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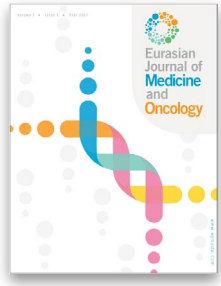

Research Article

Evaluation of MicroRNA Expressions in Ovarian Cancer

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Abstract

Objectives: The present study aims to evaluate the relationship between microribonucleic acid (miRNA) and target gene expressions with clinical and histopathological data in ovarian cancer.

Methods: We evaluated 96 archival samples of paraffin-embedded tissue. Some potentially significant miRNA and target gene expressions were evaluated in different histopathological characteristics. These were quantified using real-time–polymerase chain reaction (RT–PCR) in tumor and normal tissue. In miRNA expressions, twofold changes are accepted as significant.

Results: According to histopathological groups, 38 (39.6%) were endometroid adenocarcinoma, 11 (11.5%) were borderline serous, 29 (30.2%) were serous, and 18 (18.8%) were mucinous carcinoma. When evaluated according to their stages, 26 (27.1%) patients were stage 1A/1B. A relationship was found between miR200a and miR200c and histopathologic groups, between miR141 and estrogen receptors, between CXCL1 and survival status, and between KEAP1 and ki67. Additionally, miR200a in endometrial and miR200c in mucinous adenocarcinoma were overexpressed. When the relationship between all miRNAs and histopathological groups was evaluated, a significant change was found only in miR200c expression. It was significantly higher in serous than endometrial tumors and significantly higher in mucinous than endometroid tumors.

Conclusion: These suggested that miR200a and 200c expressions might be useful for the evaluation of histopathological subgroups of ovarian cancer.

Keywords: Biomarkers, Ovarian Cancer, MicroRNA

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Ovarian cancer is a common type of cancer in women and the fifth cause of death among gynecological cancers.[1] Despite improvements in diagnostic and treatment methods, the survival rate is low due to both establishment of diagnosis at advanced stages and resistance to treatments after a while. The five-year survival rate is 30%–90% and depends on the stage at the time of diagnosis.[2] Of the patients, 59% are diagnosed in the metastatic phase, and insufficiency of early diagnostic methods has caused high mortality rates.[3] Ovarian cancer has a highly heterogeneous histology. Epithelial origin accounts for 95% of all ovarian cancers, and serous carcinoma is the most com-

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mon subtype. The rest originates from other types of ovarian cells (germ cell tumors and sex cord-stromal tumors).[4]

Diagnostic tools for ovarian cancer include pelvic examination, vaginal ultrasound, and measurement of serum cancer antigen-125 (CA-125) level. However, these methods are often not sufficient for diagnosis at an early stage. For example, CA-125 may not be elevated in early-stage ovarian cancer and its sensitivity is low. Therefore, markers with higher sensitivity and specificity are needed for early-stage ovarian cancer. This need has led to the development of biomarkers such as the human epididymis protein 4 (HE4). HE4 whey is a glycoprotein marker, which is a member of the acidic protein family, and although it was first isolated from the epididymis, it is secreted from the epithelium of the reproductive tract and respiratory tract. It is expressed at a higher rate in ovarian cancer than in normal tissue. Especially in benign conditions such as endometrioma, the fact that the HE4 value remains stable despite an increase in the CA-125 value suggests that the HE4 biomarker is a better marker of ovarian cancer in the diagnosis.[5,6] As can be seen, new markers are required in the early stage or diagnosis for the detection of particularly high-risk patients. Therefore, the need continues for the determination of valid/reliable markers to establish a diagnosis, treatment, or follow-up strategies for the epithelial-origin cancers that account for the most prevalent part of ovarian cancer.

