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Evaluation of *miRNA-21* and CA-125 as a promising diagnostic biomarker in patients with ovarian cancer

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Abstract

Introduction/objective: Ovarian cancer is the 6th leading cause of mortality in women, killing more women than any other reproductive system cancer. We studied the expression of serum micro-ribonucleic acid-21 (*miRNA-21*) in ovarian cancer patients and explored associations with diagnosis, clinicopathological parameters, and prognosis.

Methods: Real-time fluorescence-quantitative polymerase chain reaction was used to examine the relative expression of *miRNA-21* in serum. Cancer antigen 125 (CA-125) levels were measured using an enzyme immunoassay test kit (ELISA).

Results: Serum *miR-21* expression was significantly elevated in ovarian cancer patients compared to controls ($p < 0.001$). The same was true for CA-125 serum levels, which were also significantly in cancer patients ($p < 0.001$). The sensitivity and specificity of *miR-21* detection in the diagnosis of ovarian cancer were 96%, 88% versus 74%, and 80% for CA-125.

Conclusions: *miR-21* is highly expressed in the serum of ovarian cancer patients and may be important in the development and progression of ovarian cancer, with more sensitivity and specificity than CA-125. Our results suggest that circulating serum *miRNA-21* is a promising tumor marker for use in the diagnosis and prognosis of ovarian cancer.

Keywords: *miRNA-21*, CA-125, Ovarian cancer, RT-PCR

Introduction

Ovarian cancer is the sixth most prevalent cause of cancer-related death in women in the USA, accounting for 3% of all cancers in women [1]. In women, it is the fourth most frequent cancer. According to the Egyptian National Population-Based Registry Program 2008–2011, ovarian cancer accounts for 4.12% of the population, with a crude rate of 4.6. The incidence of ovarian cancer is expected to rise steadily from 2288 in 2013 to 5957 in 2050, representing a 260% increase. Upper Egypt (6.1%) had the highest incidence, 6.1%. Lower rates were found in middle and lower Egypt (3.8% and 3.9%, respectively) [2].

Ovarian cancer is currently diagnosed using pelvic examination, ultrasound (US), and tumor biomarkers; however, the inability to detect symptoms, weak invasiveness, and treatment resistance are linked to poor prognosis [3]. Various serum and plasma biomarkers, such as CA-125, human epididymis protein 4 (HE4), mesothelin, kallikreins, and aldehyde dehydrogenase 1 (ALDH1), show higher sensitivity and specificity at the malignant stage, but lower sensitivity and specificity in early stages [4]. CA-125 levels play a significant role in monitoring patients with ovarian cancer, but its significance at first diagnosis is still debated [5]. As a result, signature biomarkers with improved specificity and sensitivity are needed to improve ovarian cancer patient survival.

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Micro-RNAs (miRNAs) are currently being investigated as hallmark biomarkers for early diagnosis [4].

miRNAs are short non-coding RNA molecules (18–25 nucleotides) that regulate the translation of specific genes by binding to the 3′ untranslated region of target mRNAs in a sequence-specific manner. miRNAs participate in cell growth, differentiation, invasion, angiogenesis, and epithelial-mesenchymal transition, all of which are common in cancer [6, 7]. Specific miRNAs play a role in carcinogenesis because of their oncogenic or tumor-suppressive characteristics [8, 9]. Plasma, serum, saliva, urine, and feces all contain miRNAs [10]. miRNAs are fundamentally stable, and their use as indicators of human disease and therapeutic targets is increasing [11, 12]. Circulating miRNAs can resist harsh physiological conditions, such as pH changes, temperature changes, and freeze/thaw cycles [13]. Further, expression levels of circulating miRNAs are consistent across physically healthy individuals [14].

miR-21 is a widely expressed miRNA in mammalian cells, and its overexpression has been linked to a variety of cancers [15]. miR-21 functions as an oncogene, with overexpression leading to malignant B-cell lymphoma, as evidenced using conditional miR-21 knock-in mice [16]. In a study of 540 clinical samples from cancer patients, miR-21 was the only consistently elevated miRNA [17].

Insufficient work on the expression of serum *miR-21* in ovarian cancer patients has been accomplished. Thus, its diagnostic value and relationships with pathological characteristics and prognosis are not completely understood. In this study, we explored the expression of *miR-21* in serum from ovarian cancer patients and assessed its value for ovarian cancer diagnosis, clinicopathology, and prognosis. Our findings may assist the development of a theoretical foundation for early clinical diagnosis and treatment of ovarian cancer.

