



# MiR-194-5p in Pancreatic Ductal Adenocarcinoma Peritoneal Washings is Associated with Peritoneal Recurrence and Overall Survival in Peritoneal Cytology-Negative Patients

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## ABSTRACT

**Background.** Peritoneal dissemination is one of the major recurrence patterns in patients with pancreatic ductal adenocarcinoma (PDAC) and is associated with poor prognosis. Here, we assessed the diagnostic potential of microRNA (miRNA) profiles in peritoneal washings for prediction of peritoneal dissemination in PDAC.

**Patients and Methods.** From January 2016 to July 2017, peritoneal washings were obtained prospectively from 59 patients with PDAC undergoing surgery the Yokohama City University Hospital. MiRNA expression was evaluated by Agilent human miRNA microarray and quantitative reverse-transcription polymerase chain reaction.

**Results.** Microarray analysis identified upregulated and downregulated miRNAs in peritoneal washings of patients with peritoneal dissemination. We validated four miRNAs (miR-141-3p, miR-194-3p, miR-194-5p, and miR-200c-3p) with high expression in peritoneal washings. The cumulative incidence rate of peritoneal recurrence in peritoneal cytology-negative patients in the miR-194-5p high group was significantly higher than that in the miR-194-5p low

group ( $p = 0.002$ ). Univariate and multivariate analyses revealed that high miR-194-5p was associated with overall survival (OS).

**Conclusions.** High expression of miR-194-5p in peritoneal washings is associated with peritoneal recurrence and poor OS in patients with peritoneal cytology-negative PDAC.

Pancreatic ductal adenocarcinoma (PDAC) is a rare cancer for which no significant improvements in diagnosis and therapy have been made in the last 30 years. Despite considerable progress in understanding of the disease at the molecular level, novel findings have not yet translated into clinical benefits, and most patients still face a grim median survival of 5–6 months. With over 43,000 PDAC-related deaths in the USA in 2017 and over 42,000 in Europe in 2016, this malignancy is currently the fourth leading cause of cancer-related death and is predicted to become the second by 2030.<sup>1–3</sup> Peritoneal dissemination of pancreatic cancer poses significant difficulties for both patients and clinicians because the associated poor general condition of affected patients and problems in assessing scattered tumors, due to ascites, jaundice, and ileus, hamper administration of standard treatment. Peritoneal recurrence of pancreatic cancer is one of the major common recurrence patterns according to previous reports.<sup>4–7</sup>

In some types of cancer, such as ovarian and gastric cancer, which are known to have a high rate of postoperative peritoneal recurrence, peritoneal wash cytology (CY) is often used to predict risk of peritoneal dissemination.<sup>8,9</sup> However, the prognostic significance of CY positivity in

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potentially resectable pancreatic cancer is controversial. Some studies have reported that CY positivity without distant metastasis should not preclude resection in resectable patients with pancreatic cancer and that, in these cases, long-term survival is possible after adjuvant chemotherapy.<sup>10–12</sup> In contrast, several other studies have shown that patients with CY (+) status are associated with advanced disease and poor prognosis and have survival rates equivalent to those of other stage IV diseases.<sup>7,13–16</sup> We previously reported that high expression of *MUC1* in peritoneal washings is a significant risk factor for peritoneal recurrence after R0 resection of CY (–) PDAC.<sup>17</sup> However, the predictive power of *MUC1* may be limited, as univariate analysis was performed in that previous study. Therefore, better prognostic predictors in pancreatic cancer are needed.

MicroRNAs (miRNAs) are small noncoding RNAs with length of 20–23 nucleotides;<sup>18</sup> they regulate the expression of more than 30% of human genes and therefore play key roles in many biological functions.<sup>19</sup> A number of miRNAs have been reported as predictors of clinical outcome in pancreatic adenocarcinoma;<sup>20</sup> For example, miR-21 and miR-1225-5p may serve as biomarkers of peritoneal recurrence after curative gastric cancer resection.<sup>21</sup> However, no studies have identified relevant miRNAs in peritoneal washings as biomarkers of peritoneal recurrence of any abdominal cancer. The purpose of this study is to identify highly expressed miRNAs in cases of peritoneal dissemination and investigate whether they are associated with peritoneal recurrence after resection.