Recently, microribonucleic acid (miRNA/MIR) has been quite a focus in cancer studies, as they have important roles in influencing various biological processes and regulating pathological situations. The miRNAs were first identified in 1993 by Ambros et al. during genetic studies on the development of *Caenorhabditis elegans (C. elegans)*, a nematode worm, and later discovered in the genome of most life forms, including human beings.[7] miRNAs are shown to be associated with cell development, differentiation, proliferation, and apoptosis.[8] As with many chronic diseases, the miRNAs are studied extensively in cancer as well. It has been reported in the literature to play a role in carcinogenesis, cell cycle, proliferation, differentiation, angiogenesis, and apoptosis. Clinical trials report that it may play a predictive role in disease prognosis and treatment processes.[7–14] miRNA 200 and 30 family are the most studied in ovarian cancer. A meta-analysis published in recent years reveals that the miR200 family and miR30 family may be promising prognostic biomarkers in ovarian cancer.[15]

The fact that the messenger RNA (mRNA) is underexpressed in cancer has suggested that it could function as a tumor suppressor and may prevent cancer by regulating the genes that control oncogenes or cell differentiation or apoptosis. The overexpression of miRNA in cancer, conversely, has shown that it functions as an oncogene, playing a role in carcinogenesis by negatively regulating the genes that control apoptosis or tumor suppressor genes; and such information may be used for treatment.[16–18]

The present study aims to evaluate the relationship of the miRNA and target gene expressions with clinical and histopathological data in ovarian cancer. To this end, we aim to assess whether the expression changes of the miR200 family and target genes, which are considered important in epithelial ovarian cancers, are associated with clinicopathological features.

Methods

All the steps of the project were carried out under the Declaration of Helsinki. Paraffin patient tissues used in this study were obtained from the archive of 96 ovarian cancer and 10 normal patient tissues operated in the Tepecik Training and Research Hospital Pathology Department. Normal patient tissue used in this study was obtained from ovarian tissue removed for noncancer purposes. Experimental studies were conducted in the laboratories of the Oncology Institute of Dokuz Eylul University, Basic Oncology Department. The ethical board approvals to conduct the study were obtained from the Ethical Board of Non-Invasive Research, Dokuz Eylul University (Ethical Board Decision no. 2017/24-19).

The study was designed as a cross-sectional cohort type and laboratory study. Ovarian cancer patients (n=96) and healthy (n=10) in the 18–80 age range were included in the study. Inclusion criteria for the study were as follows: (i) patients diagnosed with all-stage ovarian cancer, (ii) patients monitored in the clinic for at least 3 months, (iii) patients who are between 18 and 80 years old, and (iv) patients who had all histological subtype. Exclusion criteria for the study were as follows: (i) patients who had second malignancy and (ii) patients monitored in the clinic for 3 months. First, the paraffin removal from the paraffin tissues of patients, the miRNA and mRNA isolations, and then, the real-time–polymerase chain reaction (RT-PCR) work were performed. The resulting changes in miRNA and mRNA gene expression were later compared with the expression changes in healthy people, and their relationship with clinicopathological characteristics was assessed. As part of this study, to know the miRNA and target gene expressions with RT-PCR, we studied expression changes in the miR200 family (miR98, miR141, miR200a, miR200b, miR200c, and miR429) and ZEB1, ZEB2, p38, IL8, CXCL1, TUBB3, KEAP1, MAPK14, HMGA2, VEGFB, and VEGFR2, which are target genes of them.[19] Then, we attempted to establish the miRNA and the associated mRNA-gene expression changes as a marker for early- and advanced-stage epithelial ovarian cancers.

