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Increased plasma miR-370-3p expression in poor-outcome patients with pancreatic ductal adenocarcinoma

Takumi Harada [a], Kenichiro Uemura [a], Tatsuaki Sumiyoshi [a], Ryuta Shintakuya [a], Kenjiro Okada [a], Tetsuhiro Hara [a], Shinya Takahashi [a], Eiso Hiyama [b, *]

[a] Department of Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[b] Natural Science Center for Basic Research and Development, Hiroshima University, Hiroshima, Japan

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ABSTRACT

Objective: To determine whether circulating microRNAs (miRNAs) can be used as prognostic biomarkers for pancreatic ductal adenocarcinoma (PDAC).
Methods: Patients with PDAC (N = 120) who underwent surgical resection at Hiroshima University Hospital between November 2006 and January 2020 were enrolled in this study and grouped based on their overall survival (OS) into two groups: favorable prognosis group (F group; OS ≥ 18 months) and unfavorable prognosis group (U group; OS < 18 months). Blood plasma samples were collected prior to surgery. To identify candidate prognostic miRNAs, next-generation sequencing (NGS) analysis was used to evaluate the expression levels of miRNAs in seven of the plasma samples. Using quantitative real-time PCR (qRT-PCR), the expression levels of the selected miRNAs were determined in the remaining 113 patient plasma samples, and the relationship between miRNA expression and survival was statistically evaluated.
Results: NGS analysis and qRT-PCR revealed significantly upregulated plasma miR-370-3p expression in the U group compared to that in the F group ($p = 0.028$ and $p = 0.005$, respectively). Moreover, miR-370-3p expression and lymph node metastasis showed a statistically significant association ($p = 0.028$). In a multivariate analysis of OS and recurrence-free survival (RFS), the upregulation of miR-370-3p expression in plasma was identified as an independent risk factor for poor OS (HR2.13, $p = 0.004$) and RFS (HR1.84, $p = 0.015$).
Conclusions: Plasma miR-370-3p expression upregulation correlates with poor prognosis in patients with PDAC.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a fatal disease that is prevalent worldwide [1]. Although several novel clinical treatments have been recently developed, surgical extirpation is the only curative treatment option. However, most patients are diagnosed with PDAC in its advanced stages, making radical surgical resection difficult. Even when surgical resection is performed, early recurrence is often observed [2]. While carbohydrate antigen 19–9 is a widely accepted biomarker of PDAC [3], it is not perfect diagnostic and prognostic marker as CA19-9 is not applicable in Lewis-negative patients [4]. As accurate preoperative prediction of disease's prognosis enables the determination of appropriate treatment plans, highly sensitive prognostic biomarkers are required.

microRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression during the post-transcriptional phase [5]. miRNAs play important roles in crucial biological processes, including cell development, differentiation, apoptosis, and proliferation [6]. They are secreted into the blood and body fluids by cells where they exist as stable miRNAs [7]. Recent studies have revealed that circulating miRNAs have potential as both diagnostic and prognostic biomarkers for various cancers [8–13]. However, there are limited reports describing the role of miRNAs as prognostic biomarkers for PDAC.

* Corresponding author. Natural Science Center for Basic Research and Development, Hiroshima University 1-2-3, Kasumi, Minami-ku, Hiroshima city, 734-8551, Japan.
E-mail address: eiso@hiroshima-u.ac.jp (E. Hiyama).

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In this study, we aimed to identify potential circulating miRNA biomarkers that can help predict prognosis in patients with PDAC.

2. Methods

2.1. Patients

We enrolled 120 patients diagnosed with PDAC in the pancreatic head, who underwent surgical resection through the Department of Surgery at the Hiroshima University Hospital between November 2006 and January 2020. All patients had received no other treatment, including chemotherapy or radiotherapy, prior to surgery. A diagnosis of either resectable (R) or borderline resectable (BR) PDAC was made according to the 2022 National Comprehensive Cancer Network guidelines [14].

Retrospectively, the patients were divided into two groups based on their prognosis as follows: unfavorable group (U group; included patients with an overall survival [OS] of <18 months; n = 40) and favorable group (F group; included those with an OS of ≥18 months; n = 80). Informed consent was obtained according to the recommendations of the Declaration of Helsinki, and the study was approved by the Medical Ethics Committee of Hiroshima University (E20049103-06).