Methodology

The study was conducted at the Medical Biochemistry Department, Faculty of Medicine, Zagazig University, and the Gynecology and Obstetrics Department, Zagazig University Hospitals. Approval for the study was obtained from the Institutional Review Board (IRB), Faculty of Medicine, Zagazig University (reference number is 9066/27-1-2021). A case–control study was conducted with 100 adult subjects: 50 healthy adult women served as controls, and 50 were diagnosed with ovarian cancer. Informed written consent was obtained from all subjects to allow the use of samples and clinical data. Consent was provided with a dedicated form consistent with the Declaration of Helsinki. Fifty patients with histopathological confirmation of ovarian cancer, and with adequate hepatic, renal, cardiac, and respiratory function, were included. Individuals with a personal history of other malignant tumors and patients refusing to participate in the study were excluded.

Blood sampling

Participant blood samples were collected into RNase-free tubes, and serum was separated. miRNAs were extracted from serum using miRNeasy (Cat Number: Q217004; Qiagen, Germany), and serum CA-125 levels were measured using an enzyme immunoassay test kit (Catalog No. MBS454004).

RT-qPCR

TaqMan miRNA assays were used to assess levels of *miRNA-21 (miR-21)* in the blood (Applied Biosystems, Catalog Number 4427975). In a total volume of (15 µL), a fixed volume (2 µL) of total RNA was reverse transcribed using TaqMan miRNA Reverse Transcription Kits (Applied Biosystems, Catalog No. 4366596) under the following conditions: 16 °C for 30 min, 42 °C for 30 min, and 85 °C for 5 min, 4 °C maintained. miRNA Assay Kits and a TaqMan Universal Master Mix II, no UNG (Applied Biosystems, Catalog No. 4440040) were used in real-time PCR, which was carried out in duplicate on the StepOne Plus system (Applied Biosystems) under the following cycle conditions: 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Step One Software v2.3 (Applied Biosystems) was used to calculate cycle threshold (Ct) values. The $2^{-\Delta\Delta Ct}$ method was used to determine expression levels of *miRNA*, normalized to *RNU6* [18].

ΔCt was calculated as:

ΔCt = Ct (miRNA of interest) − Ct (*RNU6*). Then, ΔΔCt was calculated with a sample from a healthy volunteer as a calibrator: ΔΔCt = ΔCt (tested sample) − ΔCt (calibrator).

Statistical analysis

Data were analyzed using SPSS version 22, and data are expressed as means ± SD for quantitative parametric variables, as medians for nonparametric variables, and as frequency and percentage for categorical variables. Student's t tests, Mann–Whitney, Chi-squared tests, and Pearson correlation were used as appropriate. $p < 0.05$ was considered statistically significant. The analysis was based on the accuracy of miRNAs for diagnosis of ovarian cancer as determined using receiver operator characteristic (ROC) curves as the area under the curve (AUC) values and sensitivity and specificity.

Table 1 Risk factors and demographic data of controls and ovarian cancer patients

	Cases $n=50\%$		Control $n=50\%$		p-value
Age (years)					
X ± SD	56.3 ± 6.6		54.8 ± 6.9		
≤ 50	20	40.0%	16	32.0%	
> 50	30	60.0%	34	68.0%	0.28
Residence					
Rural	29	58%	30	60%	0.83
Urban	21	42%	20	40%	
Family history					
− ve	41	82.0%			
+ ve	9	18.0%			
Menopausal status					
Pre	22	44%	30	60%	0.1
Post	28	56%	20	40%	

Table 2 Clinical characteristics of cancer patients

	N (50)	%
FIGO stage		
I/II	30	60.0
III/IV	20	40.0
Histological type		
Serous	20	40.0
Mucinous	14	28.0
Endometrioid	16	32.0
US		
Bilateral	22	44.0
Multilocular	16	32.0
Solid	12	24.0
Metastasis		
− ve	34	68.0
+ ve	16	16.0

Results

Baseline characteristics of 50 ovarian cancer patients and 50 control subjects indicated no significant differences in age, residence, family history, and menstrual status ($p > 0.05$; Table1). Thirty patients were diagnosed with early-stage (FIGO I and II) and 20 with advanced-stage (FIGO III and IV) epithelial ovarian cancer. Twenty cases were confirmed as serous ovarian carcinoma, 14 mucinous, and 16 endometrioid. Twenty-two had bilateral, 16 multilocular, and 12 solid tumors. Thirty-four patients were negative for metastases and 16 positive (Table 2).