Analysis of Tissue Samples with Quantitative RT–PCR

Following the obtainment of tumor tissue paraffin samples for patients with epithelial ovarian cancer, the miRNA isolation (Qiagen), expressions of miR200, its family, and target genes were studied with the quantitative RT–PCR method. Again, expression changes were determined concerning ovarian tissue miRNA expressions of healthy individuals to find out whether there was a differential expression discrepancy in the miRNA expression changes of patients with ovarian cancer. miRNA and mRNA expression fold changes between the groups were calculated using the comparative 2-ΔΔCq method. Those who presented a twofold increase or decrease in the expression difference in either miRNA or mRNA were considered significant. As the cutoff point for miRNA and miRNA expression are studied, a twofold increase or decrease was accepted as the threshold in this study. The relationship of these miRNA and mRNA expressions with patients' clinical and histopathological characteristics was determined. In this way, the miR200 family and their target genes were assessed as to whether they can be important as a marker of early- and advanced-stage ovarian cancer. First, the isolation of miRNA and mRNA was performed, then the complementary deoxyribonucleic acid (cDNA) synthesis via the reverse transcriptase, and finally the RT-PCR process was implemented. As part of this study, the U6 gene was used as a control for miRNA expressions and the Actin-B gene as an internal control for mRNA studies. miRNA and targeted gene primers were used.[20,21]

miRNA and mRNA expression fold changes between the groups were calculated using the comparative 2-ΔΔCq method. Twofold changes and higher values between the groups were considered as significant.

Data Evaluation

The data were analyzed using SPSS 24.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.) package program. Continuous variables are expressed in mean±standard deviation and median, minimum and maximum values, and categoric variables in numbers and percentages. The conformity of the data to normal distribution was examined using Shapiro–Wilk test. $P<0.05$ was taken to indicate statistical significance in all analyses. Prior to statistical analysis, a logarithmic conversion has been made to the preanalytic fold change values and a new variable has been defined to ensure that samples above $\log^2$ (fold change) value 2 are considered to be positive. Kaplan–Meier survival analysis curves were also obtained over 220 months for miR220a and miR200c. Survival testing was not possible due to shortcomings in the survival data and molecular marker data. Instead, patients were considered exitus positive/negative. Receiver operating characteristic (ROC) analyses have been done for miR220a and miR200c.

Results

Different histopathological characteristics of ovarian cancer and normal paraffin-embedded tissue archive samples were obtained. The mean age of patients was 49 with an age range of 18–78. Based on histological diagnoses, patients were considered as follows: 38 (39.6%) endometroid adenocarcinoma, 11 (11.5%) borderline serous, 29 (30.2%) serous, and 18 (18.8%) mucinous carcinoma. When patients are evaluated according to staging, it was found that 24 (25%) patients were stage 1A, 2 (2.1%) stage 1B, 18 (18.8%) stage 1C, 1 (1%) stage 2A, 1 (1%) stage 2B, 4 (4.2%) stage 3A, 2 (2.1%) stage 3B, 34 (35.4%) stage 3C, and 1 (1%) patient was stage 4. When we divided patients according to hormone receptor status based on their pathology reports, 15 (15.6%) patients' estrogen receptors (ER) were positive, 7 (7.3%) patients' ER were negative, 15 (15.6%) patients' progesterone receptor (PR) were positive, and 6 (6.3%) patients' PR were negative. CA-125 and Her2 staining percentages have not been evaluated as they were very limited in the pathology reports. The abdominal wash result was negative in 42 (43.8%) patients, positive in 17 (17.7%) patients, and suspicious in 3 (3.1%) patients. Lymphovascular invasion (LVI) was detected positive in 16 (16.7%) patients and negative in 8 (8.3%) patients. As to adjuvant treatment processes, 16 (16.7%) patients received chemotherapy and/or radiotherapy, whereas 45 (46.9%) patients received no treatment at all. Information about the disease stage of nine patients could not be obtained. Therefore, no information could be given about whether these patients received adjuvant therapy. While there was an indication for adjuvant treatment in the serous subtype starting from stage 1c, adjuvant treatment was seen to be relatively less, since some patients did not apply or applied late time to us in the postoperative period. Nearly half of the patients were in the early stages. All demographic characteristics of the patients are summarized in Table 1.