The recommended treatment for patients with PDAC of the pancreatic head was pylorus preserving pancreatoduodenectomy. However, if the tumor was close to the duodenal bulb area in the superior pancreatic head, pancreaticoduodenectomy with antrectomy was performed instead. All patients underwent a systematic resection of regional and para-aortic lymph nodes. In cases where the tumor had invaded the portal vein, partial resection of the portal vein was performed. Arterial resection, including common hepatic and superior mesenteric artery resection, was performed in selected patients with BR-PDAC. Intraoperative pathological assessment of the proximal pancreatic margin was performed using frozen tissue sections. In cases where the pancreatic margin was positive for cancer cells, an additional resection of the pancreas was performed. Adjuvant gemcitabine plus S-1 (GS) chemotherapy was administered to patients who met the previously reported institutional criteria [15,16]. Patients who received more than 12 courses of GS chemotherapy were defined as receiving postoperative adjuvant chemotherapy. Tumor stage, lymph node metastasis, and final stage were determined in accordance with the 8th edition of the International Union Against Cancer guidelines [17]. If infiltration of the adenocarcinoma was observed along the proximal pancreatic transaction line or in the dissected peripancreatic soft tissue margins, the surgical margin was graded as “R1”.

Patient follow-ups were carried out at least once in the three months post-surgery, during which blood tests and computed tomography were performed. Recurrence was diagnosed based on imaging findings. For patients who had died, the postoperative survival time and cause of death were recorded. The postoperative time and recurrence status of survivors were also recorded. OS was defined as the time from the date of surgical resection to the date of death or the last follow-up visit. Recurrence-free survival (RFS) was defined as the time from the date of surgical resection to the date of recurrence or the last follow-up visit.

2.2. Plasma samples

We collected 125 plasma samples, including those from five healthy volunteers (four men and one woman, aged 33–65 years old with no evidence of malignancy). All plasma samples were collected from the patients prior to treatment, and samples and data were retrospectively collected and analyzed. With informed consent, whole blood samples were collected from patients with PDAC during anesthesia induction immediately before resection. Specifically, 8 mL of whole blood was collected in EDTA-containing tubes, and the samples were centrifuged at 3000 rpm (1500×*g*) at 23–25 °C for 10 min. Plasma samples were separated from peripheral blood cells within 4 h of sample collection.

2.2.1. RNA isolation from blood plasma samples

Total RNA, including miRNA, was isolated from 200 μL of plasma using the Qiagen miRNeasy Serum/Plasma Advanced Kit (Qiagen, Co. Ltd., Hilden, Germany) according to the manufacturer's protocol. The isolated RNAs were stored at −80 °C until further analysis.

2.3. Selection of miRNAs using next-generation sequencing (NGS)

Using NGS analysis, we evaluated the expression of miRNAs eluted from samples obtained from seven randomly selected patients (three patients from the favorable group and four from the unfavorable group) to select candidate prognostic miRNAs.

Using quantitative real-time PCR (qRT-PCR), the value of the selected candidate miRNAs as potential prognostic biomarkers was evaluated in plasma samples from the remaining 113 patients. Finally, we statistically evaluated the relationship between various clinicopathological factors and miRNA expression.

To prepare a cDNA library for small RNA sequencing, the seven selected samples were evaluated using an Ion Total RNA-Seq Kit v2 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The sizes and concentrations of the base pairs in the cDNA library were determined using a high-sensitivity DNA kit (Agilent Technologies, Santa Clara, CA, USA). The preparation steps for deep sequencing, such as emulsion PCR, bead enrichment, and chip loading, were performed using Ion Chef (Thermo Fisher Scientific). NGS was performed using Ion Proton (Thermo Fisher Scientific) and the Ion PI chip v3.

The raw sequence data were obtained from Ion Proton in the FASTQ format and were analyzed using the Strand NGS software version 2.7 (www.strand-ngs.com). Raw sequence reads were trimmed based on quality, and the reads shorter than 10 bp were discarded. The remaining reads were aligned to hg19. All types of small RNA were identified using Refseq, except for miRNA, which was removed from the data. Read counts were normalized using the Deseq algorithm. miRNAs with raw read counts of less than five were discarded.

