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REVIEW

Diagnostic Model of Serum miR-193a-5p, HE4 and CA125 Improves the Diagnostic Efficacy of Epithelium Ovarian Cancer

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Abstract

Epithelium ovarian cancer (EOC) is currently the prevalent malignant cancer worldwide. However, there is a lack of efficient biomarkers for EOC screening. Accumulating evidence reveals that serum miRNA detectable in various types of cancer. Therefore, we explore the diagnostic value of combined detection of plasma miR-193a-5p, HE4 and CA125 for EOC. Serum samples were collected from 45 patients with primary EOC, 30 patients with benign ovarian tumor patients and 40 healthy controls. The expression of serum miR-193a-5p was detected by real-time quantitative PCR, and serum HE4 and CA125 were detected by chemiluminescent immunoassay. Moreover, a diagnostic model combining miR-193a-5p, HE4 and CA125 or alone in EOC patients was evaluated by ROC curve analysis. The relative expression quantity (RQ) of serum miR-193a-5p in EOC patients, benign ovarian tumor patients and healthy control groups were 0.419 (0.093, 2.215), 3.667 (1.633, 6.691) and 1.130 (1.000, 7.087), respectively. The RQ of serum miR-193a-5p in EOC patients was significantly lower than that in benign ovarian tumor patients and healthy controls (both $P < 0.001$), and there was no significant difference between benign ovarian tumor patients and healthy controls (both $P > 0.05$). There was no significant correlation between serum miR-193-5p, HE4 and CA125 levels (both $P > 0.05$). Additionally a risk model for miR-193a-5p, HE4 and CA125 was correlated with Grading and Lymph node metastasis ($P = 0.016$, $P = 0.029$). The area under the receiver operating characteristic curve of a risk model for distinguishing EOC patients from healthy individuals was 0.996, which higher than any single biomarker. Combined detection of miR-193-5p, HE4 and CA125 by logistic regression analysis could greatly improved the diagnostic ability of EOC and may prove to be a candidate biomarker, providing new directions for further investigation.

Keywords Epithelium ovarian cancer · miRNA · miR-193a-5p · HE4 · CA125

Introduction

Epithelial ovarian cancer (EOC) has the highest mortality worldwide and is the fourth most common cause of death due to cancer among women. The overall 5-year survival rate is approximately 30% [1] .Furthermore, the majority of cases are diagnosed with ovarian cancer at later stages. Current diagnostic methods for detection and monitoring of EOC mainly include pelvic examination and transvaginal ultrasound and measurement of serum biomarker CA125 (carbohydrate

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antigen 125). However, CA125 is only elevated in approximately 50% of stage I and in 70–90% of advanced diseases [2] .Other biomarkers such as mesothelin and human epididymis 4 also have limited diagnostic utility [3] .Therefore, the identification of novel diagnostic and prognostic biomarkers for treatment response is eagerly desired.

MicroRNAs (miRNAs) are small non-protein-coding RNAs that regulate gene expression post-transcriptionally by interacting with partially complementary target sites in target mRNAs [4] .An increasing number of investigators have carried out miRNAs expression profiling in EOC. For example, miRNAs with oncogenic functions, such as miR-106b, miR-143, miR-145, miR-125b and so on [5, 6]; several miRNAs such as miR-30, miR-124 and miR-199 have tumor inhibitory effects [7, 8] .Recently many miRNAs have been proven to be predictive of EOC prognosis and metastasis and may serve as molecular biomarkers for EOC detection, and as therapy agent for EOC treatment [9, 10] .Thus, it is exceptionally urgent to identify sensitive, noninvasive biomarkers, which have great potential for use as diagnostic tools and in predicting prognosis and response to treatments.

It was found in previous study that miR-193a-5p was dysregulation in non-small-cell lung cancer [11] ,bladder cancers [12] ,esophageal squamous cell carcinoma [13] ,but the expression and function have not been reported. In the present study, we intended to explore the clinical efficacy of serum miR-193a-5p, HE4 and CA125 alone or combination in the diagnosis of EOC by detecting their expression in EOC patients, patients with benign ovarian tumors and healthy individuals.

Materials and Methods

Subjects

The study consisted of a EOC group, including 45 patients (mean age 56; range 34–70 years) who were confirmed as having EOC by biopsy and pathology on the clinical basis or during hospitalization in our hospital between August 2015 and July 2016; a benign ovarian tumor group, including 30 patients (mean age 53; range 36–72 years) who received treatment in the same hospital during the same period; and a normal control group, including 40 healthy volunteers (mean age 54; range 35–68 years) who underwent routine physical examination. All samples were anonymous and the study protocol was approved by the local ethics committee.

