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Extracellular microRNA profiling for prognostic prediction in patients with high-grade serous ovarian carcinoma

Kosuke Yoshida[1,2,3] | Akira Yokoi[1,2] | Juntaro Matsuzaki[3,4] | Tomoyasu Kato[5] | Takahiro Ochiya[6] | Hiroaki Kajiyama[1] | Yusuke Yamamoto[3]

[1]Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, Nagoya, Japan

[2]Institute for Advanced Research, Nagoya University, Nagoya, Japan

[3]Laboratory of Integrative Oncology, National Cancer Center Research Institute, Tokyo, Japan

[4]Division of Pharmacotherapeutics, Faculty of Pharmacy, Keio University, Tokyo, Japan

[5]Department of Gynecology, National Cancer Center Hospital, Tokyo, Japan

[6]Department of Molecular and Cellular Medicine, Institute of Medical Science, Tokyo Medical University, Tokyo, Japan

Correspondence
Akira Yokoi, Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, Tsuruma-cho 65, Showa-ku, Nagoya 466-8550, Japan.
Email: ayokoi@med.nagoya-u.ac.jp

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Abstract

High-grade serous ovarian carcinoma is a leading cause of death in female patients worldwide. MicroRNAs (miRNAs) are stable noncoding RNAs in the peripheral blood that reflect a patient's condition, and therefore, they have received substantial attention as noninvasive biomarkers in various diseases. We previously reported the usefulness of serum miRNAs as diagnostic biomarkers. Here, we investigated the prognostic impact of the serum miRNA profile. We used the GSE106817 dataset, which included preoperative miRNA profiles of patients with ovarian malignancies. Excluding patients with other malignancy or insufficient prognostic information, we included 175 patients with high-grade serous ovarian carcinoma. All patients except four underwent surgery and received chemotherapy as initial treatment. The median follow-up period was 54.6 months (range, 3.5-144.1 months). Univariate Cox regression analysis revealed that higher levels of miR-187-5p and miR-6870-5p were associated with both poorer progression-free survival (PFS) and overall survival (OS), and miR-1908-5p, miR-6727-5p, and miR-6850-5p were poor prognostic indicators of PFS. The OS and PFS prognostic indices were then calculated using the expression values of three prognostic miRNAs. Multivariate Cox regression analysis showed that both indices were significantly independent poor prognostic factors (hazard ratio for OS and PFS, 2.343 [$P = .015$] and 2.357 [$P = .005$], respectively). In conclusion, circulating miRNA profiles can potentially provide information to predict the prognosis of patients with high-grade serous ovarian carcinoma. Therefore, there is a strong demand for early clinical application of circulating miRNAs as noninvasive biomarkers.

KEYWORDS
circulating miRNA, high-grade serous ovarian carcinoma, miR-187-5p, miR-6870-5p, noninvasive biomarker

Abbreviations: CI, confidence interval; EOC, epithelial ovarian cancer; EV, extracellular vesicle; FIGO, International Federation of Gynecology and Obstetrics; HGSOC, high-grade serous ovarian carcinoma; HR, hazard ratio; miRNA, microRNA; NSCLC, non-small-cell lung cancer; OS, overall survival; PARP, a poly (ADP-ribose) polymerase; PFS, progression-free survival; sEV, small extracellular vesicle.

1 | INTRODUCTION

Epithelial ovarian cancer is the fifth leading cause of the estimated cancer-associated deaths that will occur in the United States in 2021, as 295 414 newly diagnosed cases and 184 799 deaths were reported worldwide in 2018.[1,2] High-grade serous ovarian carcinoma is the most common histological subtype of EOC. No screening methods have been established; thus, patients with HGSOC are typically diagnosed at an advanced stage with extensive metastatic lesions in the abdominal cavity.[3] This is one reason for the high lethality of this malignancy. Standard treatment is a combination of cytoreductive surgery and platinum-containing chemotherapy, but EOC eventually develops platinum resistance.[3] In the past decade, an antivascular endothelial growth factor Ab and a PARP inhibitor were approved for clinical use and have contributed to the prolonged survival of these patients.[4,5] However, platinum resistance is still a challenge in EOC treatment.

MicroRNAs are small noncoding RNAs that are involved in the epigenetic regulation of their target genes and play important roles in cancer progression, including that of EOC.[6-8] Moreover, miRNAs stably exist in peripheral blood because they are protected from RNase degradation and are encapsulated by extracellular vesicles, such as exosomes.[9-11] EVs contain specific nucleic acids and proteins that reflect the characteristics of the cells of origin and that mediate cell-to-cell communication in the microenvironment, which results in cancer progression.[9,12] For example, serum miR-205 was found to be abundant in patients with metastatic EOC, and miR-205 carried by sEVs derived from EOC cells was shown to contribute to angiogenesis in vitro.[13] In the peritoneal cavity, EOC-derived EVs are concentrated in malignant ascites, and EVs carrying *MMP1* mRNA were shown to play an important role during the development of peritoneal dissemination.[14] Therefore, the levels of cell-free nucleic acids might be associated with tumor burden and aggressiveness; thus, these nucleic acids have received much attention as noninvasive biomarkers.[15] We have previously investigated circulating miRNAs as noninvasive biomarkers in ovarian cancers.[16-19] Specifically, we showed that a combination of circulating miRNAs was a promising diagnostic biomarker of EOC with high accuracy (sensitivity, 0.99; specificity, 1.00).[17] However, at that time, due to short follow-up periods, we could not evaluate the impact of circulating miRNAs on platinum sensitivity and patients' prognosis.

