Plasma exosomal miRNA-205 can be used for early diagnosis and prognostic evaluation of ovarian cancer

zehua zhu (942622846@qq.com)
Hangzhou Normal University

Zehua Zhu
Hangzhou Normal University

Zhaojun Chen
Hangzhou Normal University Affiliated Hospital

Mingxin Wang
Hangzhou Normal University

Min Zhang
Zhejiang Cancer Hospital

Yiwen Chen
Hangzhou Normal University Affiliated Hospital

Xiao Yang
Hangzhou Normal University Affiliated Hospital

Changjun Zhou
Hangzhou Normal University Affiliated Hospital

Yuhua Liu
Hangzhou Normal University Affiliated Hospital

Liquan Hong
Hangzhou Normal University Affiliated Hospital

Lahong Zhang
Hangzhou Normal University Affiliated Hospital https://orcid.org/0000-0002-6629-1045

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Abstract

Background

Ovarian cancer (OC) is a serious threat to women's health around the world and new biomarkers are urgently needed for early detection. Our previous published research have confirmed that miR-205 can promote the invasion and metastasis of OC cells by inhibiting the expression of the tumor suppressor gene TCF21. This study is based on the previous work, using exosomal liquid biopsy technology to detect the expression levels of the four genes miR-205, CA125, HE4 and TCF21 in the plasma exosomes of OC patients; at the same time, combined with the clinicopathological parameters of OC patients data analysis aims to provide efficient and non-invasive laboratory testing basis for the early diagnosis of OC.

Methods

Collected 36 OC patients diagnosed in local hospitals from September 2020 to July 2021 as OC group, selected 31 cases of surgically diagnosed ovarian benign lesions for benign group and 32 healthy people who underwent physical examination during the same period as a control group, and used transmission electron microscope (TEM), western blotting (WB) and nanoparticle tracking analysis (NTA) to identify after extracting plasma exosomes from kits. The expression levels of miR-205, CA125, HE4 and TCF21 genes in plasma exosomes were detected by real-time fluorescence quantitative PCR (qRT-PCR) method. At the same time, combined with the relationship between clinical pathological parameters in OC patients, the Receiver Operating Characteristic (ROC) curves were used to evaluate the diagnostic efficacy of plasma exosomal miR-205, CA125, HE4 and TCF21 for OC.

Results

Plasma exosomes were successfully isolated; the expression level of plasma exosomal miR-205 in the OC group was higher than that of the benign group and the control group, and the difference was statistically significant ($P < 0.05$); plasma exosomal miR-205 was elevated during the III-IV period of OC and lymph node metastasis; combined detection of plasma exosomal miR-205 can improve the diagnostic ability of OC.

Conclusion

The plasma exosomal miR-205 can be used as a potential tumor biomarker to improve the diagnostic effectiveness of OC.

Background

Ovarian cancer (OC) is one of the most common gynaecological malignancies, with the leading fatality rate in the female reproductive system, posing a serious threat to women's health worldwide [1]. Due to the lack of early clinical manifestations and effective screening methods, 70%-75% of OC patients are at an advanced stage at the time of diagnosis and miss the best time for treatment, so OC is called the "silent killer" [2]. Therefore, there is an urgent need for more efficient, non-invasive, and early screening methods to improve the prognosis of OC patients. Liquid biopsy technology represented by exosomes is likely to solve this problem.

Exosomes are tiny vesicles secreted by most cells, with their unique lipid bilayer membrane structure, can well protect their contents from degradation, such as mRNA and miRNA [3]. Therefore, exosomes are much more stable than the targets contained in plasma. In the early stages of tumors, cancer cells secrete a large amount of exosomes, reaching 10^{12}/mL of blood, which makes the quantitative detection of exosomes easier and makes them possible as a new tumor marker [4]. Our previous published studies have found that miR-205 is up-regulated in OC tissues, and in the mechanism study, we have confirmed that miR-205 can promote the invasion and metastasis of OC cells by inhibiting the expression of tumor suppressor gene TCF21 [5], suggesting that miR-205 has the potential to become a new type of OC diagnostic biomarker. This study is based on the previous work, using exosomal liquid biopsy technology to detect the expression levels of the four genes miR-205, CA125, HE4 and TCF21 in the plasma exosomes of OC patients; at the same time, combined with the clinicopathological parameters of OC patients data analysis to explore the diagnostic value and clinical significance of the four exosomal genes for OC.

Methods

Study populations

A total of 99 female subjects were included in the study, 36 in the OC group, 31 in the benign group, and 32 in the control group. Cases in the OC group were all from 36 ovarian cancer patients (32 high-grade serous adenocarcinomas, 3 endometrioid adenocarcinomas, and 1 clear cell carcinoma) admitted to Zhejiang Cancer Hospital from September 2020 to July 2021, and the inclusion criteria were: (1) All met the relevant diagnostic criteria for ovarian cancer, and the histological type and the International Union of Obstetrics and Gynecology (FIGO) staging were confirmed by postoperative pathological examination [6]; (2) None of them received any anti-tumor treatment before drawing blood; (3) patients with complete medical records; (4) patients without other malignant diseases. The benign group came from 31 patients with benign ovarian lesions (including 7 serous cystadenoma, 7 endometriotic cyst, 5 mature teratoma, 3 cases of mucinous cystadenoma, 9 cases of simple ovarian cyst) who were surgically diagnosed in the Affiliated Hospital of Hangzhou Normal University during the same period. The control group consisted of 32 healthy women who participated in the physical examination in this hospital during the same period, and excluded hypertension, diabetes and other diseases, and no history of malignant tumors in all systems of the body, no dysfunction of important organs.

