EJGO European Journal of Gynaecological Oncology

Original Research

Combined measurement of miRNA-183, HE4, and CA-125 increases diagnostic efficiency for ovarian cancer

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Summary

Objective: This study aimed to determine the role of miR-205, miR-182, and miR-183 expression in the serum of ovarian cancer patients in the early diagnosis of ovarian cancer. *Materials and Methods:* The expression of miR-205, miR-182, miR-183, CA-125, and HE4 was detected in the sera of 101 patients with ovarian cancer, 50 patients with benign ovarian diseases, and 50 healthy volunteers. The results were validated in 98 patients with ovarian cancer, 50 patients with benign ovarian diseases, and 53 healthy volunteers. The expression of miR-205, miR-182, miR-183, CA-125, and HE4 was subjected to ROC analysis and binary logistic regression analysis. *Results:* The sensitivity of miR-182 and CA-125 was highest (0.901% and 0.832, respectively), but the specificity was low (both 0.27) in the early diagnosis of ovarian cancer. HE4 had the highest specificity in the early diagnosis of ovarian cancer. The sensitivity, specificity, and AUC of HE4 were 0.842, 0.81, and 0.847, respectively. Binary logistic regression analysis showed that three variables were suitable for the diagnostic model: Y=Logit(P)=-5.457+5.365*miR183+0.019*HE4+0.004*CA125. Based on the diagnostic model, ROC analysis showed that the sensitivity, specificity, and AUC were 0.97, 0.85, and 0.951, respectively. Statistical validation showed that the sensitivity, specificity and AUC were 0.941, 0.86, and 0.951, respectively. *Conclusion:* miR-183 has high specificity and sensitivity in the diagnosis of ovarian cancer. Measurement of miR-183 combined with HE4 and CA-125 is of value for the early diagnosis and evaluation of ovarian cancer.

Key words: miRNA-183; HE4; CA-125; Ovarian cancer.

Introduction

Ovarian cancer is the second most common malignancy of the reproductive system in women and the leading cause of death [1] because a majority of patients are diagnosed with ovarian cancer at an advanced stage [2]. The survival rate of patients with advanced ovarian cancer is < 15%, but early diagnosis and treatment of ovarian cancer may increase the survival rate to 90% [3]. Thus, the early diagnosis of ovarian cancer is crucial. Currently, cytologic and pathologic examinations are used for the diagnosis of ovarian cancer; however, cytologic and pathologic examinations have high specificity and low sensitivity [4]. Thus, to investigate serum biomarkers for the early diagnosis of ovarian cancer is clinically important. In recent years, micro (mi)RNAs have been shown to play important roles in the occurrence and development of cancer [5]. Indeed, there are stable miRNAs in the plasma which may serve as new biomarkers for cancers [5, 6]. Studies [7-12] have shown that the expression of miR-205, miR-182, and miR-183 in the peripheral blood increases significantly in ovarian cancer patients, but whether or not these miRNAs can serve as effective biomarkers of ovarian cancer is not clear. In the present study, qRT-PCR was used to detect serum miRNA, CA-125, and HE4 in ovarian cancer patients and healthy controls, and evaluate the clinical significance of miRNA, CA-125, and HE4 in the diagnosis of ovarian cancer.

Materials and Methods

Inpatients and outpatients were recruited from the Guangzhou General Hospital of Guangzhou Military Region and The Third Affiliated Hospital of Hunnan University of Traditional Chinese Medicine between 2012 and 2016. All of the patients had pathologically-proven ovarian cancer and had not received radiotherapy or chemotherapy. This study was approved by the Ethics Committee of the Guangzhou General Hospital of Guangzhou Military Region, and informed consent was obtained before enrollment in the study. Peripheral blood (10 ml) was obtained from each patient in the morning after an overnight fast, and stored at -80°C for further use. A chemiluminescence microparticle immunoassay was used to detect serum HE and CA-125 according to the manufacturer's instructions with a fully automatic chemiluminescence instrument and reagent kits.