2.4. qRT-PCR analysis of the selected miRNA expression levels in the plasma samples

miRNA expression in the plasma was assessed using qRT-PCR. cDNA was synthesized from the total RNA using TaqMan MicroRNA primers for miR-370-3p and miR-93-3p and the TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific), according to the manufacturer's instructions. qRT-PCR was performed using the TaqMan Advanced Master Mix, and all qRT-PCR reactions were performed in duplicate. Based on previous studies [11,12], miR-93-3p, miR-16-5p, and miR-101-3p were used as candidate reference plasma small RNAs for normalization. We examined the expression of these candidate reference miRNAs in the plasma of the healthy control, favorable, and unfavorable groups. Stability values were calculated using the NormFinder software (https://moma.dk/normfinder-software). Finally, we selected the reference endogenous miRNA based on the stability values. Quantification of target miRNAs relative to the reference endogenous control was performed using the $2^{-\Delta Ct}$ method.

2.5. Statistical analyses

The unpaired Mann–Whitney *U* test was used to compare the miRNA expressions determined using NGS and qRT-PCR analysis between favorable and unfavorable groups. The relationship between the two groups was analyzed using the Student's t-test and chi-squared test. Receiver operating curve (ROC) analysis was performed to assess the diagnostic performance of the selected miRNAs.

OS and RFS curves were analyzed using the Kaplan–Meier survival curve method. Differences in OS and RFS were compared using a univariate log-rank test. Cox proportional hazards regression analysis was used to estimate univariate and multivariate hazard ratios for OS and RFS. All statistical analyses were performed using the JMP software version 14 (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at P-value <0.05.

3. Results

3.1. Selection of candidate miRNA using NGS

Using NGS, we analyzed the plasma miRNA profiles of favorable (N = 3) and unfavorable (N = 4) prognosis groups, to determine candidate miRNAs with potential for use as new biomarkers of PDAC. The average reads of the favorable and unfavorable groups were 6,952,932 and 7,312,765, respectively. We discarded the RNAs with less than five reads. Thus, out of the 2104 identified small RNAs, 562 were selected. The unpaired Mann–Whitney *U* test analysis revealed that the expression levels of 15 miRNAs were significantly higher in the unfavorable group than those in the favorable group, and it did not detect significantly decreased microRNAs. Among these miRNAs, the expression of miR-370-3p was over 2-fold higher in the unfavorable group than in the favorable group. The average reads of miR-370-3p in the favorable and unfavorable groups were 4.3 and 0.33, respectively. Thus, miR-370-3p was identified as a promising candidate prognostic biomarker for PDAC.

3.2. Expressions of candidate miRNAs as determined by qRT-PCR

The expression levels of reference plasma miRNAs were determined to normalize the data of qRT-PCR experiments. We evaluated the expression of miR-16-5p, miR-93-3p, and miR-101-3p in the plasma of five healthy individuals and five patients from each of the favorable and unfavorable groups. The stability value of each miRNA was calculated based on their respective Ct values using NormFinder software. The stability values were 0.010 for miR-93-3p, 0.023 for miR-101-3p, and 0.030 for miR-16-5p. Therefore, miR-93-3p was selected as the reference miRNA.

We analyzed the expression of miR-370-3p and miR-93-3p in plasma samples from 77 patients in the favorable group and 36 patients in the unfavorable group. Table 1 summarizes the clinicopathological characteristics of the patients enrolled in each group. The average Ct values of plasma miR-370-3p in the favorable and unfavorable groups were 36.4 and 35.9, respectively, and the average Ct values of plasma miR-93-3p as a control in the favorable and unfavorable groups were 26.2 and 27.0, respectively. There was no significant difference in plasma miR-93-3p expression between the two groups ($p = 0.09$). Plasma miR-370-3p expression was significantly upregulated in the unfavorable group compared to that in the favorable group ($p = 0.005$) (Fig. 1).

3.3. ROC analysis

The specificity and sensitivity of miR-370-3p as a biomarker for poor prognosis of PDAC were determined using ROC analysis, revealing a sensitivity and specificity of 75 % and 58 %, respectively (Fig. 2). We set the cut-off value of relative quantification of miRNA at $\Delta Ct = 8.83$, based on the ROC analysis.

3.3.1. Association between plasma miR-370-3p level and clinicopathological factors

To assess the correlation between miR-370-3p expression and clinicopathological characteristics, 113 patients were divided into two groups (high and low) based on miR-370-3p expression (Table 2). The cut-off level was defined as the miR-370-3p expression level based on the ROC curve ($\Delta Ct = 8.83$). A statistically significant association was observed between miR-370-3p expression and lymph node metastasis ($p = 0.028$).