Serum *miR-21* expression and CA-125 serum level were significantly higher in cancer patients than in controls ($p < 0.001$) (Table 3). Significant associations were observed between high *miR-21* and family history, FIGO stage, and histological type. Correlations with age, residence, and US type were not statistically significant (Table 4). Serum CA-125 levels were significantly correlated with clinicopathological variables—FIGO stage, histological type, and US type—but not with age, residence, and family history (Table 5).

Serum *miR-21* was a reliable diagnostic marker for the detection of ovarian cancer (Table 6), with a sensitivity of 96%, specificity of 88%, and accuracy of 92%. Serum CA-125 was less dependable—sensitivity 74%, specificity 80%, and accuracy 77% (Fig. 1). Correlation coefficients for AUC were 0.99 for *miR-21* and 0 0.84 for *CA125*. There was a statistically significant positive

Table 3 Comparison of serum *miR-21* expression and CA-125 serum levels among patients and controls

	Patients ($n=50$)	Controls ($n=50$)	p
miR-21			
Mean ± SD	5.54 ± 1.87	1.1 ± 0.43	< 0.001
CA125			
Median	478	44	
Range	19–1325	10–477	< 0.001

Table 4 Associations among serum miR-21 expression and clinicopathological parameters in ovarian cancer patients

	***miR-21* expression**	
	Mean ± SD	p
Age		
≤ 50	5.92 ± 1.9	0.24
> 50	5.29 ± 1.84	
Residence		
Rural	5.79 ± 1.7	0.27
Urban	5.2 ± 2.1	
Family history		
− ve	5.18 ± 1.57	0.002*
+ ve	7.17 ± 2.3	
FIGO stage		
I/II	4.46 ± 1.13	< 0.001*
III/IV	7.16 ± 1.57	
Histological subtypes		
Serous	4.1 ± 0.72	< 0.001*
Mucinous	5.5 ± 1.67	
Endometrioid	7.6 ± 1.06	
US		
Bilateral	5.7 ± 2.02	
Multilocular	4.5 ± 0.6	0.09
Solid	6.58 ± 2.1	

*p < 0.05 when compared with control

Table 5 Relationships among serum CA-125 level and clinicopathological parameters in ovarian cancer patients

	CA-125 level median (range)	*p*
Age		
≤ 50	478 (58–1325)	0.38
> 50	409 (19–780)	
Residence		
Rural	478 (58–1325)	0.79
Urban	525 (19–1325)	
Family history		
− ve	478 (58–564)	0.7
+ ve	489 (19–1325)	
FIGO stage		
I/II	478 (19–693)	0.03*
III/IV	564 (58–1325)	
Histological subtypes		
Serous	489 (460–693)	0.006*
Mucinous	564 (354–1325)	
Endometrioid	348 (19–780)	
US		
Bilateral	354 (55–693)	
Multilocular	453 (19–780)	0.002*
Solid	564 (478–1325)	

*p < 0.05 when compared with control

correlation between CA-125 and *miRNA-21* among the studied groups as shown in Table 7 and Fig. 2).

Discussion

Ovarian cancer is the sixth leading cause of mortality in women, killing more women than any other cancer of the reproductive system. A woman's lifetime risk of developing ovarian cancer is about 1 in 78 [1]. A family history of breast or ovarian cancer is the most significant risk factor for ovarian cancer, and heritable susceptibility accounts for about 25% of all malignancies [19]. Currently, miRNAs are being investigated as serum biomarkers. These tiny non-coding RNA molecules are likely non-invasive blood biomarkers [20]. Zhang et al. reported miRNAs as diagnostic or prognostic indicators [21]. A panel of miRNAs was apparently better than traditional methods for distinguishing between malignant and reactive lesions, and among cancers with various histogenetic origins and histological subtypes of the same type of tumor. Ashrafizadeh et al. indicated that miRNAs can also function as major regulators of carcinogenesis and that targeting these molecules, or their functions, might be an effective treatment strategy [22]. Thus, our study evaluated the involvement of *miR-21* in ovarian cancer progression.