The OC group was 27–90 years old, with an average age of 57 ± 13 years; the benign group was 24–73 years old, with an average of 49 ± 13 years old; the control group was 27–77 years old, with an average age of 51 ± 14 years. There was no significant difference in age between the three groups ($P > 0.05$). The samples in this study obtained the informed consent of all subjects and were approved by the hospital ethics committee (Ethics No. 2019 (Len 02)-HS-03).

Plasma collection

All the subjects were drawn 4mL-6mL of EDTA anticoagulated venous blood on an empty stomach in the early morning, centrifuged at 3 500 r/min for 10 min at 4℃ to obtain plasma, divided into 2 tubes, one tube was stored in the refrigerator at -80℃; the other tube immediately detects the plasma protein CA125 and HE4 concentrations. If the samples cannot be detected in time within 8 hours, they should be stored in a refrigerator at 4°C, and the detection should be completed within 24 hours.

Detection of plasma protein CA125 and HE4

Plasma protein CA125 concentrations were detected using the Chemiluminescence Immunoassay Analyzer (Abbott ARCHITECT i2000SR, USA), and the plasma protein HE4 concentrations were detected using the Electrochemiluminescence Immunoassay Analyzer (Roche Cobas E602,Germany). Use the corresponding kit and strictly follow the manufacturer's protocol for testing.

Exosome isolation

Plasma exosomes were isolated using the Total Exosome Isolation Reagent (#4484450; Invitrogen, USA) according to the manufacturer’s protocol. General situation: First, the sample was thawed at room temperature, centrifuged at 2000g for 20 min, and centrifuged again at 10000×g for 20 min to completely remove the cells and debris. Next, 400 μL of plasma supernatant was mixed with 120 μL reagents, incubated at 4°C for 30 min, and centrifuged at 10,000 g for 5 min to obtain exosomal pellets. The exosomal samples were stored at -80°C for further analysis.

Transmission electron microscopy(TEM)

Resuspend the exosomal pellets in 1×PBS, and deposit the liquid on the carbon-coated copper mesh for 2 min. The excess liquid was removed, and filter paper was used to drain the grid; a drop was negatively stained with phosphotungstic acid and loaded onto the grid for 5 min. The grid was then dried at room temperature. Finally, the samples were observed by JEOL-1230 TEM at an acceleration voltage of 80 kV .

Nanoparticle tracking analysis (NTA)

The size and concentration of exosomes were used by NTA at VivaCell Biosceinces with ZetaView PMX 110 (Particle Metrix, Meerbusch, Germany) and corresponding software ZetaView 8.04.02. Specifically: The ZetaView system was calibrated with 110 nm polystyrene particles, then properly dilutes the separated exosome sample with 1× PBS buffer to measure particle size and concentration, and records

and analyzes NTA measurements at 11 locations, with temperatures remaining at around 23°C and 30°C during testing.

Western blotting analyses (WB)

The exosomal sample were treated with lysis buffer to obtain total exosomal protein, and the protein was quantified by the BCA method. Load exosomal proteins onto the SDS-PAGE and transfer it to the PVDF membrane. The PVDF membrane was blocked with BSA at room temperature for one hour, and incubated with primary antibodies including CD63 and TSG101 overnight, followed by incubation with the corresponding HRP-conjugated secondary antibodies. The proteins transferred on the PVDF membrane were finally visualized with a gel imaging system(BIO-RAD ChemiDoc XRS, USA).

Extraction of exosomal RNA and reverse transcription synthesis cDNA

Total RNA was extracted from plasma exosomes resuspended using the Multi-type Sample DNA/RNA Extraction-Purification Kit (Sansure Biotech Inc. Hunan, China) following the manufacturer's instructions. The concentration of RNA was measured using the e-spect Spectrophotometer (Beijing labaid science and technology. Ltd, China), and the OD 260/280 nm ratios of all RNA samples were ≥ 1.8. Divide the total RNA sample into two parts. Part of it was reverse-transcribed into cDNA for the following qRT-PCR of miR-205, specifically using the Mir-XTM miRNA first-strand synthesis kit (#638313; TAKARA Bio Inc, USA); the remaining RNA samples were stored at -80°C and used for qRT-PCR of CA125, HE4 and TCF21.

qRT-PCR analysis for quantification of miR-205

The reagent was TB Green Advantage qPCR Premix (#639676; TAKARA Bio Inc, USA), and the reaction was performed on the 7500 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific. Inc, USA). Specifically: two microliters cDNA products were used for qRT-PCR template, and the final volume was 25μl. U6 was used as the internal reference gene, and the reaction conditions were: 95°C pre-denaturation for 10s, 95°C denaturation for 5s, and 60°C annealing for 20s, a total of 40 cycles to get dissociation curve. Analyze the relative expression level of exosomal miR-205 using the $2^{-\Delta\Delta Ct}$ relative quantitation method[7].

qRT-PCR analysis for quantification of CA125, HE4 and TCF21

The expression levels of CA125, HE4 and TCF21 were detected by One Step TB Green®PrimeScript™Plus RT-PCR Kit (#RR096A, TAKARA Bio Inc, USA) on the 7500 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific. Inc, USA). Using β-actin as the internal control gene, the reaction conditions were: reverse transcription of RNA into cDNA at 42°C for 5 min, 95°C for 10 sec, denaturation at 95°C for 5 sec, and annealing at 60°C for 34 sec, a total of 40 cycles to abtain the products. The relative expression of genes was calculated by the $2^{-\triangle\triangle CT}$ method.