In this study, we investigated the association between serum miRNA profile and the prognosis of patients with HGSOC by assessing their clinical information.

2 | MATERIAL AND METHODS

2.1 | MicroRNA expression profile data and patient selection

In the previous study, we included 4046 serum samples from healthy controls and patients with ovarian tumors and investigated their comprehensive miRNA profiles using the 3D-Gene miRNA Labeling kit and the 3D-Gene Human miRNA Oligo Chip (Toray Industries).[17] The data are available through the NCBI database under the accession number GSE106817, and we used the pretreatment serum miRNA profile of 442 patients with ovarian tumors and 969 healthy controls in this study. In addition, we investigated clinical information, such as age, stage, histological subtype, and outcome. The study was approved by the National Cancer Center Hospital Institutional Review Board (2015-376, 2016-29), and each participant provided written informed consent.

We identified 180 patients with HGSOC by excluding 262 patients with other EOCs, other malignancies, borderline malignancies, or benign tumors (Figure 1). Then, after excluding three patients with insufficient clinical information and two patients with low-quality serum RNA samples, we assessed the association between serum miRNA profiles and the prognosis of 175 patients with HGSOC. Among them, two patients for whom details of recurrence were lost were excluded from the PFS analysis. In addition, of the 2038 miRNAs in the datasets, we selected 210 miRNAs according to the criteria used in the previous study.[17] Briefly, these miRNAs were detected in extracellular vesicles derived from ovarian cancer cells.

2.2 | In vitro analysis

A2780 and SK-OV-3 cell lines were purchased from the ATCC and maintained in RPMI-1640 (Nacalai Tesque) containing 10% FBS and antibiotics. The mirVana miRNA mimics for miR-187-5p (ID: MC12652), miR-1908-5p (ID: MC13846), miR-6870-5p (ID: MC27099), and negative control #1 were purchased from Thermo Fisher Scientific. Cells were seeded in 96-well plates and transfected with 20 nM mimic using Lipofectamine RNAi Max (Thermo Fisher Scientific). After 24, 48, and 72 h of incubation, cell viability was assessed using CellTiter-Glo 2.0 Cell Viability Assay (Promega) and a microplate reader (Gen5 Synergy H4; BioTek). For drug sensitivity analysis, after 24 h of transfection, the culture medium was replaced with cisplatin (Nichi-Iko Pharmaceutical) or docetaxel (Tokyo Chemical Industry) containing medium, and cells were incubated for 48 h. Cell viability was then assessed using CellTiter-Glo 2.0 Cell Viability Assay.

To evaluate transfection efficacy, quantitative RT-PCR was carried out. Total RNA was extracted using the miRNeasy Mini Kit (Qiagen), and cDNA was synthesized using the TaqMan Advanced miRNA cDNA Synthesis Kit (Thermo Fisher Scientific). TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific) and TaqMan Advanced miRNA Assays (assay IDs 479423_mir, 478735_mir, and 480864_mir; Thermo Fisher Scientific) were used for quantitative RT-PCR.

2.3 | Statistical analyses

Statistical analyses were carried out with SPSS version 27 (IBM). Overall survival was defined as the time from treatment initiation

