

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: $Y = \text{Logit}(P) = -5.457 + 5.365 * \text{miR}183 + 0.019 * \text{HE}4 + 0.004 * \text{CA}125$. 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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Table 1. — Frequency distribution of subject's epidemiologic and clinical characteristics by case-control status.

| Subject characteristics | Training set (n=201) | | | Validation set (n=201) | | |
|-------------------------|----------------------|------------|------------|------------------------|------------|------------|
| | 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.

centration 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 ddH₂O (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 ddH₂O (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 ddH₂O (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 training 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) |

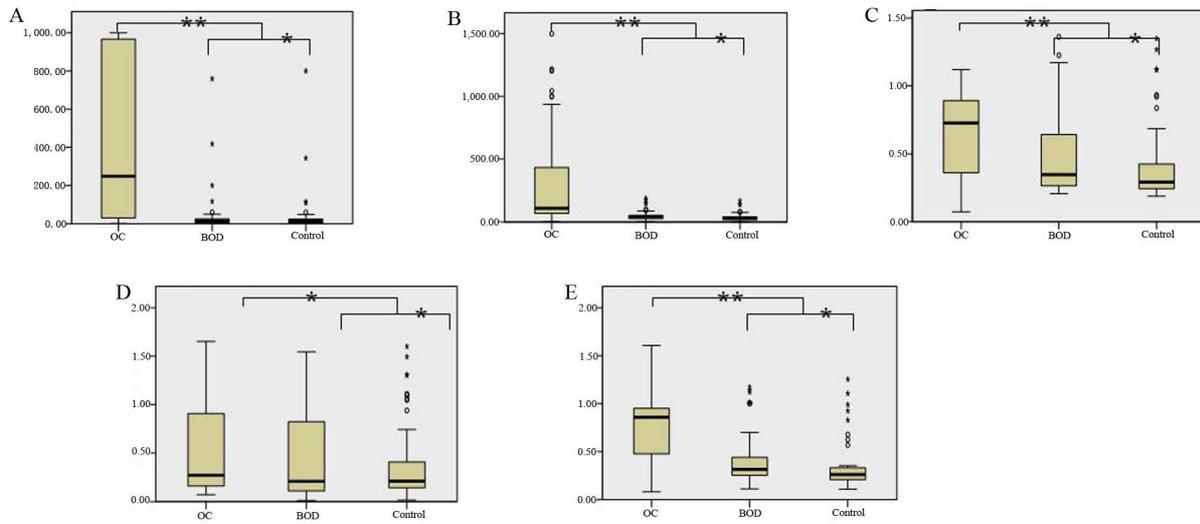


Figure 1. — Frequency distribution of candidate markers levels in training set. (A) CA125 in training set. (B) HE4 in training set. (C) miR-205 in training set. (D)miR-182 in training set. (E) miR-183 in training set. * $p < 0.05$, ** $p < 0.01$.