We found that the expression of *miR-21* was considerably elevated in sera of ovarian cancer patients compared with age-matched controls. The fold change value in serum *miR-21* expression in patients with advanced stage cancer was 7.16 ± 1.57, substantially higher than for early stages, 4.46 ± 1.13. Similarly, XU et al. reported higher blood *miR-21* levels in EOC patients, which correlated with FIGO stage and tumor grade [23]. Further, higher plasma *miR-21* levels are linked to poor long-term prognosis. Kartika et al. found that *miRNA-21* was upregulated 2.14-fold in late compared to early stages, and 6.13-fold compared to healthy controls (p 0.05) [24]. Lou et al. hypothesized that aberrant *miR-21* expression affects several biological processes in ovarian cancer cells, including proliferation, migration, and invasion [25]. *miR-21* appears to play a role in ovarian carcinogenesis and promotes invasion and metastasis. The precise mechanism of involvement of *miRNA 21* in the progression of cancer is still unknown. Lu et al. [25] and Meng et al. [26] suggested that overexpression of *miR-21* is closely linked to the negative expression of *PTEN* protein. Other tumor suppressor genes, such as *Pdcd4,* which is negatively regulated at the posttranscriptional level by *miR-21*, may also be involved [27, 28].

We found significant differences in *miR-21* expression among serous, mucinous, and endometrioid histology, with the highest expression in the endometrioid type. Nam et al. suggested that *miR-21* was the most frequently upregulated miRNA in serous ovarian carcinoma biopsies compared to normal ovarian tissue [29]. Paliwal et al. showed that RT-qPCR-calculated fold changes in *miRNA-21* expression were 3.98 times higher in serous ovarian cancer compared to controls [20]. Similarly, elevated levels of 1.99- and 1.34-fold were observed for mucinous and endometrioid ovarian carcinoma, respectively. Lou et al. reported increased *miRNA-21* expression in serous, mucinous, and endometrioid subtypes

Table 6 Validity of *miRNA 21* expression and CA-125 serum level as diagnostic markers of ovarian cancer

	AUC	Sensitivity%	Specificity%	PV%		Accuracy %
				+ ve	− ve	
miRNA 21	0.99 (0.97–1.0)	96.0	88.0	88.9	95.7	92.0
CA-125	0.84 (0.76–0.92)	74.0	80.0	78.7	75.5	77.0