The primers used in this study were purchased from Shanghai Biotech Co., Ltd. China, and the primer sequences were shown in Table 1.

Table 1
qRT-PCR primer sequences for each gene

Genes	Forward Sequence	Reverse Sequence
CA125	5 '-ACTGCCACTGAGCCAACAAGTTC-3'	5 '-GACTGTGCCAAGACTATCCGAAGC-3'
HE4	5 '-TCAACAGAAGGAGGCAATGTAT-3'	5 '-CAGCTGCTTAATCTTATGCTCG-3'
TCF-21	5 '-CAGCGATGTGGAGGACCTTCAAG-3'	5 '-TCTCCTCGGTGCTCTCGTTGG-3'
β-Actin	5 '-CTCCATCCTGGCCTCGCTGT-3'	5 '-GCTGTCACCTTCACCGTTCC-3'
miR-205	5 '-TCCTTCATTCCACCGGAGTCTG-3'	MRQ 3' Primer
U6	5 '-GGAACGATACAGAGAAGATTAGC-3'	5 '-TGGAACGCTTCACGAATTTGCG-3'

Statistical analysis

All data were analyzed using the SPSS 26.0 statistical software (IBM Corp, USA) and plotted by GraphPad Prism 7.0 (GraphPadSoftware Inc, USA). All data were expressed as mean ± standard deviation or median (interquartile range) according to data distribution. The Mann-Whitney *U* test was used to compare the two groups, and the Kruskal-Wallis *H* test or one-way ANOVA was used for comparison between multiple groups. The area under the curve (AUC) of the receiver operating characteristic curve (ROC) was used to analyze the diagnostic value of plasma exosomal miR-205, CA125, HE4 and TCF21 for OC. $P < 0.05$ was considered statistically significant.

Results

Characteristics of plasma exosomes

The exosomes extracted in this experiment were identified by three methods: TEM, NTA and WB. As we proved, the extracted clusters were observed under TEM as cup-shaped or dish-shaped vesicles with a diameter of about 100 nm (Fig. 1a); the WB results showed that the lysates of isolated plasma particles of all groups expressed the specific exosomal proteins CD63 and TSG101 (Fig. 1b); the NTA results showed that the particle size distribution range of exosomes was 20.7 ~ 345.2 nm, with an average particle size of 98.6 nm, and the total concentration of the exosomes was 1.3E + 12 particles/mL after multiplication by 20,000 times (Fig. 1c).

Comparison of expression levels of plasma exosomal miR-205, CA125, HE4 and TCF21 among the three groups of people

The expression levels of four genes in the three groups of subjects were shown in Table 2 and Fig. 2. The results showed that the expression level of plasma exosomal miR-205 in the OC group was significantly higher than that of the benign group and the control group ($P < 0.01$) (Fig. 2a); the expression level of plasma exosomes CA125 in the OC group was the highest among the three groups, which was

significantly different from the benign group ($P < 0.01$) (Fig. 2b); the expression levels of plasma exosomal HE4 in benign group was significantly lower than OC and control group($P < 0.01$)(Fig. 2c);the expression level of plasma exosomal TCF21 in the OC group and the benign group was significantly lower than that of the control group ($P < 0.05$) (Fig. 2d). In addition, there was no significant difference in the expression levels of four genes among the other groups ($P > 0.05$).

Table 2
Expression levels of plasma exosomal miR-205, CA125, HE4 and TCF21 in three groups of people [Median(Q1,Q3)]

Test Indicators	OC group (n = 36)	benign group (n = 31)	control group (n = 32)
exosomal miR-205	3.24 (1.56, 8.48) [ab]	1.93 (0.67, 3.30)	1.34 (1, 2.41)
exosomal CA125	1.97 (0.87, 3.25) [a]	0.66 (0.15, 1.79)	1 (1, 1.56)
exosomal HE4	1.57 (0.79, 2.23) [a]	0.42 (0.13, 1.19) [b]	1 (1, 1.16)
exosomal TCF21	0.94 (0.51, 1.71) [b]	0.85 (0.15, 2.04) [b]	1 (1, 2.47)
P values were calculated by the Kruskal-Wallis *H* test			
a indicated that there was a significant difference compared with the benign group ($P < 0.05$) ;			
b indicated that there was a significant difference compared with the control group ($P < 0.05$).			