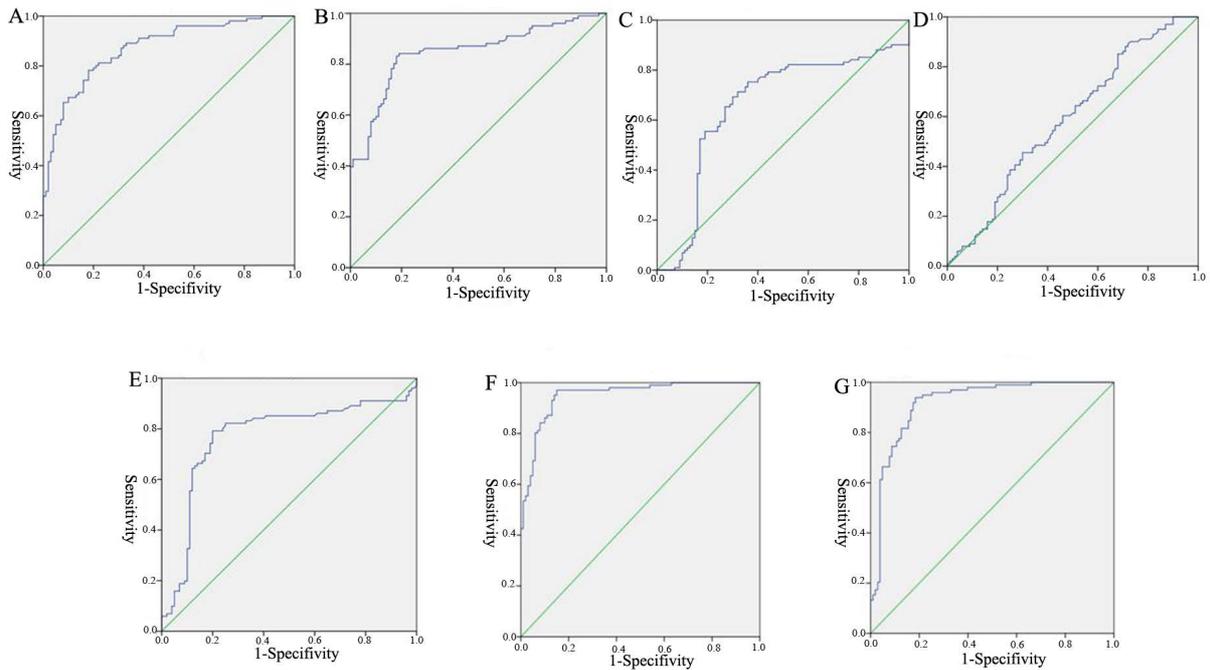


Figure 2. — The ROC curves of all candidate markers in all patients. (A) CA125 in training set. (B) HE4 in training set. (C) miR-205 in training set. (D) miR-182 in training set. (E) miR-183 in training set. (F) Combination of CA125, HE4, and miR-183 in training set. (G) Combination of CA125, HE4, and 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: $Y = \text{Logit}(P) = -$

$5.457 + 5.365 * \text{miR}183 + 0.019 * \text{HE}4 + 0.004 * \text{CA-125}$. 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-183 in 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-

regulation of PDCD4 expression and promote the growth and invasiveness of cancer cells [11], resulting in tumorigenesis and resistance to chemotherapy. Expression of miR-182 is high in ovarian cancer tissues, and high miR-182 expression is also related to a reduction in overall survival rate, which may be ascribed to the rare methylation of the miR-182 promoter in ovarian cancer [12]. This finding indicates that miR-182 may serve as an indicator for the diagnosis of ovarian cancer and predicting prognosis. The present results indicate that miR-182 is highly expressed in the serum of ovarian cancer patients, and the expression of miR-182 is significantly different between ovarian cancer patients and patients with benign ovarian diseases.

CA-125 is the most common marker used to evaluate pelvic tumors. CA-125 has been widely used in the diagnosis of malignant ovarian tumors, especially for clinical diagnosis, evaluation of therapeutic efficacy, and monitoring of disease condition in ovarian cancer patients; however, there is evidence showing that < 50% of patients with early stage ovarian cancer have elevated CA-125 levels, and 20% of patients with ovarian cancer have a CA-125 deficiency [22]. Thus, CA-125 is generally used to screen for ovarian cancer, but is not used as a specific indicator in the diagnosis of ovarian cancer. The present results showed that CA-125 has a high sensitivity (0.832), but a low specificity (0.27) in the diagnosis of ovarian cancer, which is consistent with previous findings [22]. HE4 is also known as human epididymal secretory protein 4, and is a new biomarker for ovarian cancer. It has been reported that HE4 has high specificity and high sensitivity in the early diagnosis of ovarian cancer [23]. In 2003, Hellstrom *et al.* [24] used ELISA in the detection of serum HE4 in 37 patients with ovarian cancer, 19 patients with benign ovarian diseases, and 65 healthy controls, and showed that the sensitivity of HE4 was similar to CA-125, but HE4 had a higher specificity in the diagnosis of ovarian cancer. Thus, Hellstrom *et al.* [24] proposed that HE4 can serve as a serum marker for ovarian cancer. Holcomb *et al.* [25] determined CA-125 and HE4 levels in premenopausal women with appendage masses, and showed that the sensitivity of CA-125 and HE4 was 83.3% and 88.9%, respectively, and the specificity was 59.5% and 91.8%, respectively, in the diagnosis of ovarian cancer. The present authors showed that HE4 has a high specificity without significantly compromising the sensitivity in the diagnosis of ovarian cancer compared to CA-125.