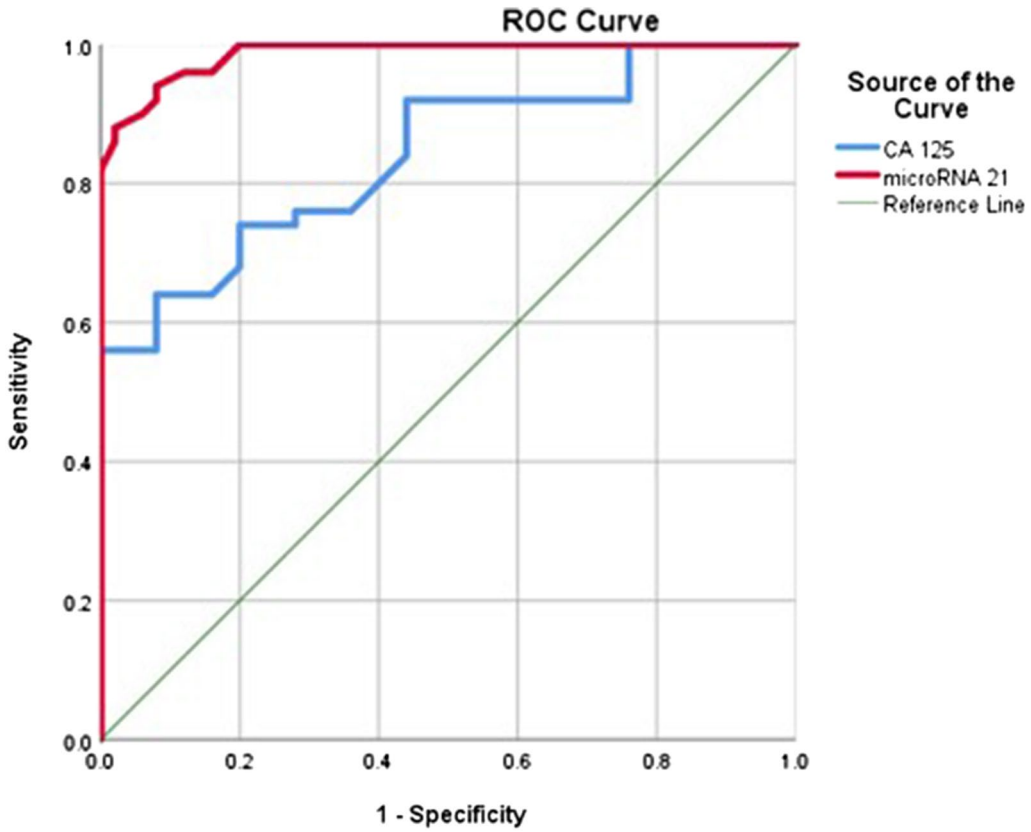

Fig. 1 ROC curve for *miR-21* as a diagnostic marker of ovarian cancer

Table 7 Correlation between CA-125 and miRNA-21 among studied groups

Variable	*R*	*p*
CA-125 with miRNA-21	0.450	< 0.001

of EOC, but did not observe any significant differences among the three histotypes [25].

Currently, pelvic examination, transvaginal ultrasonography (TVUS), and serum CA-125 levels are standard modalities for detecting ovarian cancer. CA-125 is considered a "gold standard" tumor biomarker for this disease [30, 31]. We consistently found high serum levels in the early stages of ovarian cancer in comparison with controls and higher levels in the late stages.

We also found that sensitivity, specificity, and accuracy of *miRNA-21* were superior to CA-125-96%, 88%, and 92% versus 74%, 80%, and 77%, respectively, in addition to a significant positive correlation between CA-125 and *miRNA-21* among the studied groups. Consistently, Xu et al. [23] suggested that serum *miR-21* could be used as a diagnostic and prognostic marker for EOC, as well as a therapeutic target. Overall, *miR-21* can be used for early detection and therapy planning as a tumor biomarker for ovarian cancer.

Our study's small sample size made it difficult to generalize the findings, which necessitated larger cohort studies. Additionally, the expense of the reagents prevented the study from involving more participants. We recommend future research into various populations, including high population sample sizes, in order to completely elucidate the role of *miRNA-21* gene expression in EOC. Additional studies comparing serum *miRNA-21* expression with tissue expression would unquestionably support our findings.

Conclusion

miRNA21 gene expression level significantly increases in ovarian cancer cases at higher levels in later stages. Histopathological types of ovarian cancer showed comparatively high expression levels of *miR-21*. *miRNA21* can be

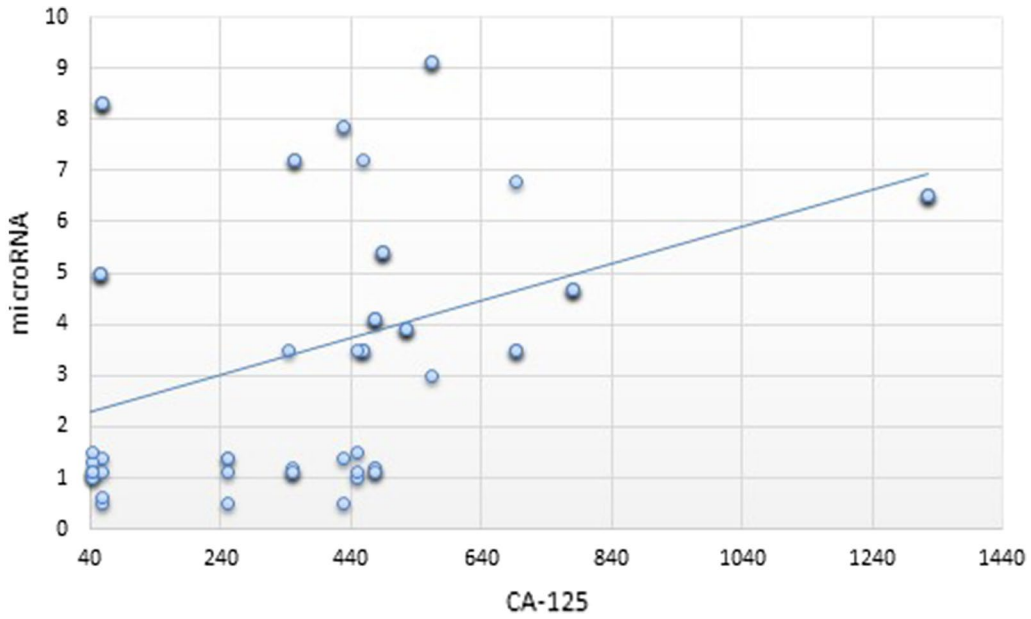

Fig. 2 Correlation between *CA-125* and *miRN-21* among the studied groups

used as a diagnostic biomarker for the early detection of ovarian cancer.

