which accounts for the vast majority of pancreatic cancer. In addition, the sample size is still small in present study, and we only conducted internal validation of the algorithm using the open source package "pROC" for R and S-PLUS. Thus, the panel identified in our study needs to be further validated using a large independent cohort.

In summary, our study shows that the combination of serum CA19-9 and PBMC miR-27a-3p level can differentiate pancreatic cancer from BPD with a higher degree of accuracy. Thus, this panel could have considerable clinical value for the diagnosis of pancreatic cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: W. Wang, D. Jin, X.-L. Wang
Development of methodology: W. Wang, L. Liu, G. Li, Y. Chen, C. Li, D. Jin, X.-L. Wang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W. Wang, L. Liu, G. Li, Y. Chen, C. Li, D. Jin, X.-L. Wang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): W. Wang, D. Jin, X.-L. Wang
Writing, review, and/or revision of the manuscript: W. Wang, X.-L. Wang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Jin, X.-L. Wang
Study supervision: X.-L. Wang

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Cancer Prevention Research

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