An assessment of the diagnostic groups, staging, ER, PRG, ki67, CA-125, and exitus status with the miRNA and cutoff values we determined for other parameters using the chi-square test (Fisher's exact test) showed the results given in Table 2. We have identified a relationship between miR200a and miR200c and diagnostic groups, between miR141, ER, CXCL1, and exitus status, and between KEAP1 and ki67. All cases (8/8) with positive miR141 cutoff variable were stained ER+, whereas 50% of the negative cases (7/14) were stained ER+. Of CXCL1 cutoff variable positive cases, 68.62% (35/51) died, which was 41.66% (10/24) for negative cases. While all (10/10) cases with positive KEAP

Table 1. Demographic characteristics of the patients

Histopathological Features and Treatments	n (%)
Histopathologic Diagnosis	
· Endometroid Adenocarcinoma	38 (39.6)
· Serous Carcinoma	29 (30.2)
· Musinous Carcinoma	18 (18.8)
· Borderline Serous Carcinoma	11 (11.5)
Patological Stage	
· Stage 1A	24 (25)
· Stage 1B	2 (2.1)
· Stage 1C	18 (18.8)
· Stage 2A	1 (1)
· Stage 2B	1 (1)
· Stage 3A	4 (4.2)
· Stage 3B	2 (2.1)
· Stage 3C	34 (35.4)
· Stage 4	1 (1)
Hormone Receptor Staining Status	
· ERPositive	15 (15.6)
· ER Negative	7 (7.3)
· PR Positive	1 (15.6)
· PR Negative	6 (6.3)
Abdominal Washing	
· Positive	17 (17.7)
· Suspicious	3 (3.1)
· Negative	42 (13.8)
Lymphovascular Invasion	
· Positive	16 (16.7)
· Negative	8 (8.3)
Neo/Adjuvant CT and/or RT	
· Received	16 (16.7)
· Not Received	45 (45.9)

Chemotherapy: CT; Estrogen Receptor: ER; Number: N; Progesterone Receptor: PR; Radiation Therapy: RT.

cutoff variable were stained ki67+, 33% (1/3) of negative cases were stained positive.

In Table 3, a statistical analysis was carried out to understand which of the diagnostic groups accounted for the difference. We found a mucinous difference in miR200a and a difference in the endometroid adenocarcinoma type in miR200c.

Table 4 shows the distribution of base-2 logarithmic conversion of miR200a and miR200c fold change values between the groups.

There are no significantly strong correlations found considering the logarithmic values of the miRNAs and other parameters using correlation analyses in terms of age and the total number of lymphatic nodes and positivity (Table 5). Moreover, logarithmic values of the miRNAs and other parameters (ER, PR, ki67, CA-125, abdominal washing, and LVI) as well as the results of other pathological reports were evaluated via statistical analysis using the Mann–Whitney U test.

In the final statistical analyses, considering the relationship between the logarithmic values of the miRNA and other

Table 3. The relationship between miR expression and histopathology

	1-2	1-3	1-4	2-3	2-4	3-4
miR200a	.196	.23	**.001***	.514	.196	**.043***
miR200c	.342	**.018**	**.003***	.345	.103	.293

*p<.05, 1: endometroid adenocarcinoma, 2: borderline serous carcinoma, 3: serous carcinoma, 4: mucinous carcinoma.

Table 2. The relationship with histopathological data of miRNA expressions and target genes

	Diagnosis	Stage	ER	PR	Ki67	Ca125	Exitus
miR-200a	**.016***	.1	.926	.169	.512	.537	.679
miR-200b	.894	.121	.134	.105	-	.6860	.072
miR-200c	**.01***	.308	.867	.577	.462	.5	.922
miR-141	.809	.825	**.015***	.776	.906	.408	.284
miR-429	.349	.406	.51	.41	.423	.5	.356
miR-98	.772	.269	.5	.771	.641	.5	.449
ZEB1	616	.937	.705	.342	.577	-	.618
ZEB2	.436	.841	.622	.228	.577	-	.745
p38	.236	.584	.268	.367	.538	.75	.507
IL8	.685	.56	.378	.053	.423	.875	.311
CXCL1	.557	.963	.5	.268	.731	.625	**.026***
TUBB3	.419	.911	.295	.658	.577	.875	.16
KEAP1	.621	.402	.349	.424	**.038***	.875	.818
MAPK14	.329	.826	.267	.576	.641	.75	1
HMGA2	.316	.931	.387	.701	.705	.5	1
VEGFB	.8	.845	.655	.55	.557	.875	.774
VEGFR2	.151	.243	.059	.094	.538	.5	.774

*p<.05, chi-square test (Fisher's Exact Test).