The relationship between detection indicators and clinicopathological parameters of OC patients

In OC patients, we detected the expression levels of plasma exosomal miR-205, CA125, HE4 and TCF21 and the plasma protein concentrations of CA125 and HE4, combined with the analysis of the patient's clinicopathological parameters, it was found that only the expression level of plasma exosomes miR-205 in stage III-IV was higher than that in stage I-II [4.59(2.23,9.39) vs (1.28(0.65, 3.35))], the group with lymph node metastasis was higher than the group without lymph node metastasis [5.15 (2.40, 10.02) vs 1.69 (0.78, 3.35)], both were statistically significant ($P < 0.05$). All other test indicators had nothing to do with age, menopausal status, FIGO stage, lymph node metastasis, and tumor site ($P > 0.05$). See Table 3 for details.

Table 3
Relationship between detection indexes and clinicopathological parameters in OC patients

Test Indicators	Age (< 50 years vs.≥50 years)	Menopause (Yes vs. No)	Metastases lymph nodes (Yes vs. No)	FIGO stage (stage I + II vs. stage III + IV)	Tumor site (unilateral vs. bilateral
	P value				
exosomal miR-205	0.689	0.768	0.007*	0.032*	0.553
exosomal CA125	0.849	0.664	0.292	0.845	0.761
exosomal HE4	0.689	0.520	0.435	0.192	0.665
exosomal TCF21	0.337	0.286	0.588	0.557	0.911
protein CA125(U/mL)	0.614	0.271	0.830	0.614	0.665
protein HE4(pmol/L)	0.374	0.286	0.934	0.362	0.170
P values were calculated by Mann-Whitney *U* test.					
* $P < 0.05$					

Evaluate the diagnostic value of the plasma exosomal four genes, plasma protein CA125, and HE4 in OC

ROC curves were used to further evaluate the diagnostic efficacy of plasma exosomal miR-205, CA125, HE4, TCF21 and plasma proteins CA125 and HE4 concentration for OC using the control group as a reference(Table 4 and Fig. 3**)**. The AUC of plasma exosomal miR-205 was 0.715 (95% *CI*: 0.590–0.841, $P = 0.002$), with a sensitivity of 66.7% and a specificity of 78.1%; the AUC of plasma exosomal CA125, HE4, and TCF21 were respectively 0.642, 0.554, and 0.673, which were all less than 0.7 and the diagnostic values of OC were not ideal. The AUC of protein CA125 was 0.915(95%*CI*:0.846–0.983, $P < 0.001$), with a sensitivity of 74.3% and a specificity of 93.7%; the AUC of protein HE4 was 0.779(95%*CI*:0.668–0.889, $P < 0.001$), with a sensitivity of 55.6% and a specificity of 100%. Combined index analysis, exosomal miR-205 combined with protein CA125 and HE4 respectively, the diagnostic AUC was 0.930 (95% CI: 0.865–0.995, $P < 0.0001$); 0.827 (95% CI: 0.726–0.929, $P < 0.0001$)); The AUC of triple diagnosis could be increased to 0.951 (95%CI: 0.899–1.004, $P < 0.0001$), the sensitivity was 100%, and the specificity was 86.1%. However, the AUC of traditional plasma protein CA125 and HE4 was 0.939 (95% CI: 0.883–0.995, $P < 0.0001$), the sensitivity was 96.9% and the specificity was 83.3%.

Table 4
Diagnostic value of single detect index and combined applications for OC patients

Test Indicators	AUC	95% CI	*P*	Sensitivity%	Specificity%	Youden's index
protein CA125 (U/mL)	0.915	0.846–0.983	< 0.001	74.3	93.7	0.68
protein HE4 (pmol/L)	0.779	0.668–0.889	< 0.001	55.6	100.0	0.56
exosomal miR205	0.715	0.590–0.841	0.002	66.7	78.1	0.45
exosomal CA125	0.642	0.501–0.784	0.044	75.0	68.7	0.44
exosomal HE4	0.554	0.403–0.704	0.446	58.3	84.4	0.43
exosomal TCF21	0.673	0.539–0.808	0.014	47.2	59.4	0.07
protein CA125 + protein HE4	0.939	0.883–0.995	< 0.0001	96.9	83.3	0.80
protein CA125 + exosomal miR-205	0.930	0.865–0.995	< 0.0001	96.9	83.3	0.80
protein HE4 + exosomal miR-205	0.827	0.726–0.929	< 0.0001	96.9	69.4	0.66
protein CA125 + protein HE4 + exosomal miR-205	0.951	0.899–1.004	< 0.0001	100	86.1	0.86

Discussion

OC is the fifth most common tumor that threatens the health of women around the world, and its mortality rate ranks first among gynecological tumors[8]. According to the latest cancer report in 2021, the incidence of OC accounts for about 3.4% of all tumors, but the mortality rate is nearly 5%, which means that there are about 310,000 new cases and 200,000 deaths in 2020 [1]. Early detection and early diagnosis are the key to improving the prognosis of OC patients. However, studies have shown that the conventional screening method of serum CA125 combined with vaginal ultrasound cannot reduce the mortality of patients[9, 10]. At present, there is no effective screening method for OC, and the diagnosis must rely on the pathological results of tissue biopsy, which brings great pain to patients [11]. The liquid biopsy technology represented by exosomes can obtain disease information through non-invasive or minimally invasive sampling methods, especially in recent years, which has received more and more attention in tumor screening, diagnosis and treatment, and prognosis evaluation [12]. This also provides new ideas for OC screening and early diagnosis.