## PATIENTS AND METHODS

### *Ethics Committee Approval*

The study protocol was approved by the Institutional Ethical Committee at the Yokohama City University (Yokohama, Japan; B111110029); written informed consent was obtained from all patients prior to enrollment in the study.

### *Clinical Samples*

From January 2016 to July 2017, peritoneal washings were obtained prospectively from 63 patients with preoperative diagnosis of pancreatic cancer undergoing surgery at Yokohama City University Hospital. Four patients diagnosed with intraductal papillary–mucinous carcinoma after surgery were excluded from the study.

We poured 600 mL of saline into the right upper abdomen, left upper abdomen, and pelvis during laparotomy and collected 300 mL of total peritoneal lavage fluid

from each site. Half of this fluid was used for routine CY examination, while the remaining half was used for quantitative polymerase chain reaction (qPCR) evaluation. In all cases, we obtained peritoneal washings at the beginning of surgery to avoid blood cell contamination. qPCR results were not used for therapeutic decision-making for any patient.

Follow-up data were obtained from patients' medical records. To confirm no recurrence, physical examination and laboratory investigations, including tests for tumor markers, were performed every month, and computed tomography (CT) scanning was performed every 3 months. If we could not diagnose recurrence through CT scanning despite findings suggesting recurrence (such as an increase in the levels of tumor markers), we performed positron emission tomography-CT (PET-CT). The gold standard for diagnosing peritoneal recurrence is positive CY for ascites accumulation after surgery. However, even if CY for ascites was not performed, peritoneal dissemination nodules were detected using CT or PET-CT. Severity of postoperative complications was graded according to the Clavien–Dindo system.<sup>22,23</sup>

### *Total RNA Extraction*

Total RNAs (including miRNA) from peritoneal washings were extracted using an miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and stored at – 80 °C until further analysis.

### *MiRNA Microarray*

MiRNAs extracted from the five peritoneal washing samples were analyzed using Agilent Human miRNA Microarrays (Santa Clara, CA, USA). Cyanine-3 (Cy3)-labeled miRNA was prepared from 100 ng total RNA using the miRNA Complete Labeling and Hyb Kit (Agilent) according to the manufacturer's instructions. The Cy3-labeled miRNA was resuspended in 18 µL of nuclease-free water, and 4.5 µL of 10× GE Blocking Agent and 22.5 µL of 2 × Hi-RPM Hybridization Buffer were added, following the manufacturer's instructions. After the sample had been incubated at 100 °C for 5 min, it was immediately transferred to an ice–water bath for 5 min and hybridized to Human miRNA Microarray Kit 8 × 60 K rel.21.0 (Agilent) for 20 h at 55 °C in an Agilent rotating hybridization oven. After hybridization, microarrays were washed for 5 min at 20–25 °C with GE wash buffer 1 (Agilent) and 5 min with 37 °C GE wash buffer 2 (Agilent), then dried. Microarrays were scanned immediately after washing on an Agilent SureScan Microarray Scanner (G2600D) using the one-color scan setting for 8 × 15 k array slides (scan area 61 × 21.6 mm<sup>2</sup>, scan resolution 5

µm; dye channel set to green; green PMT set to XDR Hi 100% and XDR Lo 5%). The scanned images were analyzed with Feature Extraction Software 12.0.3.1 (Agilent) using default parameters to obtain background-subtracted and spatially detrended processed signal intensities. The GeneView files were generated using Feature Extraction Software.