Table 4. Relation of diagnostic groups and miR expressions logarithmic values

	Endometroid (Grup 1)	Borderline Serous (Grup 2)	Serous (Grup 3)	Musinous (Grup 4)
Log (miR200a)	2.06±1.02	1.48±1.66	1.8±1.25	0.98±1.27
Log (miR200c)	1.31±1.33	1.90±1.87	2.08±1.44	2.21±1.15

parameters, and the diagnostic groups, we found a statistically significant relationship only in the miR200c (p<0.042). Table 6 shows which groups the difference originated from it. It was significantly higher in the serous carcinoma (p=0.019) compared with the endometrioid carcinoma, and in the mucinous carcinoma (p=0.007) compared with the endometrioid carcinoma. According to the ROC analysis results, miR200a and miR200c cannot be used as a marker (AUC=0.494 for miR200a and AUC=0.494 for miR200c) (data not shown). The number of patients in the study is insufficient to eliminate type 2 statistical error. Kaplan–Meier survival analysis curves comparing amplified and nonamplified for miR220a and miR200c were also obtained (Fig. 1a, b). The longest survival was 221 months. No meaningful statistical difference was observed between groups; however, the study number is too low to prevent false negative results.

Table 5. Relationship of age and lymph node involvement with miR expressions log values

	Age*	LN**	Pozitive LN**
log200a	.207	**.389*** **	.678
log200b	.158	.679	.659
log200c	.294	.262	.499
log141	.913	.806	.315
log429	.665	.574	.586
log484	.905	.238	**.317**** **
log98	.198	.301	.094
logZEB1	.055	.055	.272
logZEB2	.14	0.257	.209
logp38	.32	.368	.555
logIL8	.737	.927	.171
logCXCL1	.594	.369	.778
logTUBB3	.256	.351	.677
logKEAP1	.834	.968	.928
logMAPK14	.434	.555	.275
logHMGA2	.905	.877	**.291***** **
logVEGFB	.294	.327	.881
logVEGFR2	.924	.448	.129

log: logarithmic; lymph node: LN; *pearson correlation; **spearman correlation; ***p<.004 weak negative relationship; ****p<.02 weak positive relationship; *****p<.033 no relationship.

Table 6. Relationship between diagnostic groups and logarithmic values of miR200c expression

	1-2	1-3	1-4	2-3	2-4	3-4
log200c*	.472	**.019****	**.007****	.820	.753	.896

*p<.042 Kruskal-Wallis; **p<.05; 1: endometroid adenocarcinoma; 2: borderline; serous carcinoma; 3: serous carcinoma; 4: musinous carcinoma.

Discussion

Ovarian cancer ranks fifth among fatal cancers in women. Due to a lack of effective screening tests and uncertain symptoms, the diagnosis is often established at later stag-