In this study, we extracted the plasma exosomes of all subjects with commercial reagents, and then used three methods (TEM, WB, and NTA) to identify the extracted products. It was visually observed under TEM that the particles were in the form of cup-shaped vesicles with a diameter of about 100 nm; WB results showed that the particles expressed exosomal characteristic proteins CD63 and TSG101; NTA results showed that the average size of the particles was 98.6 nm and contained high concentration (1.3E + 12 particles/mL). These results were consistent with the characteristics of exosomes reported in the previous literature [13], so we can confirm the successful extraction of plasma exosomes. Exosomes are mainly involved in the communication between cells, and they are widely present in biological cells and various body fluids [14]. Exosomes derived from cancer cells in the early stages of tumors can be released into the blood in large quantities and are highly stable in peripheral blood, making it possible to be used as new tumor markers. A number of studies have shown that exosomes play an important role in the occurrence, development and drug resistance of ovarian cancer, and they have a certain ability to guide the diagnosis, treatment and prognosis evaluation of OC[15–18].

After successfully extracting plasma exosomes, we further analyzed the expression levels of the four genes miR-205, CA125, HE4 and TCF21 in plasma exosomes and the concentrations of plasma proteins CA125 and HE4, and found that the expression levels of plasma exosomal miR-205 in OC group were significantly higher than in benign groups and control groups, which was consistent with our previous research results in OC tissues and cell lines[5]. At the same time, combined with the analysis of the clinicopathological parameters of OC patients, it was found that the expression level of plasma exosomal miR-205 in OC patients was higher in advanced-stage(stage III-IV) than in early-stage(stage I-II), and the group with lymph node metastasis was higher than the group without lymph node metastasis, and the differences were statistically significant. However, the traditional serum markers of plasma proteins CA125 and HE4 have nothing to do with the FIGO staging of OC and lymph node metastasis. Compared with traditional markers, plasma exosomal miR-205 has shown better early diagnosis and prognostic evaluation of ovarian cancer. The big advantage suggests that plasma exosomal miR-205 is expected to become a new tumor biomarker to assist in the screening and early diagnosis of OC. In view of the fact that we found that miR-205 was highly expressed in OC tissues in our previous basic research, and miR-205 mimic in cell lines can promote the invasion and metastasis of OC cells, we highly suspect that the high expression of miR-205 in OC tissues may transmit relevant information through exosomes, thereby accelerating the overall invasion and metastasis process of OC, which also indicates that plasma exosomal miR-205 may become an early diagnostic biomarker for OC and can be used for prognostic evaluation of OC.

MiR-205 is a highly conservative miRNA, which has a dual effect, both as a tumor promote factor and as a tumor suppressor [19]. Studies have found that the expression of miR-205 was reduced in the serum of breast cancer patients [20]; while the expression of miR-205 was up-regulated in OC tissues and cells, and was related to the growth and metastasis of OC [21], which was consistent with our previous research results. In addition, studies have suggested that vascular endothelial growth factor (VEGF) through the increase of miR-205 expression leads to the down-regulation of Ezrin and Lamin A/C, thereby promoting the invasion of OC cells [22]. CHU et al. found that miR-205 regulated the proliferation and invasion of

ovarian cancer cells by inhibiting the expression of PTEN/Smad4 [23]. Other studies have shown that miR-205 can directly negatively regulate ZEB1 to promote the clinical progress of EOC patients [24]. A recent study showed that miR-205 derived from OC cell exosomes can promote the transfer of OC by inducing angiogenesis [25]. The above studies have confirmed that miR-205 had abnormal expression in OC and was related to the invasion of OC, which supported the results of our experiment.

In order to further evaluate the diagnostic value of plasma exosomal miR-205 for OC, combined with the current traditional biomarkers plasma proteins CA125 and HE4, through ROC curve analysis, it was found that the AUC of plasma proteins CA125 and HE4 were 0.915 and 0.779, respectively, the sensitivity was 74.3% and 55.6%, and the specificity was 93.7% and 100%, respectively. This experiment confirmed that traditional biomarkers have good diagnostic values for OC, but the sensitivity of both was not high, resulting in some patients missing diagnosis, so neither was an ideal means of screening OC. The plasma exosomal miR-205 alone diagnoses OC with an AUC of 0.715, a sensitivity of 66.7%, and a specificity of 78.1%. However, when combined with two traditional markers, it can significantly improve the diagnostic ability of OC. It increased the AUC of CA125 from 0.915 to 0.930, and HE4 from 0.779 to 0.827. The combination of the three detection indicators can increase the AUC to 0.951, the sensitivity to 100%, and the specificity to 86.1%.The above showed that plasma exosomal miR-205 had certain diagnostic ability for OC patients, and combined with traditional serological indicators CA125 and HE4 can effectively improve the diagnostic effectiveness of OC, the three joint tests were especially suitable for early screening of OC.