### qPCR

Peritoneal washings were centrifuged at 2000 rpm for 10 min with MX300 (TOMY SEIKO, Japan), and the supernatant was removed. After addition of 1 mL of phosphate-buffered saline, the specimens were centrifuged again at 10,000 rpm for 5 min at 4 °C. Total RNA was extracted from the remaining pellet after homogenization with QIAzol (Qiagen), followed by on-column clean up with the miRNeasy Mini Kit (Qiagen). Reverse transcription (RT) was performed using 2 µL of RNA template per 10 µL of RT reaction with the Universal cDNA Synthesis Kit II (Exiqon, Vedbaek, Denmark).

Four microliters of complementary DNA (cDNA) was used to amplify has-miR-194-3p, has-miR-194-5p, has-miR-141-3p, has-miR-200c-3p, has-miR-28-3p, has-miR-3663-5p, has-miR-4446-3p, has-miR-3121-3p, has-miR-8485, and has-miR-26b-5p endogenous control, using the miRCURY LNA<sup>TM</sup> miRNA PCR Assays system (Qiagen), in a final volume of 10 µL. The qPCR master mix contained 80-fold-diluted cDNA and 1 × ExiLENT SYBR green master mix (Exiqon #203403). All reactions were performed in triplicate with the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), and the mean values were analyzed to prevent dispersion of results. Relative quantification of miRNA expression was calculated using the  $2^{-\Delta\Delta C_t}$  method.<sup>24</sup>

### Statistical Analysis

Data were analyzed using SPSS version 25 for Windows (SPSS, Chicago IL, USA). For univariate analysis, binomial variables were compared using the  $\chi^2$  test. Continuous variables were compared using the Mann–Whitney *U* test. The continuous variables selected based on univariate analysis were converted to dichotomous variables using receiver operating characteristic (ROC) curve analysis. Multivariate survival analyses were performed by Cox proportional hazards regression. Significant parameters on univariate analyses were included in multivariate analyses for overall survival (OS). The cumulative incidence rate of peritoneal dissemination recurrence was estimated (instead of Kaplan–Meier estimates) because other recurrences followed by systemic treatment competed with the peritoneal dissemination recurrence. Survival curves were

constructed using the Kaplan–Meier method and compared using the log-rank test.  $P < 0.05$  was considered significant.

## RESULTS

### Identification of Deregulated MiRNA

We first performed miRNA array analysis using samples from five patients with PDAC to identify differentially expressed miRNA in patients with peritoneal dissemination. Three of them had unresectable PDAC because of peritoneal dissemination, one had peritoneal recurrence after surgery, and the other was relapse-free after surgery during the observation period. We compared patients with unresectable PDAC and peritoneal dissemination and relapse-free patients, and patients with peritoneal recurrence and relapse-free patients and identified upregulated (fold change > 1) and downregulated (fold change < – 1) miRNAs. We selected eight miRNAs (miR-200c-3p, miR-141-3p, miR-3663-5p, miR-194-3p, miR-194-5p, miR-4446-3p, miR-3121-3p, and miR-8485, Table 1): five upregulated miRNAs (miR-200c-3p, miR-141-3p, miR-3663-5p, miR-194-3p, and miR-194-5p) and three downregulated miRNAs (miR-4446-3p, miR-3121-3p, and miR-8485). In unresectable patients with peritoneal dissemination versus relapse-free patients, miR-200c-3p (11.5-fold), miR-141-3p (7.48-fold), miR-3663-5p (5.85-fold), miR-194-3p (5.22-fold), and miR-194-5p (3.49-fold) were increased, while miR-4446-3p (3.15-fold), miR-3121-3p (2.91-fold), and miR-8485 (2.18-fold) were decreased. In patients with peritoneal recurrence versus relapse-free patients, miR-3663-5p (5.69-fold), miR-200c-3p (5.22-fold), miR-194-3p (3.71-fold), miR-141-3p (1.42-fold), and miR-194-5p (1.1-fold) were increased, while miR-8485 (2.14-fold), miR-4446-3p (1.33-fold), and miR-3121-3p (1.08-fold) were decreased.