Identifying cancer markers with high specificity, high sensitivity, and simple detection is important for the early diagnosis of ovarian cancer. In the current study, ROC was used to evaluate the sensitivity and specificity of each marker in the diagnosis of ovarian cancer. The present results indicated that serum miR-182 and CA-125 had the highest sensitivity, but the specificity was low. Although miR-205 expression was significantly different between ovarian cancer patients and patients with benign ovarian

diseases, the sensitivity and specificity were low and the markers were not suitable for use as diagnostic markers for ovarian cancer. HE4 and miR-183 have favorable specificity and sensitivity, but any one single indicator was not good enough to be applicable in the diagnosis of ovarian cancer. Binary logistic regression analysis was then used to construct a diagnostic model for ovarian cancer in which CA125, HE4, and miR-183 are included. The results indicated that this diagnostic model had high specificity and high sensitivity in the diagnosis of ovarian cancer. These findings suggest that other cancer markers and clinical characteristics are required to increase the sensitivity and specificity of miR-183 as a marker for the diagnosis of ovarian cancer. Taken together, ovarian cancer patients have high expression of miR-182, miR-183, and miR-205 in the serum, but only miR-183 has relatively high specificity and high sensitivity in the diagnosis of ovarian cancer. The combined use of miR-183 and other markers, such as HE4, may facilitate the early diagnosis of ovarian cancer and improve the evaluation of disease status.

References

- [1] Siegel R., Naishadham D., Jemal A.: "Cancer statistics, 2013". *CA Cancer J. Clin.*, 2013, 63, 11.
- [2] Foley O.W., Rauh-Hain J.A., del Carmen M.G.: "Recurrent epithelial ovarian cancer: an update on treatment". *Oncology*, 2013, 27, 288.
- [3] Hall M., Gourley C., McNeish I., Ledermann J., Gore M., Jayson G., *et al.*: "Targeted anti-vascular therapies for ovarian cancer: current evidence". *Br. J. Cancer*, 2013, 108, 250.
- [4] Bian J., Li B., Kou X.J., Liu T.Z., Ming L.: "Clinical significance of combined detection of serum tumor markers in diagnosis of patients with ovarian cancer". *Asian Pac. J. Cancer Prev.*, 2013, 14, 6241.
- [5] Tsuchiura M., Ichikawa D., Komatsu S., Shiozaki A., Takeshita H., Kosuga T., *et al.*: "Circulating microRNAs in plasma of patients with gastric cancers". *Br. J. Cancer*, 2010, 102, 1174.
- [6] Li B.S., Zhao Y.L., Guo G., Li W., Zhu E.D., Luo X., *et al.*: "Plasma microRNAs, miR-223, miR-21 and miR-218, as novel potential biomarkers for gastric cancer detection". *PLoS One*, 2012, 7, 41629.
- [7] Chen H.X., Zhang L., Zhang L.L., Du J., Wang H.Y., Wang B.: "MicroRNA-183 correlates cancer prognosis, regulates cancer proliferation and bufalin sensitivity in epithelial ovarian cancer". *Am. J. Transl. Res.*, 2016, 8, 1748.
- [8] Marzec-Kotarska B., Cybulski M., Kotarski J.C., Ronowicz A., Tarkowski R., Polak G., *et al.*: "Molecular bases of aberrant miR-182 expression in ovarian cancer". *Genes Chromosomes Cancer*, 2016, 55, 877.
- [9] Niu K., Shen W., Zhang Y., Zhao Y., Lu Y.: "MiR-205 promotes motility of ovarian cancer cells via targeting ZEB1". *Gene*, 2015, 574, 330.
- [10] Wang L., Zhu M.J., Ren A.M., Wu H.F., Han W.M., Tan R.Y., Tu R.Q.: "A ten-microRNA signature identified from a genome-wide microRNA expression profiling in human epithelial ovarian cancer". *PLoS One*, 2014, 9, 96472.
- [11] Wang Y.Q., Guo R.D., Guo R.M., Sheng W., Yin L.R.: "MicroRNA-182 promotes cell growth, invasion, and chemoresistance by targeting programmed cell death 4 (PDCD4) in human ovarian carcinomas". *J. Cell. Biochem.*, 2013, 114, 1464.
- [12] Zheng H., Zhang L.N., Zhao Y.R., Yang D., Song F.J., Wen Y., *et al.*: "Plasma miRNAs as diagnostic and prognostic biomarkers for ovarian cancer". *PLoS One*, 2013, 8, e77853.
- [13] Xu J., Wu C., Che X., Wang L., Yu D., Zhang T., *et al.*: "Circulating