The study also found that the plasma exosomal CA125 and HE4 expression levels in the OC group were higher than those in the benign group, but there was no statistical difference from the control group, which was inconsistent with the findings of Fawzy et al. that the expression levels of CA125 and HE4 in OC tissues were higher than those of benign tumor tissues and normal ovarian tissues[26]. In addition, the expression level of plasma exosomal TCF21 in the OC group and the benign group was lower than the control group, but there was no difference between the OC group and the benign group. This was inconsistent with our previous basic research finding that the expression level of TCF21 in OC tissues was significantly lower than that of benign tissues[5]. The reason for the analysis may be due to the inherent differences in gene expression patterns in plasma exosomes and tissues. JI J et al. found that the differential expression profile of serum exosomal mRNA was not completely consistent with the tissue mRNA level, which also illustrates this point well[27]; It may also be due to the selective packaging of the mRNA molecules contained in exosomes, and it has been found that exosomes can regulate the packaging process of their internal RNA through a variety of ways[28]; In addition, the effects of differences in mRNA stability, sample source, sample size, and storage time should also be taken into account, which are for further study.

It should also be noted that although this study initially demonstrated the potential advantages of plasma exosomal miR-205 as a new biomarker for OC, this experiment also had certain limitations. Because the sample size of this study was small and may lead to deviations, in the future need to further increase the sample size, preferably multi-center, prospective research. However, in view of the literature

reports that the expression of miRNA had tissue and disease specificity [29, 30]; it can be secreted in the form of exosomes and stably exists in the peripheral blood circulation [31; and it can also be delivered to the tumor microenvironment or distant organs through exosomes, and promote tumor angiogenesis and metastasis by targeting gene expression in recipient cells;in addition, the existing research results indicated that plasma exosomal miR-205 may become a new diagnostic marker for OC after confirmation of large samples, which can provide a certain reference value for early screening of OC.

Conclusions

In summary, our research results showed that plasma exosomal miR-205 had a certain diagnostic ability for OC, and its combination with traditional serological tumor markers can improve the diagnostic efficiency of OC; at the same time, plasma exosomal miR-205 was related to OC staging and lymph node metastasis, which can provide a certain reference value for the early diagnosis and prognostic evaluation of OC patients.

Abbreviations

OC:ovarian cancer; CA125: cancer antigen 125; FIGO: International Federation of Gynecology and Obstetrics; HE4: Human epididymis protein 4; ROC: Receiver operating characteristic curves; AUC: Area under the curve; CI: confidence interval; TCF21: transcription factor 21

Declarations

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Availability of data and materials

The data supporting this study are included in this article.

Authors' contributions

Z-LH and C-ZJ conceived the idea and designed the study protocol, wrote the manuscript. Z-M and Y-X contributed with provision of study material or patients. Z-ZH, W-MX, C-YW and Z-CJ collected, assemble data and interpreted the data; L-YH and H-LQ performed statistical analysis .All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Human Ethics Committee of the Affiliated Hospital of Hangzhou Normal University, and the ethics number was 2019 (Len 02)-HS-03.

Consent for publication

Yes

Competing interests

The authors declare that they have no competing interests.

Author details

[1]The Medical School of Hangzhou Normal University ,Hangzhou, 310015 ,China;

[2] The Clinical laboratory of The Affiliated Hospital of Hangzhou Normal University, Hangzhou,

310015,China;

[3] Institute of Basic Medicine and Cancer of The Cancer Hospital of the University of Chinese

Academy of Sciences (Zhejiang Cancer Hospital), Hangzhou, 310015 ,China.