### Characteristics of Patients with Pancreatic Cancer

From January 2016 to July 2017, peritoneal washings were obtained prospectively from 59 patients with PDAC undergoing surgery at Yokohama City University Hospital. Radical resection was not performed in 17 patients because of detection of distant metastatic lesions: seven patients had liver metastasis, eight had peritoneal dissemination, and two had paraaortic lymph node metastasis. We routinely applied CY from 2010 to 2017 in our department, but CY positivity was observed in only 2 cases of 224 resected cases. Although our institution has a policy of resection even if the CY from the washing is positive, the resected cases were all cytologically negative in this study

**TABLE 1** MiRNAs significantly upregulated and downregulated in PDAC patients based on miRNA microarray

	P UR ( <i>N</i> = 3) (average)	Non rec ( <i>N</i> = 1)	Fold change [log2]	<i>P</i> rec ( <i>N</i> = 1)	Non rec ( <i>N</i> = 1)	Fold change [log2]
<i>Upregulated miRNAs</i>						
MiR-200c-3p	649.7	0.1	11.50	3.7	0.1	5.22
MiR-141-3p	1198.4	4	7.48	10.8	4	1.42
MiR-3663-5p	7.0	0.1	5.85	5.2	0.1	5.69
MiR-194-3p	4.0	0.1	5.22	1.3	0.1	3.71
MiR-194-5p	745.2	53.3	3.49	114.6	53.3	1.1
<i>Downregulated miRNAs</i>						
MiR-4446-3p	5.9	46.4	- 3.15	18.5	46.4	- 1.33
MiR-8485	30.9	121	- 2.18	27.6	121	- 2.13
MiR-3121-3p	2.1	7.6	- 2.91	3.6	7.6	- 1.08

The continuous variable represents the total gene signal

miRNA micro RNA, PDAC pancreatic ductal adenocarcinoma, *P UR* unresectable with peritoneal dissemination, *Non rec* relapse-free patients, *P rec* patients with peritoneal recurrence

period. In other words, of the 42 resected patients included in this study, CY was negative in all cases, 10 (23.8%) had peritoneal recurrence and were therefore classified into the peritoneal recurrence group, while the remaining 32 (76.2%) were classified into the non-peritoneal recurrence group. In the non-peritoneal recurrence group, 17 patients experienced recurrence with distant metastasis: seven patients had metastasis in the liver, five in the lungs, and two in the paraaortic lymph nodes. Three patients had local recurrence, while 15 patients did not experience recurrence within the follow-up period.

For the 42 patients who underwent resection, we reviewed the demographic information and clinicopathological characteristics, including tumor size, location, and grade (Table 2). There were no significant differences in age, sex, tumor marker level at operation, tumor location, tumor size, or clinical TNM stage between the peritoneal recurrence and non-peritoneal recurrence groups. The proportion of neural invasion ( $p = 0.083$ ) and vascular invasion ( $p = 0.026$ ) was higher in the peritoneal recurrence group than in the non-peritoneal recurrence group. The median observation period after surgery was 19.8 months.

#### *Expression of MiR-141-3p, MiR-194-3p, MiR-194-5p, and MiR-200c-3p in Peritoneal Washings*

Peritoneal washing samples were collected from the 59 patients with PDAC, and the levels of the eight selected miRNAs were assessed by qRT-PCR. We validated four of the miRNAs (miR-141-3p, miR-194-3p, miR-194-5p, and miR-200c-3p).

We then investigated whether these four miRNAs were associated with peritoneal recurrence after resection. Resected

patients with peritoneal recurrence had higher levels of miR-141-3p ( $p = 0.040$ ), miR-194-5p ( $p = 0.019$ ), and miR-200c-3p ( $p = 0.030$ ) than patients with no recurrence or non-peritoneal recurrence (Fig. 1).