3.4. Survival analysis

The median follow-up time for the 113 patients after surgery was 40 months (a range of 2–149 months). The OS and RFS survival

Table 1
Clinicopathologic characteristics of patients.

Factor		Favorable (n = 77) (%)	Unfavorable (n = 36) (%)
Age, years	Median, IQR	71,63-75	74,67-80
Sex	Male/female	35(45)/42(55)	22(61)/14(39)
Body weight, kg	Median, IQR	54.7, 48.9–62.7	55.1, 46-63
Diabetes mellitus	Yes/no	25(32)/52(68)	14(39)/22(61)
Biliary drainage	Yes/no	39(51)/38(49)	19(53)/17(47)
Preoperative CA19-9, U/l	Median, IQR	102, 23–420.5	158, 37–886.8
Postoperative CA19-9, U/l	Median, IQR	9, 4-22	26, 10-74
Tumor diameter, mm	Median, IQR	26, 19.5–30	35, 28.5–45
Resectability status	R/BR	67(87)/10(13)	16(46)/19(54)
Adjuvant chemotherapy	Yes/no	63(82)/14(18)	21(58)/15(42)
Operative time, min	Median, IQR	328, 289-384	400, 332-445
Extent of blood loss, ml	Median, IQR	610, 395-1014	1227, 826-1962
Blood transfusion	Yes/no	10(13)/67(87)	13(36)/23(64)
PV/SMV resection	Yes/no	22(29)/55(71)	3(8)/33(92)
Postoperative complication	Yes/no	9(12)/68(88)	4(11)/32(89)
Lymph node metastasis	Yes:/no	52(68)/25(32)	28(78)/8(22)
Histological grade	Grade1/Grade2 or 3	25(32)/52(68)	7(19)/29(81)
Residual tumor	R0/R1	61(79)/16(21)	19(53)/17(47)
UICC pT factor	pT1 or 2/pT3 or 4	4(5)/73(95)	1(3)/35(97)
Recurrence	Yes/no	41(53)/36(47)	33(92)/3(8)

IQR:Interquartile range, R:Resectable, BR:Borderline resectable.

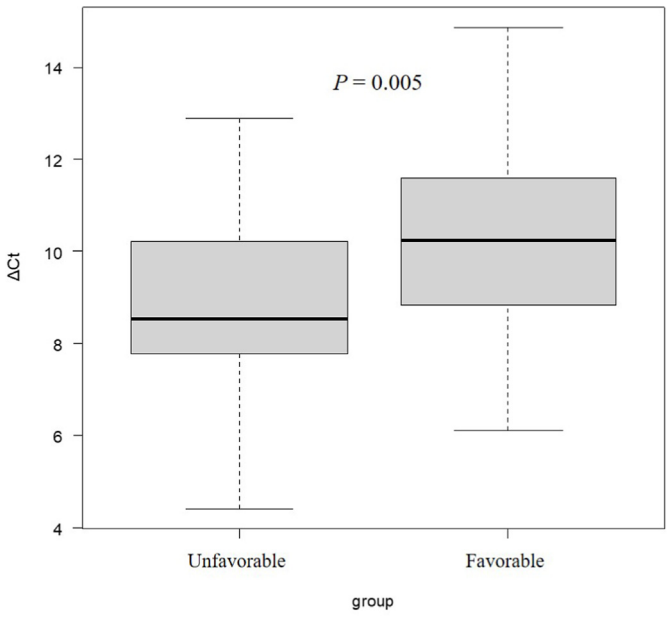

Fig. 1. Plasma miR-370-3p expression levels, quantified by qRT-PCR, in favorable and unfavorable prognosis groups to validate the use of miR-370-3p as a prognostic biomarker for PDAC.
Plasma miR-370-3p expression was significantly upregulated in the unfavorable group compared to that in the favorable group ($p = 0.005$). The average Ct values of plasma miR-370-3p in the favorable and unfavorable groups were 36.4 and 35.9, respectively.

curves stratified by miR-370-3p expression are shown in Fig. 3. The median survival time of patients with upregulated miR-370-3p levels was shorter than that of patients with downregulated miR-370-3p levels (16.7 vs 59.9 months). Upregulated miR-370-3p levels were significantly correlated with poor prognosis in both the OS ($p < 0.001$) and RFS ($p < 0.001$) analyses.