Specimen Collection and Serum RNA Extraction

Blood samples were collected and centrifuged at 1000 rpm for 10 min. The separated serum were placed in RNase-free centrifuge tubes and stored at −80 °C for use. Total RNA was extracted using the serum RNA extraction kit (Life Technologies, US), and stored at −80 °C after confirmation of the concentration and the purity by ultraviolet spectrophotometry.

Real-Time Quantitative PCR

Firstly, cDNAs were synthesized from total RNA using gene-specific primers. Reverse transcriptase reactions contained 2 ng of RNA samples, 10 μl of RT master mix including 2 μl of 100 mM dNTP mix, 1 μl Reverse AidRNAse Reverse Ensyme, 4 μl of 5 × Reactive Buffer, 1 μl of 20 U/μl Ribolock RNase Inhibitor and 1 μl of Nuclease-free water, and 1 μl of stem-loop RT primer. The 20 μl of reactions were incubated for 60 min at 42 °C, 5 min at 72 °C, and then held at 4 °C. Secondly, real-time PCR was performed using an ABI 7500 PCR Detection System (ABI, USA). The miR-193a-5p primer sequences are as follows: forward primer: 5'-ACACTCCAGCTGGGTGGGTCTTTGCGGGCG-3′, and backward primer: 5'-TGGTGTCGTGGAGTCG-3′; U6 primer sequences: forward primer: 5'-CTCGCTTCGGCAGC ACA-3′, and backward primer: 5'-AACGCTTCACGAAT TTGCGT-3′. The 20 μl of PCR included 10 μl SYBR Green I mix, 3 μl cDNA, 1 μl forward primer, 1 μl reverse primer and 5 μl RNase-free H_2O. Reactions were incubated in optical tubes at 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 31 s. The relative expression quantity of miRNA was expressed as $RQ = 2^{-\triangle\triangle CT}$. $\triangle\triangle CT$ = study group (CT_{miRNA}-CT_{U6}) – mean value of control group (CT_{miRNA}-CT_{U6}).

Detection of HE4 and CA125 Levels

The concentrations of HE4 and CA125 in the serum samples from EOC patients were detected by I4000SR chemiluminescence apparatus (Abbott, Chicago, IL, USA). The reagents and products for calibration and quality control were all original products, and all procedures were performed strictly according to the operating manuals. The content of HE4 > 105 ng/ml or CA125 > 35 U/ml was considered abnormal.

Statistical Treatment

Statistical analyses were performed using the SPSS 17.0 statistical software (SPSS, Chicago, IL, USA) and GraphPad Prism 5 (GraphPad Software Inc., CA, USA). Data were expressed as medians (lower quartile and upper quartile). Comparison between two groups was performed by Mann-Whitney U test. Kruskal-Wallis H-test was used for multiple comparisons between the groups. Correlations between miR-193a-5p, HE4 and CA125 levels in EOC patients were analyzed by Spearman correlation analysis. Multivariate classification models were constructed to determine the combination

of miR-193a-5p, HE4 and CA125 with the greatest predictive ability for cancer. Their diagnostic efficacy as a diagnostic marker was assessed by receiver-operating characteristics (ROC) and the area under the curve (AUC). Values of $P < 0.05$ were considered statistically significant.

Results

Serum miR-193a-5p Expression in EOC, Benign Ovarian Tumor and Healthy Control Groups

MiR-193a-5p in serum was detected by real-time quantitative PCR. It was found that the relative expression level of serum miR-193a-5p in EOC, benign ovarian tumor and healthy control groups were 0.419 (0.093, 2.215), 3.667 (1.633, 6.691) and 1.130 (1.000, 7.087), respectively. It was significantly lower in EOC group than that in other two groups (U = 278.0, 490.0, both $P < 0.001$), and there was no significant difference between benign ovarian tumor and healthy control groups (U = 477.5, $P = 0.147$) (Fig. 1). These results suggested that serum miR-193a-5p could be helpful in the auxiliary diagnosis of EOC.

Correlation between Serum miR-193a-5p and HE4, CA125 Levels in EOC Patients

Correlations between the relative expression of miR-193a-5p and HE4, CA125 in EOC patients were analyzed by Spearman correlation analysis. The results showed that the relative expression of miR-193a-5p was not significantly correlated with HE4 (r = −0.225, $P = 0.137$) or CA125 concentration (r = −0.154, $P = 0.311$) in EOC patients (Fig. 2).