The cutoff value for the selected continuous variable for predicting peritoneal recurrence on univariate analysis was estimated using ROC curves. The best cutoff value was 63.4 for miR-141-3p/miR-26b-5p, 3.11 for miR-194-5p/miR-26b-5p, and 9.80 for miR-200c-3p/miR-26b-5p. Using this cutoff value, we converted the quantity of miR-141-3p/miR-26b-5p, miR-194-5p/miR-26b-5p, and miR-200c-3p/miR-26b-5p to a binary variable (high or low). Of the 42 patients, 18 were miR-141-3p high and 24 were miR-141-3p low, while 16 were miR-194-5p high and 26 were miR-194-5p low. In the same way, 18 were miR-200c-3p high and 24 were miR-200c-3p low.

In this study, 28 of 42 patients received neoadjuvant chemoradiotherapy. Among 17 cases with high miR-194-5p, 12 received neoadjuvant chemoradiotherapy. Out of 25 cases with low miR-194-5p, 16 received neoadjuvant chemoradiotherapy. The Chi square test gave a *P* value of 0.747; That is, no correlation was found between miR-194-5p expression and neoadjuvant chemoradiotherapy.

#### *Association of MiR-194-5p with Peritoneal Recurrence and OS*

The cumulative incidence rate of peritoneal recurrence in the miR-194-5p high group was significantly higher than that in the miR-194-5p low group ( $p = 0.001$ ). Median peritoneal recurrence-free survival for the miR-194-5p low group was 19.0 months. Comparison of OS between the miR-194-5p high and low groups revealed that OS was

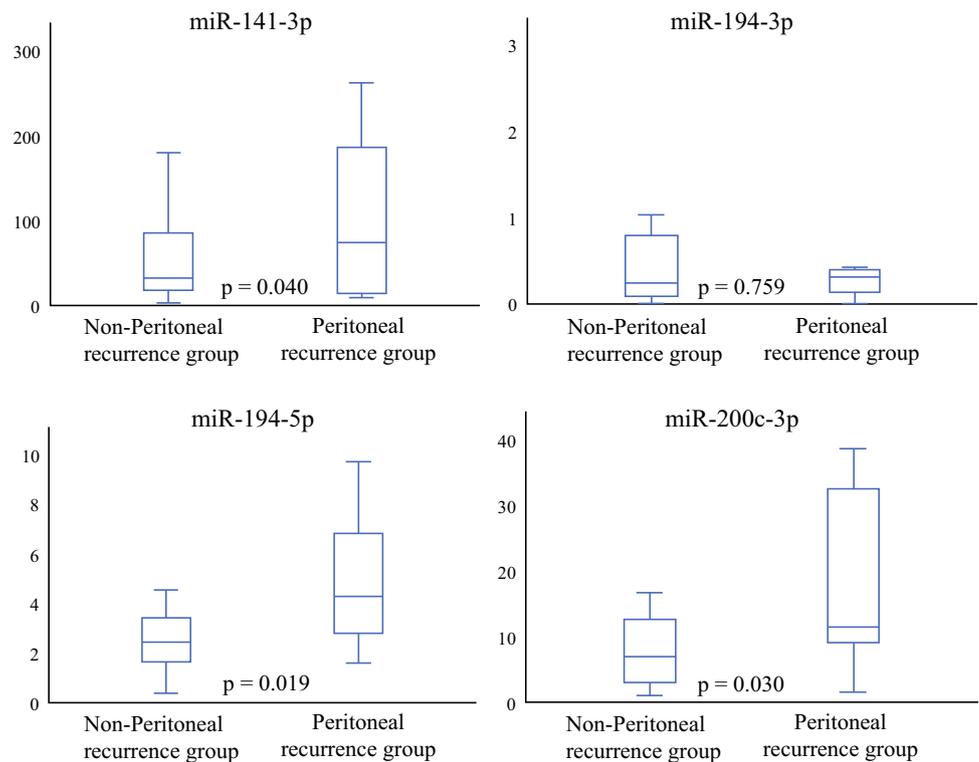
**TABLE 2** Histopathological characteristics of the two groups stratified by peritoneal recurrence status