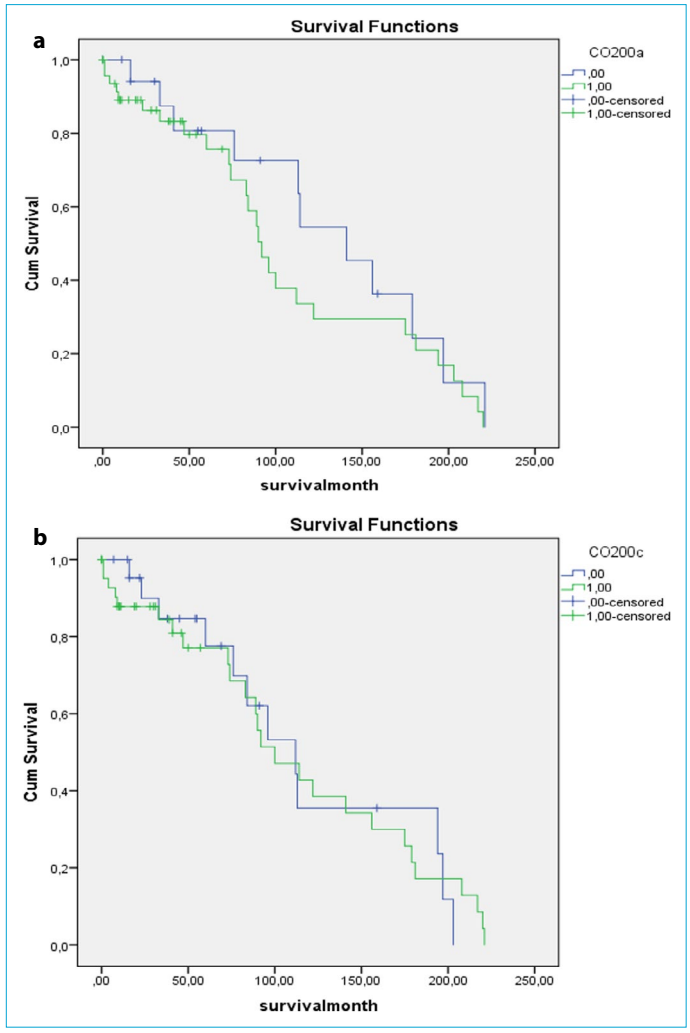

Figure 1. (a) Survival curve for miR200a. **(b)** Survival curve for miR200c.

es. In the early stages, 5 years of survival is over 90%, but it drops below 20% in the advanced stages. Therefore, we need both prognostic and predictive markers to provide early diagnosis and more optimized approaches with personalized treatments. In this effort, we worked on the tissue samples of 96 women with ovarian cancer. Their mean age was 49 years and the age range was 18–78. When we look at the literature, about half of the epithelial ovarian cancers are aged >64 and 25% are aged 74 and older.[18] Our population included younger patients.

Based on histological diagnoses, patients were considered as follows: 38 (39.6%) endometroid adenocarcinoma, 11 (11.5%) borderline serous, 29 (30.2%) serous, and 18 (18.8%) mucinous carcinoma. About 90% of the primary malignant ovarian tumors are of epithelial origin and originate in the ovary's serous surface epithelium. When we divided patients according to hormone receptor status based on their pathology reports, 15 (15.6%) patients' ER were positive, 7 (7.3%) patients' ER were negative, 15 (15.6%) patients' PRG were positive, and 6 (6.3%) patients' PRG were found to be negative. Due to the study's retrospective design and the circumstances regarding the coronavirus disease pandemic, the patients' file data were inaccessible for plasma levels. CA-125 and Her2 staining percentages have not been evaluated as they were very limited in the pathology reports. The abdominal wash result was negative in 42 (43.8%) patients, positive in 17 (17.7%) patients, and suspicious in 3 (3.1%) patients. LVI was detected positive in 16 (16.7%) patients and negative in 8 (8.3%) patients. As to adjuvant treatment processes, 16 (16.7%) patients received CT and/or RT, whereas 45 (46.9%) patients received no treatment at all. Nearly half of the patients were in the early stages, considering that the study was taken from patients with better prognoses in terms of stage and histological type.

We assessed the cutoff values determined for the miRNAs, the relevant mRNAs, other parameters, and diagnostic groups, stage, ER, PR, ki67, CA-125, and survival. A significant correlation was found between the diagnostic groups and miR200a and miR200c, and miR141 and ER. The miR200 family consists of the five members (miR200 a, b, c, 141, and 429) studied in many cancers. These were also considered in the present study. We found that the difference between the diagnostic groups was due to the mucinous type in miR200a and the endometrioid adenocarcinoma in miR200c. The endometrioid was expressed more in tumor tissue in miR200a and the mucinous in miR200c.