Abbreviations

ALDH1: Aldehyde dehydrogenase 1; AUC: Area under the curve; CA-125: Cancer antigen 125; Ct: Cycle threshold; ELISA: Enzyme-linked immunosorbent assay; EOC: Epithelial ovarian cancer; FIGO: International Federation of Gynecology and Obstetrics; HE4: Human epididymis protein 4; IRB: Institutional Review Board; miRNA-21/miR-21: Micro-ribonucleic acid-21; PTEN: Phosphatase and tensin homolog; PV: Predictive value; ROC: Receiver operator characteristic; RT-qPCR: Real-time fluorescence-quantitative polymerase chain reaction; SD: Standard deviation; SPSS: Statistical Package for the Social Sciences; TVUS: Transvaginal ultrasonography; UNG: Uracil-N-glycosylase gene; US: Ultrasound.

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Author contributions

AT prepared the idea and designed the study. SFS did the data statistical analysis. AT and SFS performed all the laboratory investigations and interpreted the patients' data regarding each studied group. MAH selected the patients and the control group. All authors wrote, read, and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

This study has been approved by the Faculty of Medicine, Zagazig University, IRB, for human studies (reference number is 9066/27-1-2021), and the patients have signed informed written consent.

Consent for publication

Not applicable.

Competing interests

All authors declared that they have no competing interests.

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References

1. Siegel R, Miller K, Jemal A (2018) Cancer statistics, 2018. CA A Cancer J Clin 68(1):7–30. https://doi.org/10.3322/caac.21442
2. Ibrahim A, Khaled H, Mikhail N, Baraka H, Kamel H (2014) Cancer incidence in Egypt: results of the national population-based cancer registry program. J Cancer Epidemiol 2014:1–18. https://doi.org/10.1155/2014/437971
3. Qu H, Xu W, Huang Y, Yang S (2011) Circulating miRNAs: promising biomarkers of human cancer. Asian Pac J Cancer Prev 12(5):1117–1125
4. Resnick K, Alder H, Hagan J, Richardson D, Croce C, Cohn D (2009) The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol 112(1):55–59. https://doi.org/10.1016/j.ygyno.2008.08.036