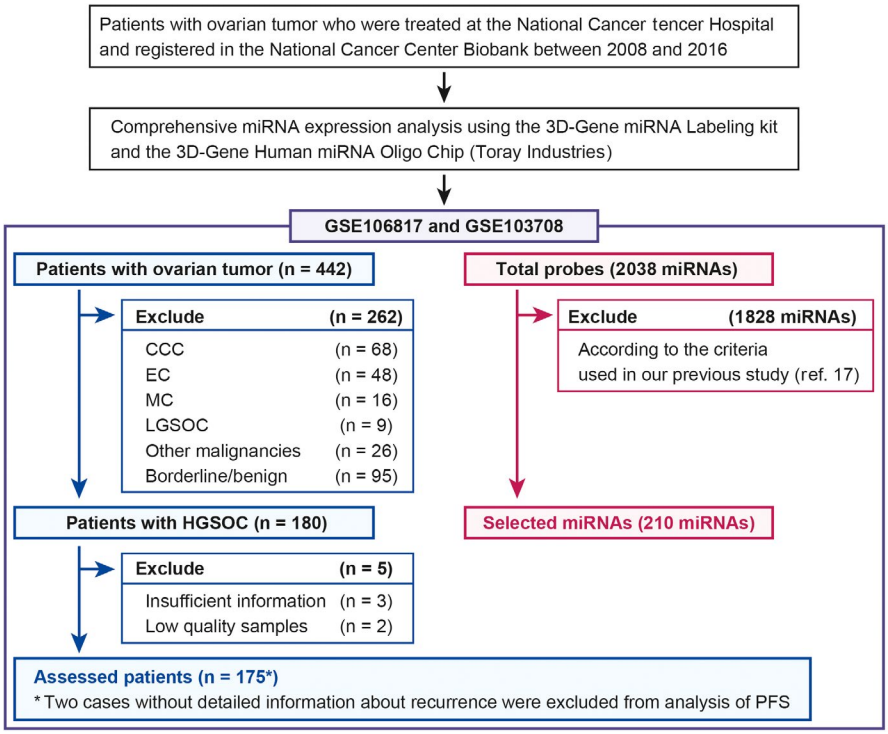

FIGURE 1 Flowchart of patients and microRNA (miRNA) selection. This study was based on the GSE106817 dataset, which was compiled using serum miRNA profiles of patients with ovarian tumors used in our previous study.[17] CCC, clear cell carcinoma; EC, endometrioid carcinoma; HGSOC, high-grade serous ovarian carcinoma; LGSOC, low-grade serous ovarian carcinoma; MC, mucinous carcinoma; PFS, progression-free survival

to death from any cause; PFS was defined as the time from treatment initiation to tumor progression. Kaplan-Meier curves were used for the analysis of OS and PFS and were compared using the log-rank test. Univariate and multivariate Cox regression analyses were used to calculate HRs and 95% CIs. The prognostic indices for OS and PFS were separately calculate based on a multivariate Cox regression model for miRNA candidates. The indices for OS (index-OS1 and index-OS2) and for PFS (index-PFS1 and index-PFS2) were as follows: (index-OS1) = 0.218 × (miR-187-5p) + 0.280 × (miR-6870-5p); (index-OS2) = 0.148 × (miR-187-5p) + 0.273 × (miR-6870-5p) + 0.186 × (miR-1908-5p); (index-PFS1) = 0.034 × (miR-187-5p) + 0.236 × (miR-6870-5p) + 0.504 × (miR-6727-5p) + 0.048 × (miR-1908-5p) − 0.251 × (miR-6750-5p); and (index-PFS2) = 0.031 × (miR-187-5p) + 0.231 × (miR-6870-5p) +0.351 × (miR-6727-5p), respectively. The Pearson correlation coefficient was used to evaluate the correlation between the two indices. The expression of miRNAs and cell viability was compared using Welch's *t* test. Differences at $P < .05$ were considered significant.

3 | RESULTS

In this study, we used 210 pretreatment serum miRNA levels from 175 patients with HGSOC (Figure 1), the characteristics of whom are shown in Table 1. The median age of the patients was 60 years (range, 28-82 years), and 161 patients (92.0%) were diagnosed with FIGO stage III or IV disease. As approximately half of the patients received neoadjuvant chemotherapy, 151 patients (86.3%) underwent complete or optimal cytoreductive surgery. Additionally, 150 patients (85.7%) received adjuvant chemotherapy, which was typically carboplatin plus paclitaxel combination chemotherapy. However, despite neoadjuvant chemotherapy, four patients with FIGO stage IV disease could not undergo surgery due to disease progression. Therefore, all the patients except four underwent surgery

TABLE 1 Clinical characteristics of 175 patients with high-grade serous ovarian carcinoma

Characteristic	Case (n = 175)
Age, y	
Median (range)	60 (28-82)
Stage	
I	4 (2.3)
II	10 (5.7)
III	104 (59.4)
IV	57 (32.6)
Neoadjuvant chemotherapy	
Yes	99 (56.6)
No	76 (43.4)
Surgery and residual tumor volume	
Complete/optimal surgery	151 (86.3)
Suboptimal surgery	20 (11.4)
No surgery	4 (2.3)
Adjuvant chemotherapy	
Yes	150 (85.7)
No	25 (14.3)

Note: Data are shown as n (%) unless otherwise noted.

and received chemotherapy as initial treatment. The median follow-up period was 54.6 months (range, 3.5-144.1 months).

First, to investigate the prognostic impact of 210 miRNAs on OS, we generated Kaplan-Meier curves after patients were stratified into high and low expression groups based on the median level of each miRNA. Thirteen miRNAs were associated with significantly poorer OS (miR-187-5p, $P = .040$; miR-711, $P = .033$; miR-1229-5p, $P = .024$; miR-1908-5p, $P = .011$; miR-1914-3p, $P = .041$; miR-4513, $P = .017$; miR-4656, $P = .017$; miR-4787-3p, $P = .040$; miR-6727-5p, $P = .044$; miR-6850-5p, $P = .012$; miR-6870-5p, $P = .024$; miR-7107-5p, $P = .034$; miR-7150, $P = .014$; Figure 2). In contrast, miR-6787-5p was the only miRNA associated with a favorable OS ($P = .045$; Figure 2). We then undertook a Cox regression analysis for OS and PFS using the 14 miRNAs as a continuous variable. Univariate Cox regression analysis for OS revealed that only two miRNAs were significantly associated with shorter OS and that the HRs of miR-6870-5p and miR-187-5p were 1.383 ($P = .011$) and 1.394 ($P = .046$), respectively (Figure 3A). Similarly, a univariate Cox regression analysis for PFS showed that the two miRNAs were also significantly associated with shorter PFS and that the HRs of miR-6870-5p and miR-187-5p were 1.280 ($P = .027$) and 1.375 ($P = .029$), respectively (Figure 3B). In addition, three more miRNAs were significantly related to poor PFS (miR-6727-5p [HR 1.469; $P = .009$], miR-6850-5p [HR 1.641; $P = .012$], and miR-1908-5p [HR 1.489; $P = .043$]; Figure 3B). Therefore, miR-187-5p and miR-6870-5p were associated with both PFS and OS, whereas miR-1908-5p, miR-6727-5p, and miR-6850-5p were related to PFS.