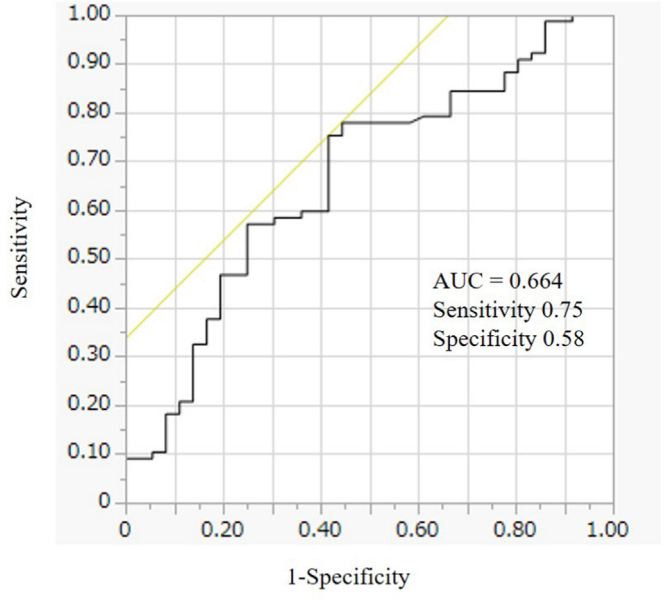

Fig. 2. Receiver operating curve (ROC) analysis of plasma miR-370-3p expression for predicting poor prognosis in patients with PDAC.
ROC analysis showed that the sensitivity and specificity of this marker in predicting poor prognosis were 75 % and 58 %, respectively.

3.5. Univariate and multivariate analyses

The results of the univariate and multivariate OS analyses of clinicopathological factors and miR-370-3p expression are shown in Table 3. In the univariate analysis, poor OS was significantly associated with tumor diameter ($p < 0.001$), resectability status ($p < 0.001$), operative time ($p = 0.049$), portal vein resection ($p = 0.032$), lymph node metastasis ($p = 0.012$), residual tumor ($p = 0.003$), and plasma miR-370-3p expression upregulation ($p < 0.001$). The multivariate analysis for OS showed that the independent prognostic factors were tumor diameter, resectability status, and miR-370-3p expression upregulation. The results of the univariate and multivariate RFS analyses of clinicopathological factors and miR-370-3p expression are shown in Table 4. In the univariate analysis of RFS, poor RFS was significantly associated with tumor diameter ($p = 0.002$), resectability status ($p = 0.001$), lymph node metastasis ($p = 0.002$), portal vein resection ($p = 0.028$), residual tumor ($p = 0.04$), and plasma miR-370-3p expression upregulation ($p = 0.002$). Furthermore, the multivariate analysis for RFS revealed that the independent prognostic factors were tumor diameter and miR-370-3p expression upregulation. Therefore, these results indicate that plasma miR-370-3p expression upregulation is an independent risk factor for poor OS and RFS.

4. Discussion

This study demonstrated that miR-370-3p plays a progressive role in PDAC tumor proliferation. miR-370 is dysregulated in various human malignancies and is involved in many organ system diseases [18–21]. Lo et al. reported that miR-370 expression was upregulated in both the tumor tissue and plasma samples of patients with gastric cancer, and higher expression levels of miR-370 in plasma were linked to higher clinical stages [22]. Moreover, Fan et al. found that the overexpression of miR-370 in gastric cancer cells promoted cell proliferation and anchorage-independent growth [23]. In patients with breast cancer, miR-370-3p expression is also upregulated in both the tumor cells and sera, and high serum miR-370-3p expression is remarkably correlated with lymphatic metastasis and tumor node metastasis (TNM) stages [24]. In contrast, miR-370 expression is downregulated in hepatocellular carcinoma tumor tissue, with lower expressions of miR-370 associated with shorter OS [25]. Serum miR-370 expression is reduced in patients with early-stage cervical cancers, and low serum miR-370 expression is correlated with a high chance of lymph node metastasis and recurrence [13]. In these tumors, miR-370 appears to play a suppressive role in tumor proliferation. This discrepancy may suggest that the function of miR-370 differs between cancer types.