Variable	Peritoneal recurrence group (N = 10)	Non-peritoneal recurrence group (N = 32)	P value
Age, years	70.5 (58–74)	68 (65.5–74.5)	0.531
Sex			
Male	7	17	0.473
Female	3	15	
Tumor markers			
CEA (ng/mL)	4.1 (3.5–5.6)	3.0 (2.15–4.30)	0.201
CA19-9 (U/mL)	138 (12–182)	24 (12.5–75.0)	0.742
SPan-1 (U/mL)	51 (11–67)	19 (10–32)	0.524
DUPAN-2 (U/mL)	49 (25–430)	58 (25–345)	0.670
Tumor location			0.404
Head	9	23	
Body/tail	1	9	
Tumor size (cm)	2.7 (2.2–3.7)	2.40 (1.70–2.95)	0.932
Neoadjuvant chemoradiotherapy			0.451
No	2	12	
Yes	8	20	
Adjuvant chemotherapy			0.657
No	1	5	
Yes	9	27	
Operative procedures			0.404
PD	9	23	
DP/DP-CAR	1	9	
UICC T-stage			0.506
T1	0	1	
T2	0	0	
T3	5	21	
T4	5	10	
Lymph node metastasis			0.679
Negative	3	18	
Positive	7	14	
Neural invasion			0.083
No	2	15	
Yes	8	17	
Vascular invasion			0.026
No	2	21	
Yes	8	11	
UICC stage			0.665
IA	0	1	
IB	0	0	
IIA	2	12	
IIB	3	8	
III	5	11	

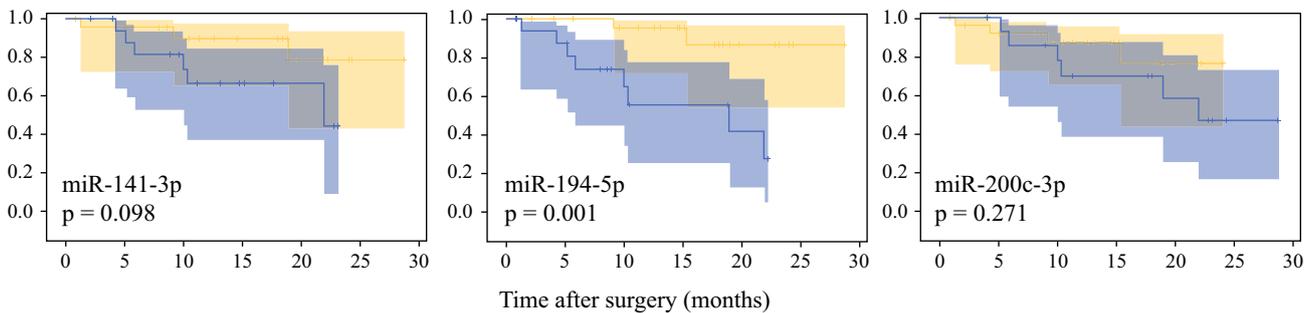
Continuous variables shown as median (interquartile range)

CEA carcinoembryonic antigen, CA19-9 carbohydrate antigen 19-9, PD pancreaticoduodenectomy, DP distal pancreatectomy, DP-CAR distal pancreatectomy with en bloc celiac axis resection