According to our previous report, 10 circulating miRNAs (miR-320a, miR-665, miR-3184-5p, miR-6717-5p, miR-4459, miR-6076, miR-3195, miR-1275, miR-3185, and miR-4640-5p) were used for the diagnostic model for discrimination between ovarian cancer and healthy control samples.[17] However, none of them were significantly associated with the prognosis of patients with HGSOC in this study. Conversely, the levels of the prognostic miRNAs in healthy controls were evaluated, and miR-187-5p and miR-6870-5p were significantly higher in patients with HGSOC than healthy controls ($P = .042$ and $P < .001$, respectively; Figure S1A). However, the levels of miR-1908-5p, miR-6727-5p, and miR-6850-5p were significantly lower in HGSOC ($P < .001$ in the three miRNAs; Figure S1B).

To improve the accuracy of prognostic miRNAs, the prognostic index for OS was calculated. First, index-OS1 was calculated using the expression values of two miRNAs (miR-187-5p and miR-6870-5p), but the Kaplan-Meier curves for OS showed no significant difference between the high and low groups ($P = .294$; Figure S2A). Thus, we calculated the index-OS2 using miR-187-5p, miR-6870-5p, and miR-1908-5p, which was the third OS-related miRNA with marginal significance ($P = .082$; Figure 3A). The patient characteristics stratified by index-OS2 are shown in Table 2. The Kaplan-Meier curves for

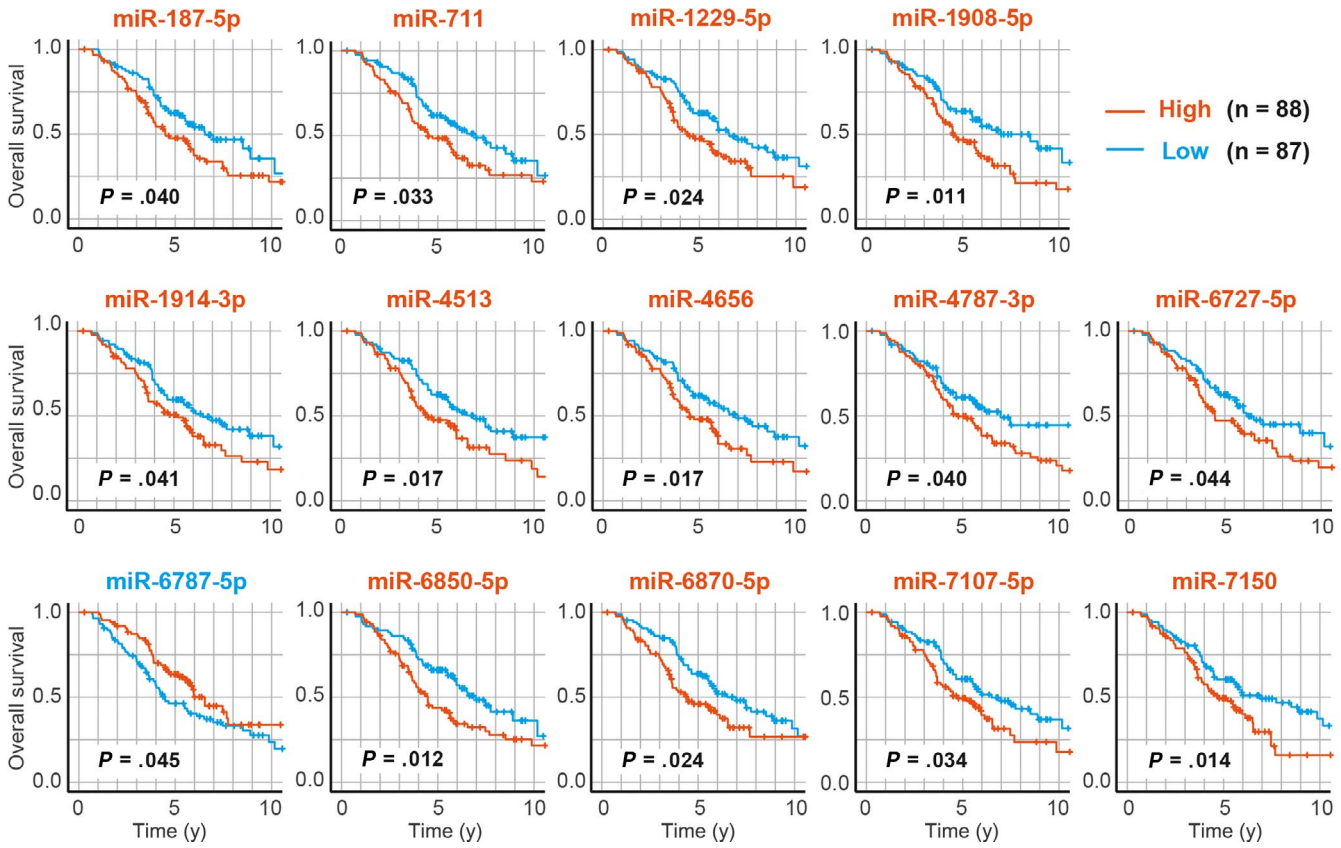

FIGURE 2 Kaplan-Meier curves showing overall survival of patients with high-grade serous ovarian carcinoma stratified according to serum microRNA (miR) levels. Patients were stratified according to the median level of each miRNA; orange and blue lines represent high- and low-level groups, respectively. Survival curves were compared using the log-rank test