- ng microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis". *Mol. Carcinog.*, 2011, *50*, 136.
- [14] Zhu W., He J., Chen D., Zhang B., Xu L., Ma H., *et al.*: "Expression of miR-29c, miR-93, and miR-429 as potential biomarkers for detection of early stage non-small lung cancer". *PLoS one*, 2014, *9*, 87780.
- [15] Chen X., Ba Y., Ma L., Cai X., Yin Y., Wang K., *et al.*: "Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases". *Cell Res.*, 2008, *18*, 997.
- [16] Lu J., Getz G., Miska E.A., Alvarez-Saavedra E., Lamb J., Peck D., *et al.*: "MicroRNA expression profiles classify human cancers". *Nature*, 2005, *435*, 834.
- [17] Eitan R., Kushnir M., Lithwick-Yanai G., David M.B., Hoshen M., Glezerman M., *et al.*: "Tumor microRNA expression patterns associated with resistance to platinum based chemotherapy and survival in ovarian cancer patients". *Gynecol Oncol.*, 2009, *114*, 253.
- [18] Sorrentino A., Liu C.G., Addario A., Peschle C., Scambia G., Ferlini C.: "Role of microRNAs in drug-resistant ovarian cancer cells". *Gynecol Oncol.*, 2008, *111*, 478.
- [19] Yang N., Kaur S., Volinia S., Greshock J., Lassus H., Hasegawa K., *et al.*: "MicroRNA microarray identifies Let-7i as a novel biomarker and therapeutic target in human epithelial ovarian cancer". *Cancer Res.*, 2008, *68*, 10307.
- [20] Fang L., Du W.W., Yang W., Rutnam Z.J., Peng C., Li H., *et al.*: "MiR-93 enhances angiogenesis and metastasis by targeting LAT52". *Cell cycle*, 2012, *11*, 4352.
- [21] Yang M., Chen J., Su F., Yu B., Su F., Lin L., *et al.*: "Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells". *Mol. Cancer*, 2011, *10*, 117.
- [22] Jacobs I., Bast R.C., Jr.: "The CA 125 tumour-associated antigen: a review of the literature". *Hum. Reprod.*, 1989, *4*, 1.
- [23] Kirchoff C.: "Molecular characterization of epididymal proteins". *Rev. Reprod.*, 1998, *3*, 86.
- [24] Hellstrom I., Raycraft J., Hayden-Ledbetter M., Ledbetter J.A., Schummer M., McIntosh M., *et al.*: "The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma". *Cancer Res.*, 2003, *63*, 3695.
- [25] Holcomb K., Vucetic Z., Miller M.C., Knapp R.C.: "Human epididymis protein 4 offers superior specificity in the differentiation of benign and malignant adnexal masses in premenopausal women". *Am. J. Obstet. Gynecol.*, 2011, *205*, 351.

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