RNA extraction, cDNA synthesis, and detection of miRNAs by qRT-PCR.A miRcute serum/plasma miRNA extraction and separation kit were used to extract total RNA from serum samples (200 dl). A spectrophotometer was used to detect the co-

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Revised manuscript accepted for publication June 11, 2018

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Table 1. — *Frequency distribution of subject's epidemiologic and clinical characteristics by case–control status.*

	Training set (n=201)			Validation set (n=201)		
Subject characteristics	OC (n=101)	BOD (n=50)	Con (n=50)	OC (n=98)	BOD (n=50)	Con (n=53)
Age, years						
≤ 50	46.000	21	19	44	22	20
> 50	55.000	29	31	54	28	33
Histology						
Serous	26.000			29		
Mucinous	12.000			13		
Others	63.000			56		
Stage						
I	18.000			19		
II	22.000			20		
III	29.000			33		
IV	32.000			26		

OC: epithelial ovarian cancer; BOD: benign ovarian diseases; Con: control.

ncentration and purity of RNA. RNA samples with an absorbance ratio of 1.8-2.2 were used in subsequent experiments. A miRcute miRNA cDNA first strand synthesis kit was used to add poly(A) to the 3' end of miRNA, and the oligo(dT)-universal tag commonly-used primers were used for reverse transcription of the cDNA first strand.

The mixture used for the addition of poly(A) to the 3' end of miRNA was as follows: total RNA (5 µl); *E.coli* poly(A) polymerase (5 U/µl [0.4 µl]); 10× poly(A) polymerase buffer (2 µl); 1× 5×rATP solution (4 µl); and 1× RNase-free ddH$_2$O (8.6 µl). This mixture was incubated at 37°C for 60 minutes and then stored at -20°C. The mixture used for reverse transcription with poly(A) containing miRNA was as follows: poly(A) reaction solution (2 µl); 10× RT primer (2 µl); 10× RT buffer (2 µl); Super Pure dNTPs (2.5 mM each [1 µl]); RNasin (40 U/µl [1 µl]); Quant RTase (0.5 µl); and RNase-free ddH$_2$O (11.5 µl). A miRcute miRNA fluorescence quantitative detection kit (SYBR Green) was used to quantitatively detect the relative expression of miRNAs in the serum by qPCR. The mixture used for qPCR was as follows: 2× miRcute miRNA Premix (10 µl); 1× Forward Primer (1 µl); Reverse Primer (10 µM [0.4 µl]); miRNA first strand cDNA (2 µl); and RNase-free ddH$_2$O (6.6 µl). The conditions for qPCR were as follows: 94°C for 7 minutes, 94°C for 20 seconds, and 60°C for 35 seconds × 40 cycles. Real-time quantitative PCR was performed using a strata-gene Mx3005p. U6 served as an internal reference, and the Ct value was used for calculation of the relative expression of target genes with the 2^{-Ct} method [7-9].

Statistical analysis.Statistical analysis was performed with SPSS (version 23.0). Non-normally distributed data are expressed as medians (P25-P75) and compared with the Mann-Whitney U test or Kruskal-Wallis test. All of the variables were subjected to logistic regression analysis, and a diagnostic model was established. A ROC was delineated, and the diagnostic performance of each variable was calculated. The AUC was used to evaluate the sensitivity and specificity in the diagnosis of ovarian cancer.

Results

A total of 201 subjects were included in the present study (101 patients with ovarian cancer, 50 patients with benign ovarian diseases, and 50 healthy volunteers). For validation, there were 98 patients with ovarian cancer, 50 patients with benign ovarian diseases, and 53 healthy volunteers. The patient characteristics are shown in Table 1.

Serum biomarkers of ovarian cancer in different groups. Non-parametric testing was used for paired comparisons. The median expression of HE4 and CA-125 in ovarian cancer patients was 109.9 pmol/L (range, 68.5-434.5), and 248.8 U/ml (range, 21.30-788.40), respectively, which were significantly different from patients with benign ovarian diseases and healthy controls (all $p < 0.01$). When compared with the healthy control group, the expression of miR-205, miR-182, and miR-183 was increased in ovarian cancer patients and patients with benign ovarian diseases ($p < 0.01$ and 0.05, respectively; Table 2, Figure 1).