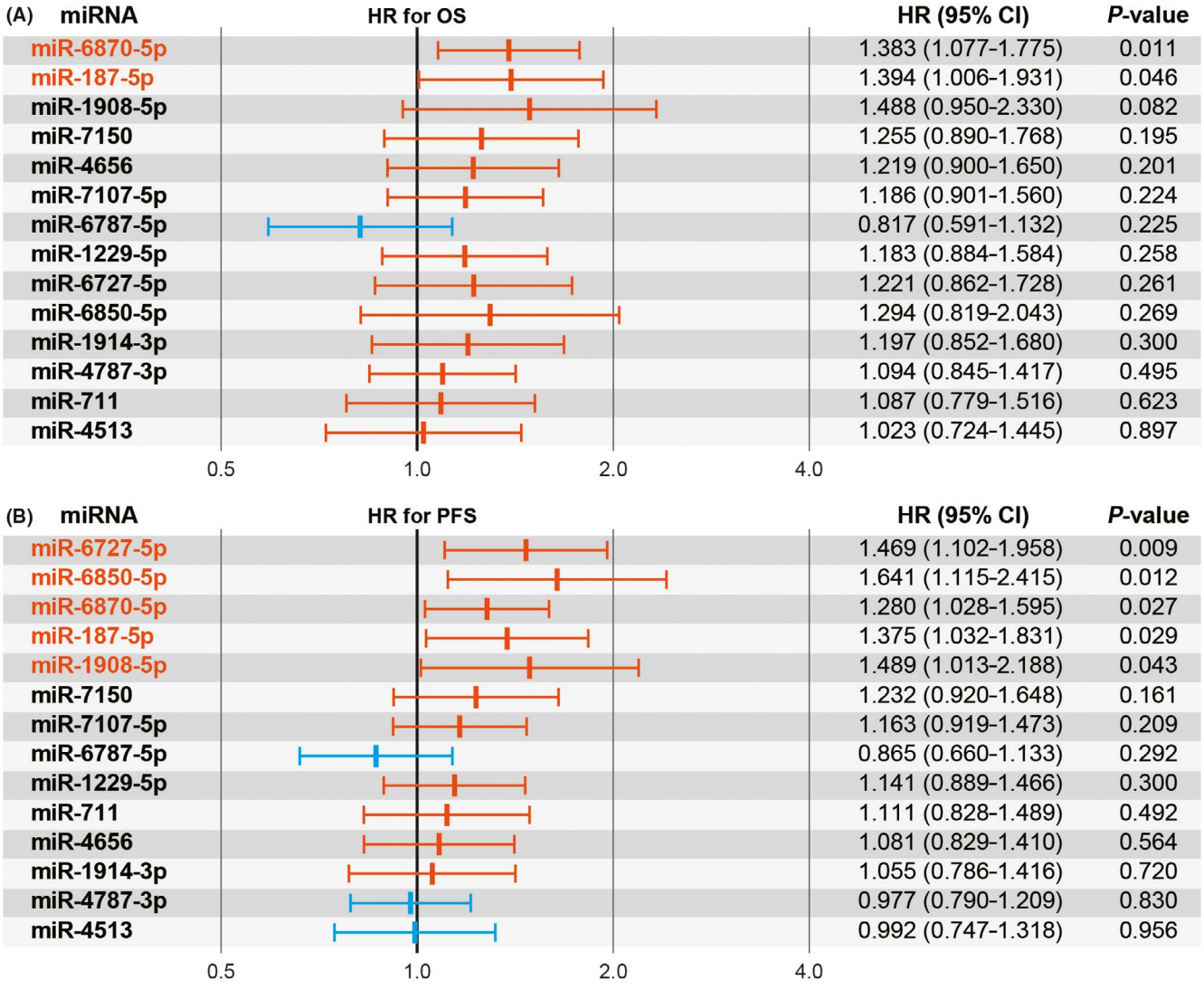

FIGURE 3 Univariate Cox regression analysis of survival of patients with high-grade serous ovarian carcinoma according to microRNA (miRNA) levels. Hazard ratios (HRs) and 95% confidence intervals (CIs) for (A) overall survival (OS) and (B) progression-free survival (PFS) were calculated using the levels of miRNAs as a continuous variable