Various reports have investigated the potential targets of miR-370. In gastric cancer, miR-370 targets the transforming growth factor-β receptor [21] and Forkhead Box O subfamily of transcription factors (FOXO) − 1 [23]. FOXO-1 is also reported to be a target of miR-370 in prostate cancer [26]. Mao et al. reported that miR-370 promoted breast cancer progression by inhibiting fibulin-5 (FBLN5) expression and activating the NF-κB signaling pathway [24]. Ji et al. reported that miR-370-5p could bind to the p21 promoter region and regulate its activity in pancreatic cancer, leading to cancer progression [27]. However, there are few reports on the function and target genes of miR-370 in PDAC, a topic that requires further investigation.

This study revealed that plasma miR-370-3p expression levels are correlated with lymph node metastasis. Mao et al. reported that increased expression of miR-370-3p in the sera of breast cancer patients was associated with lymph node metastasis and TNM

Table 2
Relationship between clinicopathological factors and miR-370 expression.

Factor		All patients (n = 113)		p value
		miR-370		
		High (n = 41 (36 %))	Low (n = 72 (64 %))	
Age, Median(years)		73	71	0.112
Sex	Male	22	35	0.606
	Female	19	37	
Preoperative CA19-9, Median(IU/U)		157.5	93	0.469
Tumor diameter, Median(mm)		30	28	0.276
Resectability Status	Resectable	26	57	0.072
	Borderline	15	15	
Adjuvant chemotherapy	Yes	28	56	0.271
	No	13	16	
Operative time, Median(min)		377	332.5	0.11
Extent of blood loss, Median(ml)		940	748	0.953
Portal vein resection	Yes	18	23	0.206
	No	23	49	
Postoperative complication	Yes	4	9	0.657
	No	37	63	
Lymph node metastasis	Yes	34	46	0.028
	No	7	26	
Histological grade	Grade1	16	16	0.059
	Grade2/3	25	56	
Residual tumor	R0	26	54	0.196
	R1	15	18	

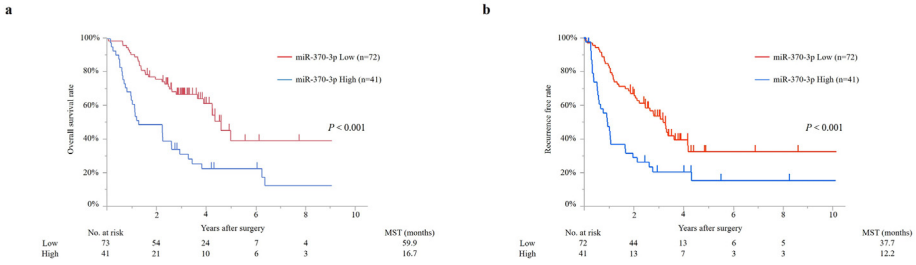

Fig. 3. (a) Overall survival (OS) and (b) recurrence-free survival (RFS) curves stratified by the plasma miR-370-3p expression level. Upregulated miR-370-3p levels were significantly associated with poor prognosis in both OS ($p < 0.001$) and RFS ($p < 0.001$) analyses.

Table 3
Univariate and multivariate analyses of the prognostic factors for OS.

Factor		Univariate analysisn	Univariate analysis		Multivariate analysis	
			MST (months)	*P*	HR (95 % CI)	*P*
Age	≧70	65	44.7	0.316		
	<70	48	49.8			
Sex	Male	57	50.7			
	Female	56	36.2	0.347		
Preoperative CA19-9,IU/U	≧37	80	44.7	0.391		
	< 37	33	81.2			
Tumor diameter,mm	≧30	57	21.8	<0.001	1.89 (1.10−3.25)	0.021
	< 30	56	64.9			
Resectability Status	Resectable	83	56.7			
	Borderline	30	14.5	<0.001	2.07 (1.06−4.04)	0.034
Adjuvant chemotherapy	Yes	84	49.8			
	No	29	17.1	0.23		
Operative time,min	≧300	85	34	0.049	1.35 (0.65−2.80)	0.42
	< 300	28	55.2			
Portal vein resection	Yes	41	34	0.032	0.87 (0.46−1.63)	0.67
	No	72	55.2			
Postoperative complication	Yes	13	47.5			
	No	100	44.7	0.99		
Histological grade	Grade1	32	59.9			
	Grade2/3	81	34	0.164		
Residual tumor	R0	80	55.2			
	R1	33	19.9	0.003	1.42 (0.83−2.43)	0.19
miR-370	high	41	16.7	<0.001	2.25 (1.36−3.72)	0.002
	low	72	59.9			

Table 4
Univariate and multivariate analyses of the prognostic factors for RFS.