Diagnostic performance of serum biomarkers in ovarian cancer patients. Serum miR-182 and CA-125 had the highest sensitivity for the early diagnosis of ovarian cancer (0.901% and 0.832, respectively), but low specificity (both 0.27). HE4 had the highest specificity for the early diagnosis of ovarian cancer, and the sensitivity, specificity, and AUC were 0.842, 0.81, and 0.847, respectively. Thus, HE4 is an ideal biomarker for the diagnosis of ovarian cancer. The sensitivity, specificity, and AUC of miR-183 were 0.752, 0.80, and 0.77, respectively, suggesting moderate performance in the diagnosis of ovarian cancer; however, miR-183 alone cannot be used for the diagnosis of ovarian cancer. miR-205 had different levels of expression between ovarian cancer patients and patients with benign ovarian diseases, but the sensitivity and specificity were low (< 0.7; Figure 2 and Table 3).

Establishment of a diagnostic model for ovarian cancer and evaluation of combined use of biomarkers. The variables with significant differences between ovarian cancer patients and patients with benign ovarian diseases were used for lo-

Table 2. — *All candidate markers levels in traning set.*

	OC	BOD	Con
n	101	50	50
miR-205	0.726 (0.361-0.892)	0.346 (0.264-0.641)	0.292 (0.243-0.425)
miR-183	0.859 (0.478-0.952)	0.316 (0.254-0.442)	0.262 (0.210-0.333)
miR-182	0.271 (0.162-0.910)	0.208 (0.110-0.825)	30.98 (17.18-41.19)
HE4 (pmol/L)	109.9 (68.5-434.5)	42.05 (27.2-52.28)	30.98 (17.18-41.19)
CA125 (U/ml)	248.8 (30.9-966.85)	13 (5.09-24.95)	12.63 (4.88-24.35)

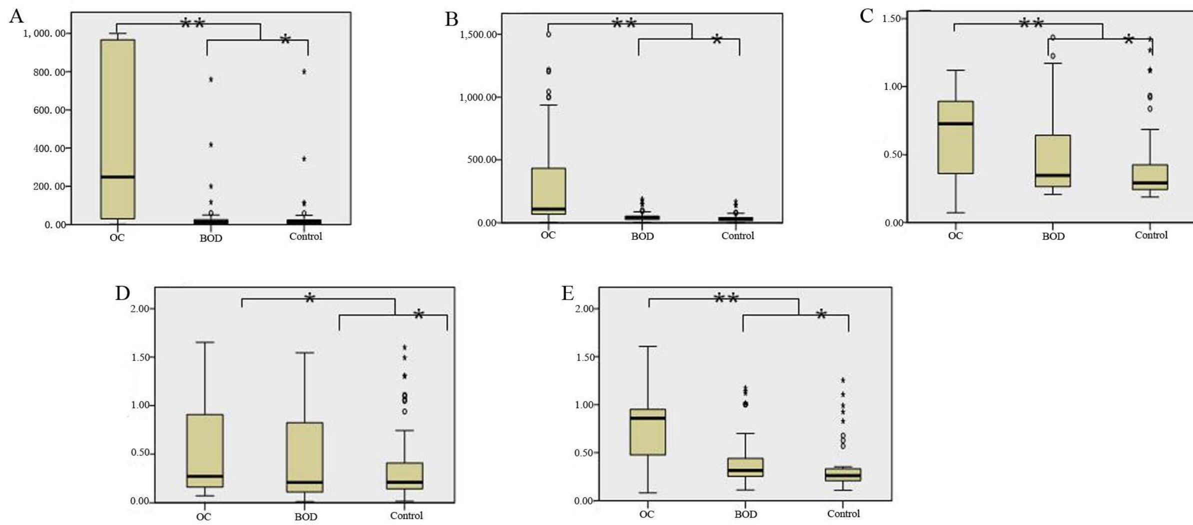

Figure 1. — Frequency distribution of candidate markers levels in traning set. (A) CA125 in traning set. (B) HE4 in traning set. (C) miR-205 in traning set. (D)miR-182 in traning set. (E) miR-183 in traning set. $*p < 0.05$,$**p < 0.01$.