OS showed that patients with a high index had significantly shorter OS than those with a low index ($P = .036$; Figure 4A). Similarly, the index for PFS was calculated using the five miRNAs (miR-187-5p, miR-6870-5p, miR-6727-5p, miR-1908-5p, and miR-6850-5p), and the Kaplan-Meier curves for PFS showed that patients with high index-PFS1 had significantly shorter PFS than those with low index-PFS1 ($P = .003$; Figure S2B). Index-PFS1 was then simplified, and index-PFS2 was created using miR-187-5p, miR-6870-5p, and miR-6727-5p. Patient characteristics stratified by index-PFS2 are shown in Table 3. The Kaplan-Meier curves for PFS showed that patients with a high index had significantly shorter PFS than those with a low index ($P = .006$; Figure 4B). Therefore, index-PFS2 was enough to predict early recurrence. In addition, we stratified the patients into three groups (low, intermediate, and high) based on index-OS2 and index-PFS2. The Kaplan-Meier curves for OS showed that patients with a high index had significantly poorer OS than those with low and intermediate indices ($P = .003$ and $P = .033$, respectively; Figure S3A). Moreover, the Kaplan-Meier curves for PFS showed that patients with a low index had significantly more favorable PFS than those with high and intermediate indices ($P < .001$ and $P = .003$, respectively; Figure S3B). The correlation between index-OS2 and index-PFS2 was evaluated using the Pearson correlation coefficient, which revealed a significant correlation ($R^2 = 0.859$, $P < .001$; Figure S3C). Therefore, patients with intermediate indices were considered to have relatively poor PFS but favorable OS.

Then, to evaluate whether the indices were independent prognostic factors, a multivariate Cox regression analysis for OS and PFS was carried out. According to the univariate analysis, FIGO stage and residual tumor volume at the time of debulking surgery (complete or optimal surgery vs suboptimal surgery or no surgery) were also significantly associated with poor OS ($P = .012$ and $P < .001$, respectively; Table 4). A multivariate analysis for OS showed that

TABLE 2 Characteristics of patients with high-grade serous ovarian carcinoma, stratified by index-OS2

Characteristic	Index-OS2[a]		
	Low (n = 87)	High (n = 88)	*P* value
Age, y			
Median (range)	59 (34-82)	61.5 (28-82)	.189
Stage			
I	3 (3.4)	1 (1.1)	.082
II	8 (9.2)	2 (2.3)	
III	51 (58.6)	53 (60.2)	
IV	25 (28.7)	32 (36.4)	
Surgery and residual tumor volume			
Complete/optimal surgery	78 (89.7)	73 (83.0)	.199
Suboptimal surgery/No surgery	9 (10.3)	15 (17.0)	
miRNA levels			
Median (range)			
miR-187-5p	7.836 (6.762-9.113)	8.297 (6.400-9.945)	<.001
miR-6870-5p	7.162 (5.543-8.084)	8.234 (7.362-10.688)	<.001
miR-1908-5p	11.237 (10.314-11.993)	11.691 (9.520-12.455)	<.001
Index-OS2[a]	5.237 (4.501-5.455)	5.658 (5.456-6.094)	<.001

Note: Data are shown as n (%) unless otherwise noted.

Abbreviation: miRNA, microRNA.

[a]Index-OS2 = 0.148 × (miR-187-5p) + 0.273 × (miR-6870-5p) + 0.186 × (miR-1908-5p).

residual tumor volume and index-OS2 were independent poor prognostic factors (HR 2.165 [95% CI, 1.277-3.669], $P = .004$; and HR 2.343 [95% CI, 1.182-4.641], $P = .015$, respectively). Similarly, according to the univariate analysis for PFS, FIGO stage and residual tumor volume at the time of debulking surgery were also significantly associated with worse prognosis ($P = .001$ and $P = .008$, respectively; Table 4). The multivariate analysis for PFS confirmed that both FIGO stage and residual tumor volume were independent poor prognostic factors (HR 1.390 [95% CI, 1.069-1.809], $P = .014$; and HR 1.771 [95% CI, 1.077-2.914], $P = .024$, respectively). Moreover, index-PFS2 was also a significant independent poor prognostic factor (HR 2.357 [95% CI, 1.289-4.311], $P = .005$; Table 4). Therefore, both indices were significant independent poor prognostic factors.

Finally, we investigated the potential functions of miR-187-5p, miR-6870-5p, and miR-1908-5p in EOC cells. These miRNAs were successfully transfected in A2780 and SK-OV-3 cell lines (Figure S4A). Proliferation assay revealed that miR-1908-5p significantly reduced cell viability in both cell lines (A2780, $P < .05$; and SK-OV-3, $P < .01$; Figure S4B). The remaining two miRNAs slightly inhibited cell proliferation in SK-OV-3 cells, although there was no growth inhibitory effect in A2780 cells. We then evaluated the impact of the miRNAs on treatment resistance. However, the miRNAs were not associated with sensitivity to cisplatin or docetaxel in either cell line (Figure S4C).

4 | DISCUSSION

In this study, we evaluated the association between circulating miRNA profiles and the prognosis of patients with HGSOC. Interestingly, none of the diagnostic circulating miRNAs identified in our previous reports were associated with the prognosis of the patients.[17] This implies that the biological characteristics required for diagnostic and prognostic biomarkers are quite different and should be evaluated individually.