5. Sölétormos G, Duffy M, Othman Abu Hassan S, Verheijen R, Tholander B, Bast R et al (2016) Clinical use of cancer biomarkers in epithelial ovarian cancer: updated guidelines from the European group on tumor markers. Int J Gynecol Cancer 26(1):43–51. https://doi.org/10.1097/igc.0000000000000586
6. Calin G, Croce C (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6(11):857–866. https://doi.org/10.1038/nrc1997
7. Esquela-Kerscher A, Slack F (2006) Oncomirs: microRNAs with a role in cancer. Nat Rev Cancer 6(4):259–269. https://doi.org/10.1038/nrc1840
8. Wiemer E (2007) The role of microRNAs in cancer: no small matter. Eur J Cancer 43(10):1529–1544. https://doi.org/10.1016/j.ejca.2007.04.002
9. Lu J, Getz G, Miska E, Alvarez-Saavedra E, Lamb J, Peck D et al (2005) MicroRNA expression profiles classify human cancers. Nature 435(7043):834–838. https://doi.org/10.1038/nature03702
10. Chevillet J, Lee I, Briggs H, He Y, Wang K (2014) Issues and prospects of microRNA-based biomarkers in blood and other body fluids. Molecules 19(5):6080–6105. https://doi.org/10.3390/molecules19056080
11. Kanaan Z, Rai S, Eichenberger M, Roberts H, Keskey B, Pan J, Galandiuk S (2012) Plasma MiR-21. Ann Surg 256(3):544–551. https://doi.org/10.1097/sla.0b013e318265bd6f
12. Mishra P (2014) Non-coding RNAs as clinical biomarkers for cancer diagnosis and prognosis. Expert Rev Mol Diagn 14(8):917–919. https://doi.org/10.1586/14737159.2014.971761
13. Mitchell P, Parkin R, Kroh E, Fritz B, Wyman S, Pogosova-Agadjanyan E et al (2008) Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci 105(30):10513–10518. https://doi.org/10.1073/pnas.0804549105
14. Duttagupta R, Jiang R, Gollub J, Getts R, Jones K (2011) Impact of cellular miRNAs on circulating miRNA biomarker signatures. PLoS ONE 6(6):e20769. https://doi.org/10.1371/journal.pone.0020769
15. Feng Y, Wu C, Tsao C, Chang J, Lu P, Yeh K et al (2011) Deregulated expression of sprouty2 and microRNA-21 in human colon cancer: correlation with the clinical stage of the disease. Cancer Biol Ther 11(1):111–121. https://doi.org/10.4161/cbt.11.1.13965
16. Medina P, Nolde M, Slack F (2010) OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. Nature 467(7311):86–90. https://doi.org/10.1038/nature09284
17. Volinia S, Calin G, Liu C, Ambs S, Cimmino A, Petrocca F et al (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci 103(7):2257–2261. https://doi.org/10.1073/pnas.0510565103
18. Livak K, Schmittgen T (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25(4):402–408. https://doi.org/10.1006/meth.2001
19. Walsh T, Casadei S, Lee M, Pennil C, Nord A, Thornton A et al (2011) Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci 108(44):18032–18037. https://doi.org/10.1073/pnas.1115052108
20. Paliwal N, Vashist M, Chauhan M (2020) Evaluation of miR-22 and miR-21 as diagnostic biomarkers in patients with epithelial ovarian cancer. 3 Biotech. https://doi.org/10.1007/s13205-020-2124-7
21. Zhang W, Dahlberg J, Tam W (2007) MicroRNAs in tumorigenesis. Am J Pathol 171(3):728–738. https://doi.org/10.2353/ajpath.2007.070070
22. Ashrafizadeh M, Hushmandi K, Hashemi M, Akbari M, Kubatka P, Raei M et al (2020) Role of microRNA/Epithelial-to-mesenchymal transition axis in the metastasis of bladder cancer. Biomolecules 10(8):1159. https://doi.org/10.3390/biom10081159
23. Xu Y, Xi Q, Ge W, Zhang X (2013) Identification of serum MicroRNA-21 as a biomarker for early detection and prognosis in human epithelial ovarian cancer. Asian Pac J Cancer Prev 14(2):1057–1060. https://doi.org/10.7314/apjcp.2013.14.2.1057
24. Kartika A, Chasanah S, Fitriawan A, Tanjung D, Trirahmanto A, Pradjatmo H et al (2018) MicroRNA-21 as a biomarker for ovarian cancer detection. Indones J Biotechnol 23(1):35. https://doi.org/10.22146/ijbiotech.35692
25. Lou (2010) MicroRNA-21 promotes the cell proliferation, invasion, and migration abilities in ovarian epithelial carcinomas through inhibiting the expression of PTEN protein. Int J Mol Med. https://doi.org/10.3892/ijmm_00000530
26. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob S, Patel T (2007) MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 133(2):647–658. https://doi.org/10.1053/j.gastro.2007.05.022
27. Asangani I, Rasheed S, Nikolova D, Leupold J, Colburn N, Post S, Allgayer H (2007) MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation, and metastasis in colorectal cancer. Oncogene 27(15):2128–2136. https://doi.org/10.1038/sj.onc.1210856
28. Chan J, Blansit K, Kiet T, Sherman A, Wong G, Earle C, Bourguignon L (2014) The inhibition of miR-21 promotes apoptosis and chemosensitivity in ovarian cancer. Gynecol Oncol 132(3):739–744. https://doi.org/10.1016/j.ygyno.2014.01.034
29. Nam E, Yoon H, Kim S, Kim H, Kim Y, Kim J et al (2008) MicroRNA expression profiles in serous ovarian carcinoma. Clin Cancer Res 14(9):2690–2695. https://doi.org/10.1158/1078-0432.ccr-07-1731
30. Azzam A, Hashad D, Kamel N (2013) Evaluation of HE4 as an extrabiomarker to CA125 to improve detection of ovarian carcinoma: is it time for a step forward? Arch Gynecol Obstetr 288(1):167–172. https://doi.org/10.1007/s00404-013-2722-2
31. Park Y, Lee J, Hong D, Lee E, Kim H (2011) Diagnostic performances of HE4 and CA125 for the detection of ovarian cancer from patients with various gynecologic and non-gynecologic diseases. Clin Biochem 44(10–11):884–888. https://doi.org/10.1016/j.clinbiochem.2011.04.011

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