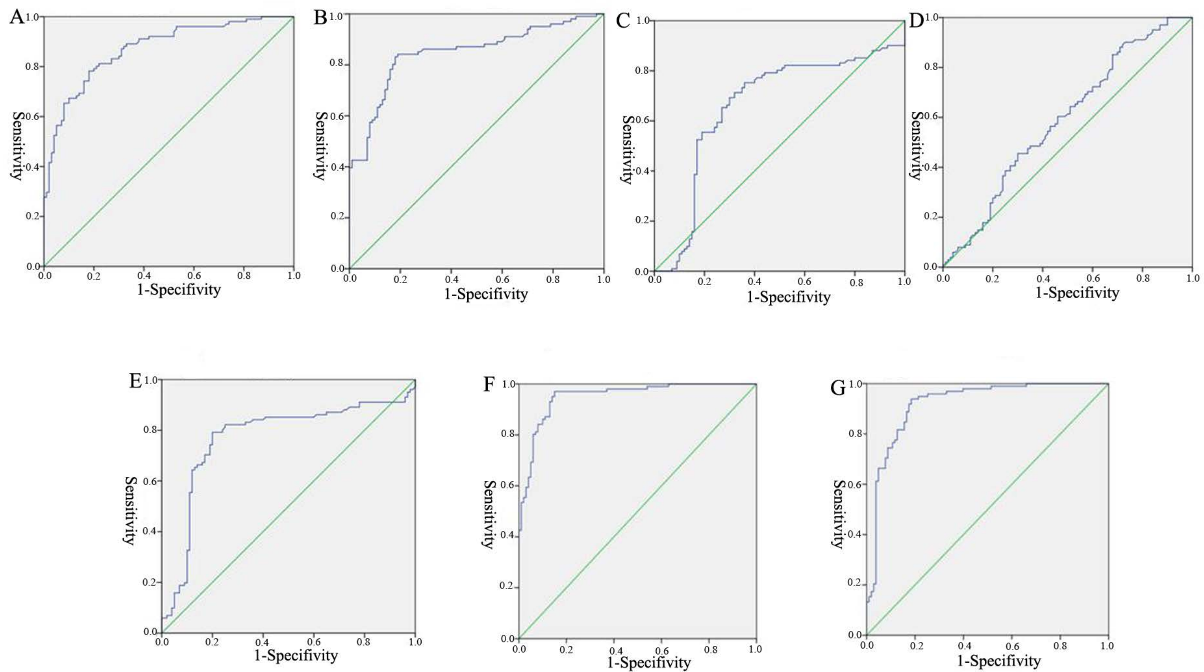

Figure 2. — The ROC curves of all candidate markers in all patients. (A) CA125 in traning set. (B) HE4 in traning set. (C) miR-205 in traning set. (D) miR-182 in traning set. (E) miR-183 in traning set. (F) Combination of CA125, HE4, and miR-183 in traning set. (G) Combination of CA125, HE4, and miR-183 in validate set.

gistic regression analysis. Three variables were included in developing the diagnostic model equation (miR-183, HE4, and CA-125; Table 4), as follows: Y=Logit(P)=-5.457+5.365*miR183+0.019*HE4+0.004*CA-125. ROC analysis showed that the AUC was 0.951, and the 95% confidence interval (CI) was 0.924-0.979. When the cut-off

Table 3. — *Diagnostic value of all candidate markers levels in training set.*

Candidate marker	Cut-off value	Sensitivity (%)	Specificity (%)	Youden index§	AUC (95% CI)	*p*
CA125	23.49	0.832	0.27	0.562	0.871 (0.823-0.919)	<0.001
HE4	53.55	0.842	0.81	0.032	0.847 (0.791-0.902)	<0.001
miR-205	0.415	0.693	0.70	0.393	0.661 (0.58-0.741)	<0.001
miR-183	0.4775	0.752	0.80	0.592	0.77 (0.698-0.841)	<0.001
miR-182	0.1245	0.901	0.27	0.171	0.589 (0.51-0.668)	<0.05
CA125+HE4+miR-183in training set	0.3372	0.97	0.85	0.082	0.951 (0.924-0.979)	<0.001
CA125+HE4+miR-183 in validate set	0.4671	0.939	0.816	0.755	0.920 (0.881-0.960)	<0.001

Table 4. — *Binary logistic regression analysis of all candidate markers levels in training set.*