The 210 miRNAs used in this study were selected based on miRNA profiles of sEVs from EOC cells, thus these circulating miRNAs can be derived from EOC tissues.[17] Cancer-derived sEVs are believed to be increased as cancer develops, and circulating miRNA could predict surgical outcomes in HGSOC.[20] Moreover, low levels of serum miR-125b predicted chemoresistance in EOC, although serum miR-125b levels were low in chemotherapy responders with breast cancer.[21,22] Other reports also indicated the usefulness of combining circulating miRNAs as predictors of response to chemotherapy and prognosis of patients with colorectal and NSCLC.[23-25] Therefore, the circulating miRNAs' profile might reflect the treatment sensitivity of the cancer cells, but the optimal combination of circulating miRNAs depends on the cancer type. Moreover, the prognostic impact of circulating miRNAs can be affected by therapeutic agents. For example, the circulating miR-200c level was reported to be predictive of bevacizumab treatment effects in EOC.[26]

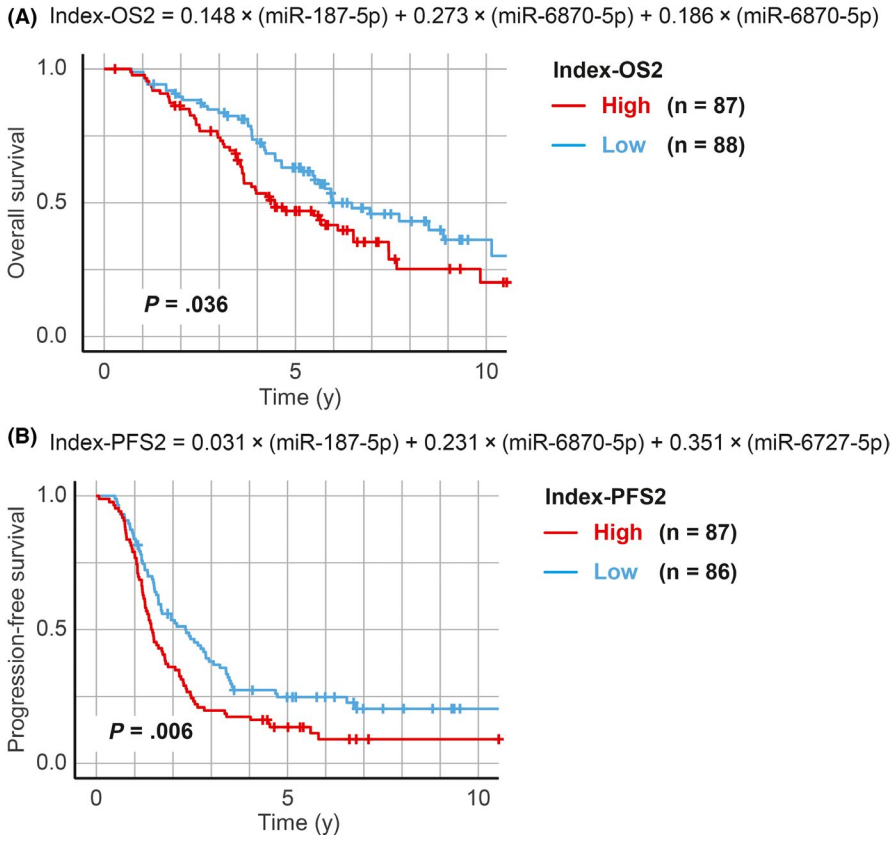

FIGURE 4 Prognostic impacts of the indices. A, Kaplan-Meier curves showing overall survival (OS) stratified with the median of index-OS2. B, Kaplan-Meier curves showing progression-free survival (PFS) stratified with the median of index-PFS2. Red and blue lines represent high- and low-level groups, respectively. Survival curves were compared using the log-rank test

TABLE 3 Characteristics of patients with high-grade serous ovarian carcinoma, stratified by index-PFS2

Characteristic	Index-PFS2[a]		*P* value
	Low (n = 87)	High (n = 86)	
Age, y			
Median (range)	60 (34-82)	61 (38-82)	.356
Stage			
I	2 (2.3)	2 (2.3)	.032
II	8 (9.2)	2 (2.3)	
III	54 (62.1)	48 (55.8)	
IV	23 (26.4)	34 (39.5)	
Surgery and residual tumor volume			
Complete/optimal surgery	77 (88.5)	73 (84.9)	.484
Suboptimal surgery/no surgery	10 (11.5)	13 (15.1)	
miRNA levels			
Median (range)			
miR-187-5p	7.819 (6.400-9.113)	8.348 (7.212-9.945)	<.001
miR-6870-5p	7.236 (5.543-10.688)	8.084 (6.606-10.039)	<.001
miR-6727-5p	11.664 (10.048-12.787)	12.262 (11.279-13.473)	<.001
Index-PFS2[a]	6.041 (5.553-6.204)	6.396 (6.205-6.983)	<.001

Note: Data are shown as n (%) unless otherwise noted.

Abbreviation: miRNA, microRNA.

[a]Index-PFS2 = 0.031 × (miR-187-5p) + 0.231 × (miR-6870-5p) + 0.351 × (miR-6727-5p).