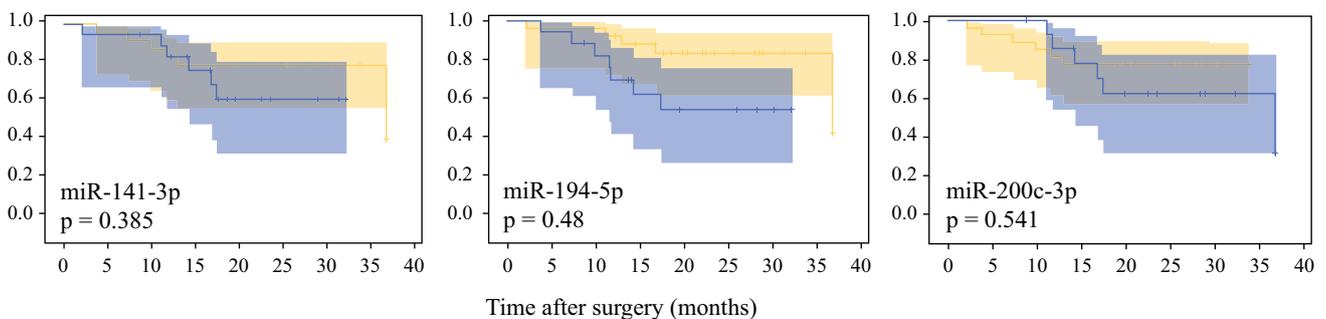
**FIG. 1** MiR-194-5p, miR-141-3p, and miR-200c-3p in peritoneal washings were associated with peritoneal recurrence. Box plots comparing expression of four miRNAs in the peritoneal recurrence group and non-peritoneal recurrence group. Expression of miR-194-5p, miR-141-3p, and miR-200c-3p in the peritoneal recurrence group was significantly higher than in the non-peritoneal recurrence group



**A** Peritoneal recurrence free survival



**B** Overall survival



— High Expression level of cut-off value      — Low Expression level of cut-off value  
 ■ 95% range of High Expression level      ■ 95% range of Low Expression level

**FIG. 2** MiR-194-5p in peritoneal washings was associated with peritoneal recurrence and OS: Kaplan–Meier plots of **a** peritoneal recurrence and **b** OS for miR-141-3p, miR-194-5p, and miR-200c-3p

significantly shorter for the former ( $p = 0.048$ ; Fig. 2). The median OS of miR-194-5p high patients was 36.9 months.

Univariate analysis showed that DUPAN-2, miR-194-5p, and vascular invasion were associated with peritoneal recurrence. Multivariate analysis showed that miR-194-5p and vascular invasion were independent factors of peritoneal recurrence (Table 3). Additionally, univariate analysis showed that neoadjuvant chemoradiotherapy, adjuvant chemotherapy, and miR-194-5p were associated with OS. Multivariate analysis, miR-194-5p, and adjuvant chemotherapy were independent factors of OS (Table 3).

## DISCUSSION

Unlike previous reports, which assessed the levels of prognostic biomarkers in pancreatic cancer tissues, this study focused on detection of factors in peritoneal washings from patients with PDAC. To discover factors for early peritoneal recurrence of PDAC, we performed miRNA microarray analysis and identified three miRNAs that were specifically downregulated (miR-4446-3p, miR-8485, and miR-3121-3p) and five that were specifically upregulated (miR-200c-3p, miR-141-3p, miR-3663-5p, miR-194-3p, and miR-194-5p) in PDAC patients with peritoneal dissemination. Four of these miRNAs (miR-200c-3p, miR-141-3p, miR-194-3p, and miR-194-5p) were validated by qRT-PCR, and one of them (miR-194-5p) was associated with peritoneal recurrence after radical resection on Kaplan–Meier analysis and with OS on univariate and multivariate analyses.

The American Joint Committee on Cancer and treatment guidelines from the National Comprehensive Cancer Network consider CY (+) as stage IV metastatic cancer and a contraindicator for pancreatic resection.<sup>25,26</sup> However, many patients develop peritoneal recurrence, despite negative CY.<sup>27</sup> This study showed that miR-194-5p in peritoneal washings is a significant risk factor for both peritoneal recurrence and OS.

A study showed that upregulation of miR-194 promotes tumor growth and progression in PDAC, possibly via suppression of Dachshund homolog1 (DACH1).<sup>28</sup> Similarly, Mees et al.<sup>29</sup> reported that upregulation of miR-194 correlates with reduced expression of tumor suppressor EP300 in metastatic PDAC cells. These findings suggest that miR-194 plays a favorable role in PDAC progression. Accordingly, we demonstrated here that high expression of miR-194-5p is associated with peritoneal recurrence.