Variable	B	S.E.	Wald	df	Sig.	Exp (B)
miR-183	6.137	1.568	15.324	1	0	462.738
HE4	0.02	0.005	13.537	1	0	1.02
CA125	0.004	0.001	12.504	1	0	1.004
miR-205	-0.937	1.678	0.312	1	0.577	0.392
miR-182	-0.031	0.649	0.002	1	0.962	0.97
Constant	-5.36	0.836	41.07	1	1 0	0.005

value was set at 0.3372, the sensitivity and specificity were 0.97 and 0.85, respectively (Table 3). This diagnostic model improved the diagnostic performance in ovarian cancer patients, characterized by increased sensitivity and specificity.

The diagnostic model was further validated in ovarian cancer patients, patients with benign ovarian diseases, and healthy controls, and ROC analysis was also performed. The AUC was as high as 0.920, and the 95% CI was 0.881-0.960 (Figure 2G). When the cut-off value was set at 0.4671, the sensitivity and specificity were 0.939 and 0.816, respectively, for the diagnosis of ovarian cancer (Table 3). This finding suggests that the diagnostic model is good in differentiating ovarian cancer patients from patients with benign ovarian diseases.

Discussion

The miRNAs belong to endogenous non-encoding RNA with approximately 22 nucleotides. The miRNAs bind to the 3' untranslated region of mRNA in a complementary manner, then cause mRNA rupture and inhibition of translation or mediate mRNA degradation via a miRNA-induced de-adenosine effect, which may negatively regulate gene expression. In recent years, studies have confirmed that miRNA may cause a variety of biological responses and regulate cell apoptosis, differentiation, and proliferation [13, 14]. It has been confirmed that miRNAs play important roles in the occurrence and development of cancers. The miRNAs can be divided into oncogenic and anti-oncogenic types according to the role in cancer. There are stable miRNAs in the plasma and serum, and the expression is similar between individuals of the same species, which suggests the possibility for miRNAs as potential biomarkers of cancers. Although different hypotheses have been proposed for the explanation of cancer miRNA in blood, cancers may definitely affect miRNAs in blood [15]. It has been reported that a cancer has a specific miRNA profile [16] which is closely related to resistance to chemotherapy [17-19]. Several studies have confirmed that miRNA in plasma, which are more stable compared to traditional cancer markers, may be used to diagnose cancer and predict therapeutic efficacy [20, 21].

It has been reported that miR-205 expression is significantly higher in ovarian cancer tissues than normal tissues [9], and ZEB1 mRNA expression in ovarian cancer tissues is increased markedly compared to normal tissues. Moreover, miR-205 is negatively related to ZEB1 expression. Thus, miR-205 acts as an oncogene and may negatively regulate ZEB1 expression, leading to the occurrence of ovarian cancer. Zheng *et al.* evaluated plasma samples from 360 ovarian cancer patients and 200 healthy controls, and found that miR-205 expression in the peripheral blood of ovarian cancer patients was significantly higher than healthy controls. When miR-205 and CA-125 were combined, the diagnostic performance was increased. The present results also showed high expression of miR-205 in the plasma of ovarian cancer patients, suggesting that miR-205 acts as an oncogene in ovarian cancer, which is consistent with a previous report. [8] A study involving whole genomic miRNA screening [9] showed that miR-183 expression is markedly up-regulated in ovarian cancer compared to normal ovarian tissues, suggesting that miR-183 is involved in the progression of ovarian cancer. Another study [10] used qRT-PCR for the detection of serum miR183. The results showed that serum miR183 in ovarian cancer patients was related to clinical stage and lymph node metastasis, and ovarian cancer patients with high miR-183 expression had a significantly reduced survival rate. This finding implies that miR-183 is a potential biomarker for ovarian cancer. The present results showed that serum miR-183 expression increased significantly in ovarian cancer patients, indicating that miR-183 acts as an oncogene in ovarian cancer. Moreover, there was a significant difference in miR-183 expression between ovarian cancer patients and those with benign ovarian diseases, suggesting that miR-183 may serve as a potential good biomarker for ovarian cancer. Expression of miR-182 is up-regulated in ovarian cancer tissues and cell lines, and may induce down-

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