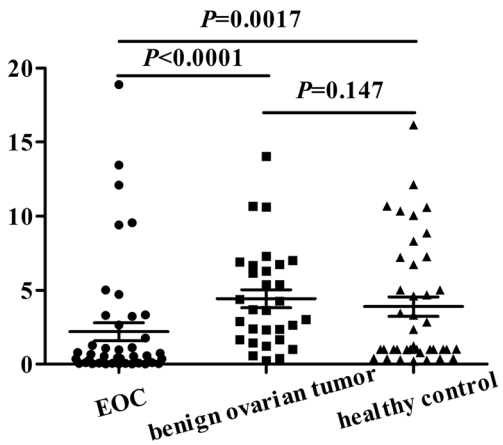

Fig. 1 The relative expression of serum miR-193-5p in EOC ($n = 45$), benign ovarian tumor patients ($n = 30$) and healthy control groups ($n = 40$). * $P < 0.05$

Diagnostic Model Construction and Predictive Power of Serum miR-193a-5p, HE4 and CA125

Receiver operating characteristics (ROC) curves and the area under the curve (AUC) were constructed to assess diagnostic efficiency. In terms of comparison between EOC and healthy control groups, at the cutoff value of 0.979 for miR-193a-5p, the related sensitivity, specificity and accuracy were 66.7%, 72.5% and 69.4%, respectively vs. 15.6%, 72.5% and 42.4% for HE4, and 51.1%, 67.5% and 58.8% for CA125 (Table 1). Furthermore, we explore the diagnostic ability of their combined detection, a diagnostic model combining miR-193a-5p, HE4 and CA125 with a Logist regression analysis were constructed (Table 2). The model showed that the combination of miR-193a-5p, HE4 and HE4 had the greatest predictive ability for EOC. The AUC value was the highest of 0.996 (Table 1).

Correlation between the Model and Clinical Characteristics

To identify the clinical relevance between the model and clinicopathological parameters such as FIGO stage, Grading, Lymph node metastasis, Histological types (Table 3). It was showed that there was no significant difference in the expression of model combining miR-193a-5p, CA125 and HE4 between the 45 EOC patients in terms of FIGO stage and Histological types ($P = 0.664$, $P = 0.768$), while there were significant differences in terms of different Grading and Lymph node metastasis ($P = 0.016$, $P = 0.029$).

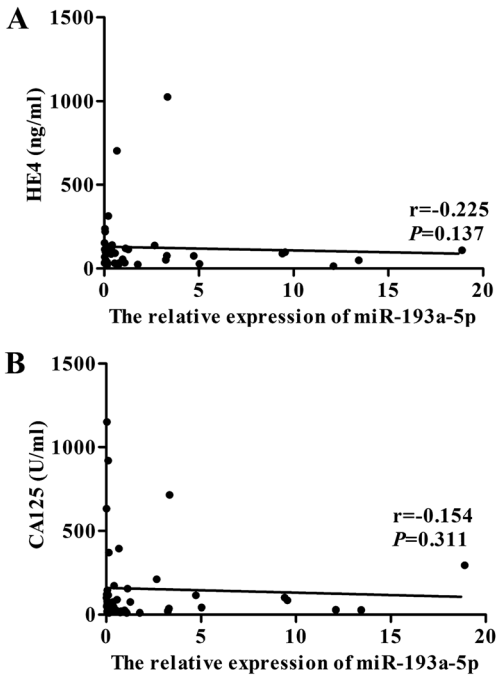

Fig. 2 Correlations of serum miR-193-5p level with HE4 and CA125. **a** HE4 **b** CA125

Table 1 Comparison of the diagnostic efficacy of miR-193a-5p, HE4 and CA125 in EOC

lncRNA	AUC	SEN	SPE	ACCU	PPV	NPV
miR-193a-5p	0.708	66.7%(30/45)	72.5%(29/40)	69.4%(59/85)	73.2%(30/41)	65.9%(29/44)
HE4	0.877	15.6%(7/45)	72.5%(29/40)	42.4%(36/85)	38.9%(7/18)	65.9%(29/67)
CA125	0.900	51.1%(23/45)	67.5%(27/40)	58.8%(50/85)	63.9%(23/36)	54.0%(27/50)
model	0.996	93.3%(42/45)	97.5%(39/40)	95.3%(81/85)	97.7%(42/43)	92.9%(39/42)