Factor		Univariate analysisn	Univariate analysis		Multivariate analysis	
			MST (months)	*P*	HR (95 % CI)	*P*
Age	≧70	65	31.5	0.85		
	<70	48	28			
Sex	Male	57	31.5	0.83		
	Female	56	25.7			
Preoperative CA19-9,IU/U	≧37	80	25.3	0.9		
	< 37	33	30.3			
Tumor diameter,mm	≧30	57	12.6	0.002	1.80 (1.09–2.97)	0.021
	< 30	56	37.9			
Resectability Status	Resectable	83	36.5	0.001	0.53 (0.27–1.02)	0.059
	Borderline	30	10.8			
Adjuvant therapy	Yes	84	28	0.91		
	No	29	30.3			
Operative time,min	≧300	85	22.9	0.1		
	< 300	28	37.9			
Portal vein resection	Yes	41	15.8	0.028	0.90 (0.50–1.64)	0.74
	No	72	37.7			
Postoperative complication	Yes	13	N/A	0.69		
	No	100	25.7			
Histological grade	Grade1	32	33.3	0.27		
	Grade2/3	81	23.6			
Residual tumor	R0	80	32.1	0.04	0.75 (0.45–1.24)	0.26
	R1	33	13.1			
miR-370	high	41	12.2	0.002	2.00 (1.25–3.22)	0.004
	low	72	37.7			

N/A not available.

stages [24]. Additionally, they found that miR-370-3p activates the NF-κB signaling pathway by targeting FBLN5 to promote the proliferation, metastasis, and stemness of breast cancer cells. A similar mechanism of action may exist between miR-370-3p expression and lymph node metastasis in PDAC. However, this study showed that there was no significant relationship between the high expression of miR-370-3p and portal vein invasion or tumor size. Further study is warranted to investigate whether other mechanisms of miR-370-3p unique to lymph node metastasis exist in PDAC.

This study demonstrated that the upregulation of plasma miR-370-3p expression was an independent poor prognostic factor in patients with PDAC. Moreover, this study indicated that the upregulation of plasma miR-370-3p expression was correlated with recurrence. Based on these results, serum miR-370-3p expression levels may help evaluate the risk of recurrence prior to surgical resection. If the prognosis can be predicted precisely, appropriate treatments can be selected according to the risk of recurrence. Adjuvant chemotherapy also plays an important role in PDAC treatment. CA19-9 is also useful for chemotherapy monitoring in patients of PDAC [28]. However, as some Lewis antigen-negative patients show a false negative result for CA19-9, it may not be suitable for all patients. Meijer et al. reported that downregulation of plasma miR-181a-5p expression predicted the response of PDAC to the FOLFIRINOX chemotherapy regimen [12]. Our findings indicated that the upregulation of plasma miR-370-3p expression might also be a useful biomarker for monitoring chemotherapeutic responses. Further clinical trials using more patients are needed to investigate whether plasma miR-370-3p is a useful biomarker for monitoring the response of PDAC to various chemotherapies. In the future, we plan to investigate plasma miR-370-3p expression before and after chemotherapy in patients with PDAC.

Some limitations of this study must be reviewed. First, it was performed retrospectively at a single institution with a small sample size. Thus, a large, multicenter study is required to clarify the usefulness of plasma miR-370-3p expression levels as a prognostic biomarker for PDAC. Second, only patients with PDAC of the pancreatic head were enrolled in this study. Hence, future studies evaluating the clinical usefulness of plasma miR-370-3p expression levels as a biomarker for PDAC of the pancreatic tail or body are needed.

To the best of our knowledge, this is the first study to demonstrate that upregulation of miR-370-3p expression in plasma is associated with poor prognosis in patients with PDAC. Notably, a significant association was observed between miR-370-3p expression and lymph node metastasis. Therefore, this study indicated that plasma miR-370-3p expression upregulation correlates with a highly malignant grade of PDAC.

Declaration of competing interest

The authors have no conflicts of interest to disclose concerning this paper.

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