MiR-194-5p is associated with epithelial-to-mesenchymal transition (EMT) in gastric cancer,<sup>30</sup> and EMT plays a crucial role in the invasion and metastasis of diverse carcinomas, including PDAC.<sup>31</sup> EMT is characterized by loss of epithelial cell-to-cell contacts and acquisition of motile mesenchymal features by epithelial cells, leading to cell migration and invasion,<sup>32–35</sup> resulting in distant metastasis and peritoneal dissemination. Based on these findings, we speculate that miR-194-5p may promote EMT of PDAC cells and cause peritoneal dissemination.

In the present study, CY was negative in all cases, but peritoneal recurrence was observed in 10 cases. Nine of these 10 cases received tegafur/gimeracil/oteracil (S-1; Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) as adjuvant chemotherapy. Although 8 of 17 miR-194-5p high patients had peritoneal recurrence, their survival was significantly better than that of patients with miR-194-5p high expression without adjuvant chemotherapy. Also, miR-194-5p low patients who received adjuvant chemotherapy showed much higher survival curves than patients with miR-194-5p low without adjuvant chemotherapy (Supplementary Fig. 3). These results suggest that S-1 adjuvant therapy has a role in the improvement of survival. Conroy et al. reported that adjuvant therapy with a modified FOLFIRINOX regimen led to significantly longer survival than gemcitabine among patients with resected pancreatic cancer, but with higher incidence of toxic effects.<sup>36</sup> Considering this high toxicity, selection of patients is

**TABLE 3** Multivariate analysis for peritoneal recurrence and overall survival

Factor	Peritoneal recurrence		Overall survival	
	Multivariate analysis		Multivariate analysis	
	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value
MiR-194-5p expression level		0.010		0.047
Low	1.00		1.00	
High	7.792 (1.64–46.99)		3.61 (1.01–12.87)	
Vascular invasion		0.040		–
Negative	1.00		–	
Positive	5.10 (1.08–24.12)		–	
Adjuvant chemotherapy		–		0.001
Yes	–		1.00	
No	–		10.15 (2.59–39.75)	

HR hazard ratio, CI confidence interval

important, and miR-194-5p in peritoneal washings might be useful for this purpose. In addition, use of neoadjuvant therapy for treatment of potentially resectable pancreatic cancer remains controversial.<sup>37–39</sup> In Japan, Motoi et al. reported neoadjuvant chemotherapy for resectable pancreatic cancer in the PREP-01 study.<sup>40</sup> We suppose that neoadjuvant chemotherapy may become more common even for resectable pancreatic cancer in the near future. We hope that use of findings of miR-194-5p in peritoneal washings will be beneficial to improve patients' survival. We are currently preparing a prospective study concerning miR-194-5p in peritoneal washings for future individualized treatment strategies for pancreatic cancer.

Liquid biopsies, a recently adopted term for blood-based molecular analyses for cancer diagnostics, enables monitoring of disease evolution and response to treatment. One major advantage of liquid biopsies is the ability to compare serial samples from the same patient and thus generate a molecular readout of disease progression and therapy response or resistance in real time. Additionally, blood collection is minimally invasive and provides a biospecimen of comparable composition from a homogeneous compartment, i.e., the bloodstream.<sup>41</sup> Although miR-194-5p has been reported as a biomarker for osteoporosis and hepatitis B in liquid biopsies, there are no similar reports for pancreatic cancer.<sup>42,43</sup> In the near future, prediction of early relapse in patients may be possible by detecting relevant biomarkers in more convenient samples, including blood.

This study has limitations worth noting. Only a single institute was included in this retrospective analysis. In addition, the sample size was small, and the follow-up period was relatively short. Furthermore, the effect of neoadjuvant chemotherapy on miRNAs in peritoneal washings is also unknown. However, this study identified the possibility that miR-194-5p is associated with not only peritoneal recurrence but also OS. Further studies on a larger number of patients with longer follow-up periods are warranted.

In conclusion, miR-194-5p in peritoneal washings is associated with peritoneal recurrence and OS in patients with PDAC and may provide guidance for pancreatic cancer treatment.

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**DISCLOSURE** The authors declare that they have no conflicts of interest.

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