Discussion

Accumulating studies have identified a number of miRNAs with aberrant expression in EOC associated with multiple genetic factors such as tumor suppressor genes and oncogenes during cell proliferation, apoptosis, migration and invasion. Molecular signaling pathways involved in miRNA-mediated regulation of EOC. Let-7 targets c-Myc, ras, high-mobility group A (HMGA), Janus protein tyrosinekinase (JAK), signal transducer and activator of transcription3 (STAT3), and NIRF [14] .The miR-200 family played an important role in transition by targeting ZEB-1 and ZEB-2, affecting epithelial-mesenchymal transition (EMT) in EOC [15] .MiR-21 was the activator of the PTEN/AKT pathway, leading to cisplatin resistance in ovarian cancer cells by down-regulation of PTEN protein and activation of the PI3K/AKT/mTOR pathway [16] .Nakano et al. [17] found miR-124, miR-192 and miR-193 were over-expression in A2780 cells that may be as therapeutic tools to treat ovarian cancer. MiR-193a also potentially targets the ARHGAP19, CCND1, ERBB4, KRAS, and MCL1 genes. Multiple miRs appear to be dysregulated in EOC but it is unclear whether circulating miRs can be used to identify women with EOC compared to controls. The discovery of robust predictive biomarkers and the development of new molecularly targeted drugs are essential for effective treatment EOC.

Recent studies found that detection of serum miR-193a-3p could be used for accessory diagnosis of NSCLC [18] .Serum miR-141, miR-214, miR-146b-5p, and miR-193a-3p were as novel biomarkers for Parkinson's disease [19] .MiR-193a-5p also expressed in a group of South African patients diagnosed with chronic myeloid leukaemia [20] .But serum miR-193a is not detected in EOC. In this study, we successfully established a method of detecting serum miR-193a-5p by RT-qPCR, and found that the relative expression level of serum miR-193a-5p in EOC was lower than that in benign ovarian tumor and healthy control groups, indicating that it may has some value in accessory diagnosis of EOC.

CA125 has been so far the best-performing single tumor marker in early detection and prediction of prognosis of ovarian cancer. However, 20% of ovarian cancer patients presented normal or only slightly elevated serum CA125, especially in early stage disease [21] .In addition, number of studies reported that Human Epididymis-specific protein 4 (HE4) was frequently over-expressed in ovarian cancers [22] .Moreover, HE4 showed better sensibility and specificity in the diagnosis of ovarian cancer recurrence with respect to CA-125 [23] .Thus, combing CA-125, HE4 or other markers to discriminate ovarian cancer from other benign gynaecological diseases will be used widely in the future.

In the present study, ROC showed that AUC of miR-193-5p, HE4 and CA125 for the diagnosis of EOC was 0.708, 0.877 and 0.900 respectively. But the sensitivity and specificity of miR-193a-5p were higher than those of HE4 and CA125. Therefore, it is necessary to use a combination to

Table 2 Binary logistic regression analysis of the miR-193a-5p, CA125 and HE4 in sera of patients with EOC

lncRNA	Regression coefficient	OR (95% CI)	*P*
miR-193	−0.134	0.875(0.305–2.510)	0.044
HE4	0.054	1.055(0.971–1.147)	0.021
CA125	0.484	1.623(1.123–2.345)	0.010

The regression equation: The relative expression level of the panel of 2 lncRNAs = −7.268-0.134 × miR-193 + 0.054 × HE4 + 0.484 × CA125

CI: confidence interval. OR: odds ratio

Table 3 Correlation between the model and clinicopathologic features of EOC patients

Clinicopathologic features	n	*P*
FIGO stage		
I–II	12	0.664
III–IV	26	
Unknown	27	
Grading		
Low grade	24	0.016*
High grade	14	
Unknown	7	
Histological types		
Serous adenocarcinoma	22	0.768
Mucinous	15	
Endometrioid	8	
Lymph node metastasis		
N0	17	0.029*
N1	23	
Unknown	5	

The relative expression levels of the panel of miR-193a-5p, CA125 and HE4 were calculated using a Logist regression analysis. * $P < 0.05$

improve both sensitivity and specificity. Knowing that logistic regression analysis is an ideal method in assessing the diagnostic efficacy of multiple indexes, we constructed a diagnostic model of miR-193-5p, HE4 and CA125 combination with a Logist regression analysis. The model showed a more powerful ability to distinguish EOC patients from healthy controls, which the AUC was 0.996 much higher than any single traditional biomarkers. The sensitivity and specificity of the model was also highest, indicating that combined detection of miR-193-5p, HE4 and CA125 may help early screening of EOC. Furthermore, the expression of the three-marker panel was significantly associated with clinical characteristic of EOC, such as grading, lymph node metastasis. These observations suggested that serum expression of the model may prove to be useful in auxiliary diagnosis of EOC. However, further examination with long-term follow-up is necessary to evaluate predictability of serum miR-193-5p.

In summary, the expression level of serum miR-193-5p was decreased in EOC. Combined detection of miR-193-5p, HE4 and CA125 by logistic regression analysis could improve the diagnostic efficacy of EOC, and may prove to be a reliable mode for early screening and diagnosis of EOC.

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Compliance with Ethical Standards

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Ethical Approval All samples in the manuscript were anonymous and the study protocol was approved by the local ethics committee.

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