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Figures

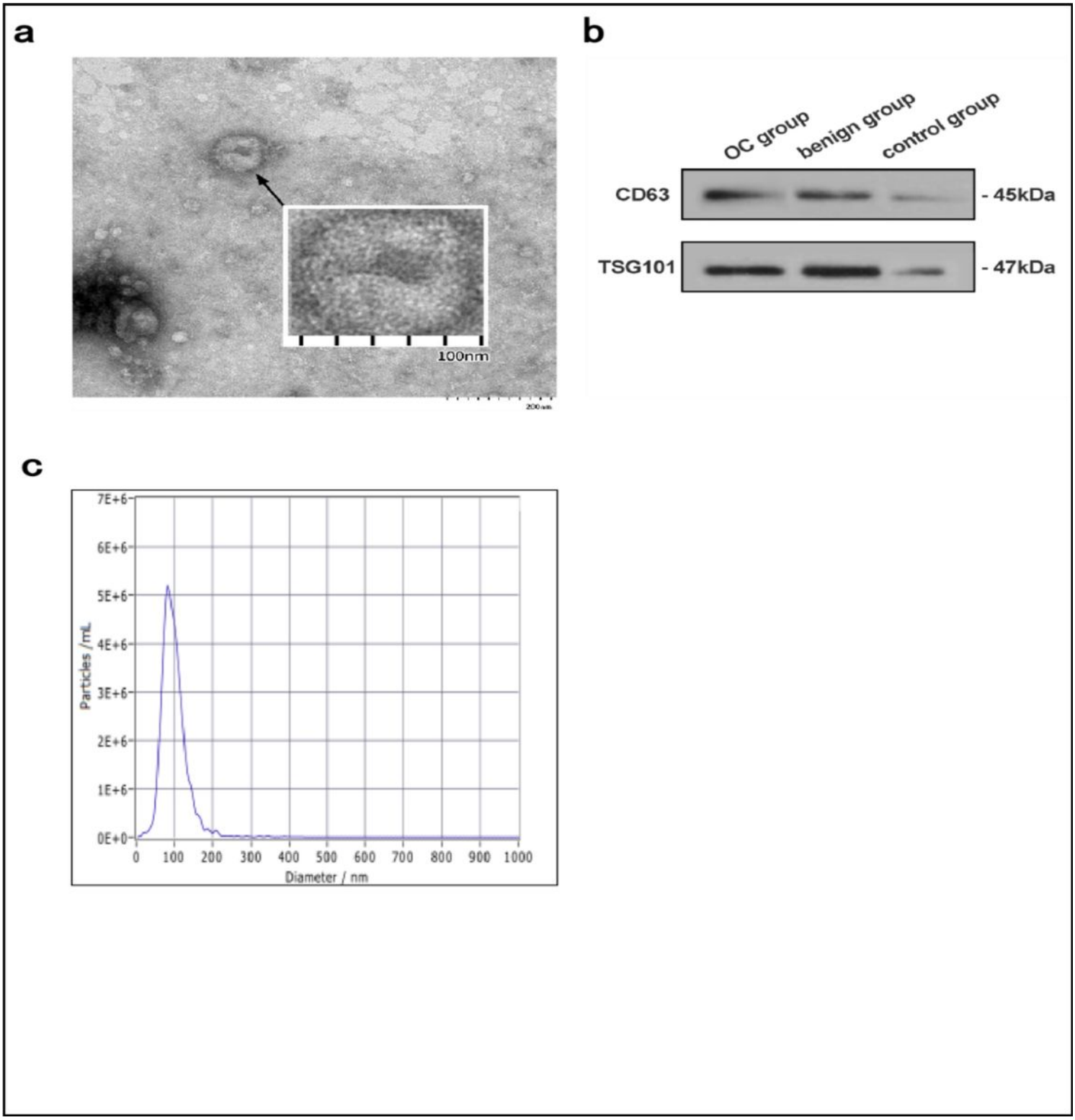

Figure 1

Plasma exosome characteristics. a Transmission electron microscopy (TEM) revealed the round shape of exosomes. The bar represents 200 nm. b The marker proteins of exosomes (CD63 and TSG101) were detected by western blotting (WB). c The Nanoparticle tracking analysis (NTA) presents the size and concentration distribution.