In the earlier studies, plasma, acid, and serum expressions were detected in the miR200 family.[20–22] The roles of miR in invasion, metastasis, and angiogenesis in cancer were shown. Since the present study is retrospective, miRNA and related gene expressions in the tissue were investigated. Furthermore, type 1 ovarian cancers were investigated instead of type 2 ovarian cancers, which are more aggressive and have a high number of mutations and high-grade serous carcinomas that progress with p53 mutations. They are a group of histologically and low-degree serous, mucinous, endometrioid, and clear cell groups, with BRAF, KRAS, and PTEN mutations, and present a better prognosis. In an earlier study, the findings were particularly similar concerning the evaluation of histopathological data.[20] In the case series of Iorio et al., the miR200a and c were shown to be commonly overexpressed in serous, endometrioid, and clear cell histology.[23] In the study of Zuberi et al. that investigated miR200a, b, and c in ovarian cancer patients regarding a candidate biomarker and the correlation with histopathological findings, the overexpression of miR200a, b, and c were found significant for disease progress and aggressive tumor histology.[20,22] It was indicated that further studies were necessary to predict prognosis and survival and to prove to be an appropriate biomarker. In the present study, we have not identified a relationship with survival. We associated our results with both heterogeneous histologies of patients and the excess of early stages. Moreover, we have shown that all three family members (a, b, c) are overexpressed in tumors with mucinous histology. We have not used the degree of differentiation due to patients' heterogeneous distribution and the presence of early-stage cases.

In the oxidative stress response in miR141 and 200a ovarian cancer tumorigenesis, the p38 target protein was demonstrated to play a role.[24] There was no correlation between p38 levels and clinicopathological data and survival in our study. miR200a also has prooncogenic functions.[25,26] miR200c and niR429 are potentially involved in the epithelial–mesenchymal transition (EMT) using the E-cadherin regulatory protein ZEB2.[27] In recent publications, EMT has played a critical role in ovarian cancer progression and survival.[28] The loss of E-cadherin expression is fundamental to EMT. Additionally, SNAIL, SLUG, TWIST, and ZEB play an important role in this mechanism. In type 2 high-grade ovarian cancers, the EMT pathway, survival, and metastasis are frequently studied, but the mechanisms remain unclear. Similarly, there is little information yet on the EMT relationship between low-grade and borderline ovarian tumors. We were unable to find a relationship in our study group because early-stage, low-grade, and borderline patient groups were included. Further studies are required in this respect.

The effect of estrogen is especially important in the development of hormone-dependent tumors such as endometrial and breast cancer carcinogenesis. In the present study,

there was a relationship between the miR141 expression and the ER level of staining. All cases (8/8) with positive miR141 cutoff variable were ER (+) stained, whereas 50% of negative cases (7/14) were ER (+) stained. In the study of Chen et al., estrogen was shown to influence the cycle by a negative effect between miR200c and PTENP1 to inhibit the PTEN expression in the development of endometrial carcinoma.[29] Further research is needed to interpret that phenomenon concerning our results.

The prognostic and predictive role of Nrf2, KEAP1, p16, and E-cadherin in epithelial ovarian cancers has also been explored.[30–32] In that study, 108 ovarian cancer patients were included. Serous carcinoma and high KEAP1 expression were reported to occur in women with older age, advanced stage, and poor prognosis. The results of multiple factor analysis particularly showed a significant correlation between survival and Nrf2 and p16. In the present study, we determined a significant correlation between the proliferation of KEAP1 and ki67. In those with KEAP1 expression, ki67 staining can be predicted to be associated with a highly probable poor prognosis. While all cases (10/10) with positive KEAP1 cutoff variable were ki67(+) stained, 33% (1/3) of negative cases were positively stained. A higher number of homogeneous groups should be used to evaluate these results in combination with the literature. However, the present study work may shed light on future study designs since it has shown this even in early-stage and low-grade ovarian cancers.