TABLE 4 Univariate and multivariate Cox regression analysis for overall survival (OS) and progression-free survival (PFS) in patients with high-grade serous ovarian carcinoma

	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
<OS>				
Age	0.995 (0.975-1.015)	.595	0.998 (0.978-1.019)	.850
Stage (I, II, III, or IV)	1.501 (1.095-2.057)	.012	1.294 (0.932-1.796)	.124
Residual tumor volume[a]	2.530 (1.527-4.190)	.000	2.165 (1.277-3.669)	.004
Index-OS2[b]	2.720 (1.382-5.352)	.004	2.343 (1.182-4.641)	.015
<PFS>				
Age	1.009 (0.993-1.025)	.268	1.013 (0.997-1.029)	.119
Stage (I, II, III, or IV)	1.532 (1.189-1.974)	.001	1.390 (1.069-1.809)	.014
Residual tumor volume[a]	1.913 (1.187-3.081)	.008	1.771 (1.077-2.914)	.024
Index-PFS2[c]	2.716 (1.521-4.851)	.001	2.357 (1.289-4.311)	.005

Abbreviations: CI, confidence interval; HR, hazard ratio; miR, microRNA.

[a]0 for Complete/optimal surgery; 1 for Suboptimal surgery/no surgery.

[b]Index-OS2 = 0.148 × (miR-187-5p) + 0.273 × (miR-6870-5p) + 0.186 × (miR-1908-5p).

[c]Index-PFS2 = 0.031 × (miR-187-5p) + 0.231 × (miR-6870-5p) + 0.351 × (miR-6727-5p).

Furthermore, higher expression of serum miR-622 is an independent poor prognostic biomarker of response to platinum-based chemotherapy, and miR-622 overexpression was known to induce resistance to PARP inhibitor and cisplatin.[27,28]

In this study, the higher levels of miR-187-5p and miR-6870-5p were significantly associated with shorter PFS and OS. In general, patients with HGSOC received multiple regimens until disease progression; hence, it was difficult to investigate the associations between circulating miRNAs and the efficacy of specific regimens in this retrospective setting. However, five circulating miRNAs were significantly associated with PFS. As most patients received paclitaxel and carboplatin combination chemotherapy as initial treatment, these miRNAs might reflect platinum and taxane sensitivity. Hence, we evaluated the effects of miR-187-5p, miR-6870-5p, and miR-1908-5p on treatment resistance. However, at least in A2780 and SK-OV-3 cell lines, these miRNAs could not enhance treatment resistance. In addition, miR-1908-5p was a tumor suppressor in both cell lines. It should be noted that poor prognostic circulating miRNAs do not always have oncogenic functions in cancer cells. For example, cellular miR-1246 was downregulated and acted as a tumor suppressor, whereas a higher level of circulating miR-1246 was associated with prostate cancer aggressiveness.[29] In other words, tumor-suppressive miRNAs were considered to be selectively released by exosomes to maintain the aggressiveness of prostate cancer cells. Moreover, according to previous reports, miR-187-5p promoted proliferation and inhibited apoptosis of cancer cells, and the higher expression of cellular miR-187-5p was associated with poor survival of patients with bladder cancer.[30,31] In contrast, the function of miR-187-5p was reversed in NSCLC, as it acted as a tumor supprssor.[32] In addition, miR-6727-5p contributed to cervical cancer progression by targeting *TBX1*, and miR-1908-5p had oncogenic functions in osteosarcoma cells.[33,34] However, no reports on miR-6870-5p and miR-6850-5p in cancer have been published. Therefore, further studies are essential to elucidate how these miRNAs are involved in the development of EOC, including treatment resistance.

Circulating miRNAs are derived from not only cancer cells but all the cells in the human body. Hence, it is difficult to identify the origin of circulating miRNAs experimentally, but the origin of prognostic circulating miRNAs might be immune cells. Reportedly, the levels of circulating miRNAs are dysregulated and can reflect specific immune-mediated inflammatory disorders or infections.[35,36] Recently, PBMCs were reported to secrete tumor-suppressive EVs in patients with melanoma, and a circulating miRNA signature was correlated with the status of tumor-infiltrated lymphocytes in breast cancer.[37,38] Moreover, we previously indicated that dysregulated circulating miRNAs might be associated with interferon-related pathways in ovarian clear cell carcinoma.[19] These results suggested that circulating miRNAs derived from immune cells might reflect the host immune status. Therefore, circulating miRNAs might be predictive biomarkers for immunotherapy, and two independent studies showed that circulating miRNA signatures were associated with survival of patients with NSCLC treated with anti-PD1 drugs.[39,40]

This study was based on the reanalysis of datasets and therefore had several limitations. First, the extracted RNA samples were not preserved; thus, it is impossible to undertake validation

experiments using the same sample sets. Moreover, it was difficult to evaluate the origin and functions of the circulating miRNAs in HGSOC tissue. Second, one of the clinical interests is the prediction of PARP resistance, but only a few patients receive PARP inhibitors as a treatment for recurrence. Therefore, further studies are needed to evaluate the effect of miRNAs as predictive biomarkers of PARP inhibitors.

In conclusion, we showed that several circulating miRNAs were useful as predictive biomarkers in patients with HGSOC. This study and our previous report indicated that circulating miRNA profiles can be useful clinical tools for a highly accurate diagnosis and prognostic prediction using a single blood test.[17] Further optimization of circulating miRNA analysis is expected for early clinical application.

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DISCLOSURE

The authors have no conflict of interest.

ORCID

Akira Yokoi https://orcid.org/0000-0003-0789-5102
Juntaro Matsuzaki https://orcid.org/0000-0002-3204-5049
Yusuke Yamamoto https://orcid.org/0000-0002-5262-8479

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SUPPORTING INFORMATION

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