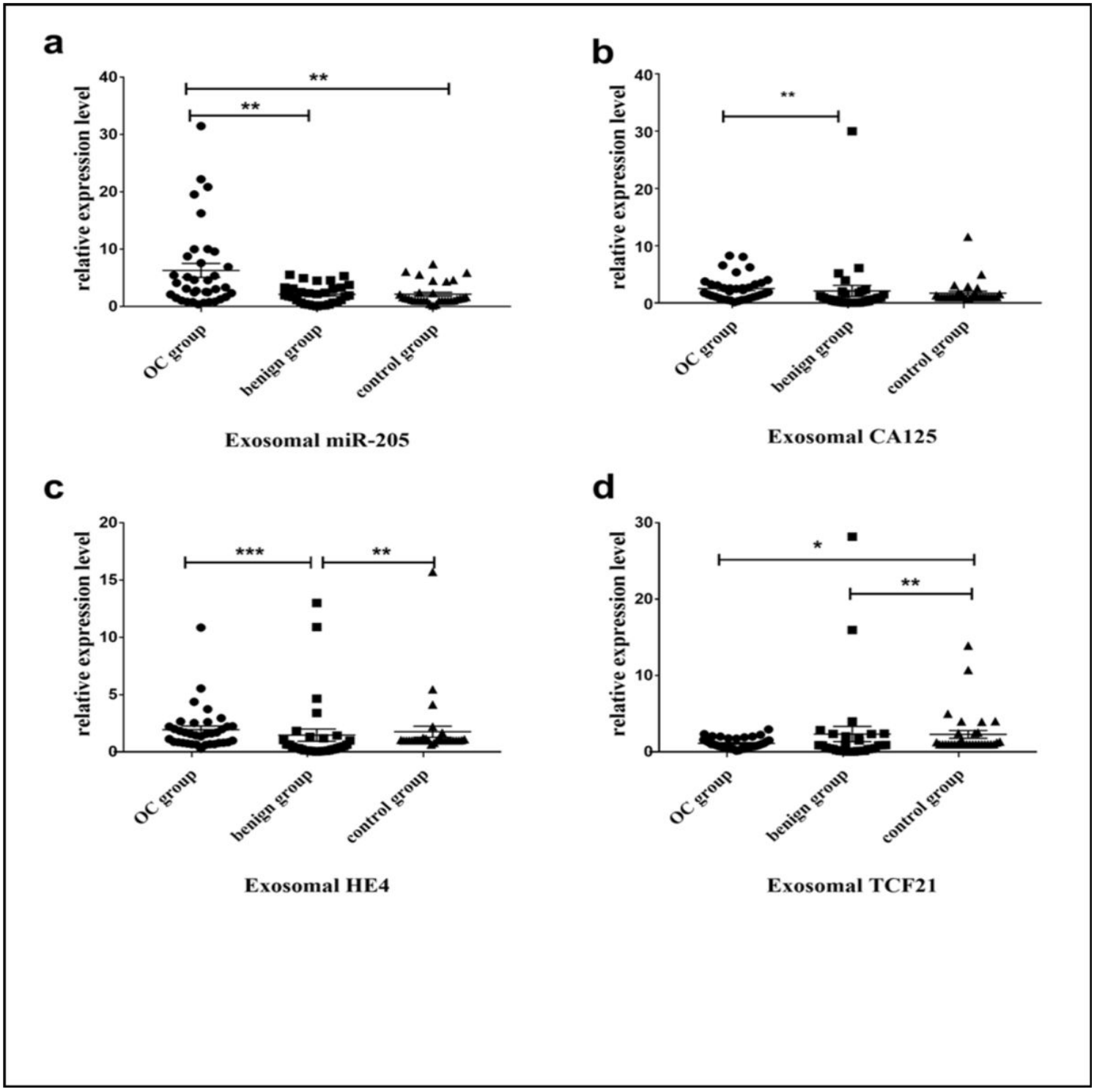

Figure 2

The expression levels of plasma exosomal miR-205, CA125, HE4 and TCF21 among three groups of people(Kruskal-Wallis H test) . *p<0.05; **p<0.01; ***p<0.001

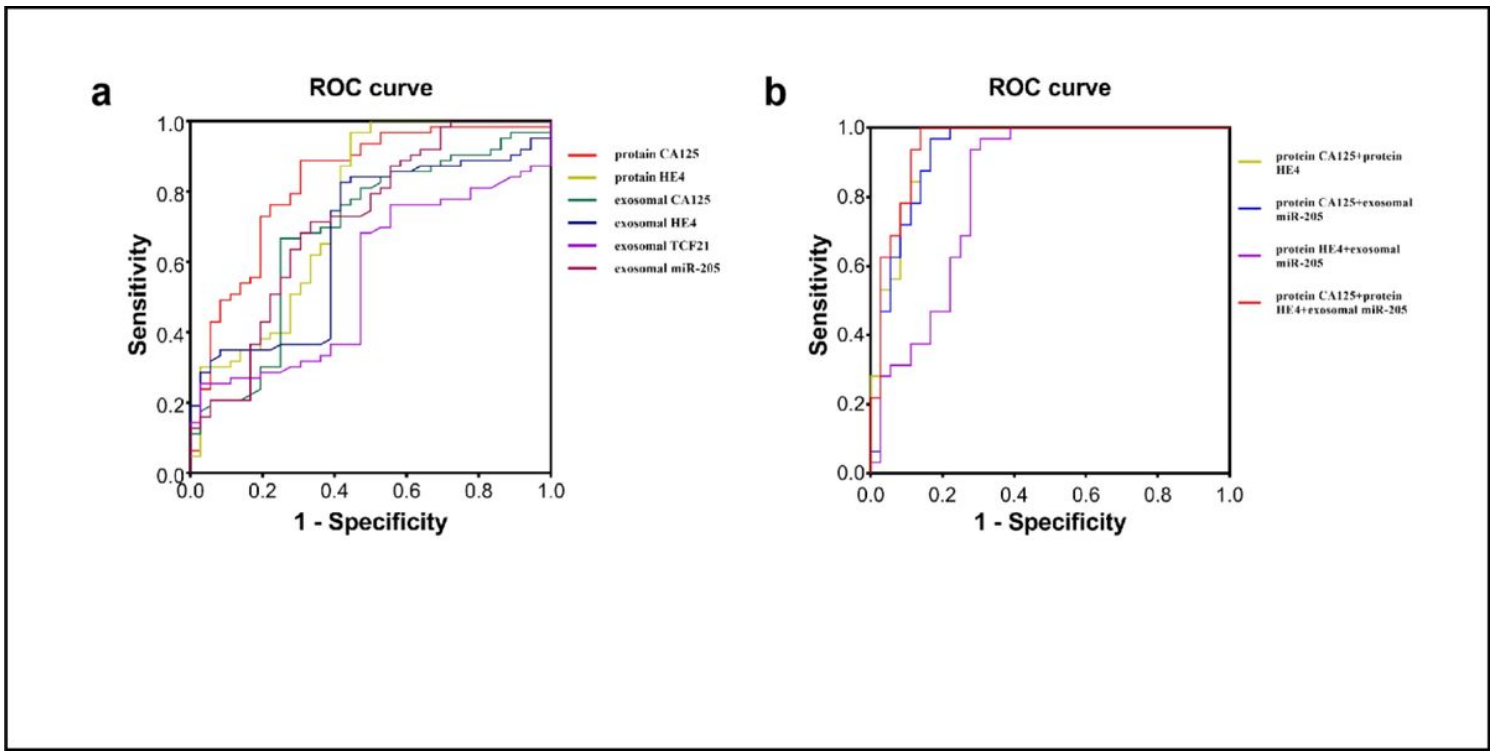

Figure 3

Receiver operator characteristics(ROC) curves for prediction of OC by plasma exosomal miR-205, CA125, HE4 and TCF21 alone or combined with traditional biomarkers protein CA125 and HE4