Chemokines (CXC) are small proteins that control lymphoid organ development and immune cell movements. They play a role in the angiogenesis phase in the steps of carcinogenesis. In the present work, we have identified a significant relationship between CXCL1 and survival. Of the CXCL1 cutoff variable positive cases, 68.6% (35/51) died, which was 41.7% (10/24) for negative cases. Adiponectin regulates cytokine secretion from the fat tissue, cell proliferation, inflammation, and energy homeostasis. The role of adiponectin in carcinogenesis in obesity-associated cancers such as ovarian cancer is intensively studied, but the relationship is still unclear. In their in vitro study, Ouh et al. investigated the mediation of CXC ligand 1 in adipokine-related angiogenesis in ovarian cancer.[33] The study indicated that adiponectin is proangiogenic in ovarian cancer, and CXCL1 secretion increases in cancer cells (independent of VEGF). Although there were contradictory results in the literature regarding the adiponectin and carcinogenesis relationship, we found that adiponectin is related to survival.

We determined no relationship between age and the number of lymph nodes and gene expressions associated with miRNAs and other parameters. This may be because patient histologies were heterogeneous, and early-stage cases were included.

Our study's main limitation was the difficulty in evaluating ovarian cancers in general, since the hospital where the tissue samples were taken and where the patients were studied were different and the cases mostly consisted of early-stage ovarian cancers. Among other limitations are the study's retrospective design, exclusion of recurrent treatments, low number of patients, lack of genetic assessments, and heterogeneity of histopathologies and stages. Both in the world and our country, the changes in hospitals' work environment during the pandemic, especially the difficulties in access to clinical data, have also been a constraint for our study.

Our study's strengths include the examination of tissue samples and the inclusion of low-grade, early-stage, and borderline tumors, which are studied to a lesser extent in the literature. In the existing literature, there are many studies on different miRNAs in ovarian cancer investigating both their predictive and prognostic roles. In future studies on ovarian cancer, different results and heterogeneous data from different tumor regions should be considered, and the most accurate targets should be identified, keeping in mind the complex mechanisms in which epigenetic and genetic mechanisms are also involved. In future studies, it will continue to be treated as a hypothesis that various miRNAs regulate various target molecules and that they can guide both the formation and prevention of cancer, as well as the determination of treatment and prognosis stages. Although it is not yet possible to use miRNA or anti-miRNA in the treatment for future studies, the data in the literature will provide us with useful information in terms of ovarian cancer biology, its treatment, and prognosis. Tissue and circulating miRNAs may be novel and noninvasive biomarkers for detecting ovarian cancer, particularly multiple miRNA panels, which have potential diagnostic value as screening tools in clinical practice in the future.

Disclosures

Ethics Committee Approval: The ethical board approvals to conduct the study were obtained from the Ethical Board of Non-Invasive Research, Dokuz Eylul University (Ethical Board Decision no. 2017/24-19).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – H.T.Y., Z.S.A., S.A.; Design – Z.S.A., S.A., G.D.; Supervision – Z.S.A., S.A.; Materials – G.D., S.S., D.A.; Data collection &/or processing – G.D., S.S., D.A., Z.S.A., S.A.; Analysis and/or interpretation – S.A., H.T.Y.; Literature search – H.T.Y., T.Y.; Writing – H.T.Y., T.Y., B.S., İ.Ç.; Critical review – H.T.Y., T.Y., S.A., Z.S.A.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. A Cancer J Clin 2021;71:7–33. [CrossRef]
2. Noone AM, Howlader N, Krapcho M, Miller D, Brest A, Yu M, et al. SEER cancer statistics review, 1975-2015, National Cancer Institute. Based on November 2017 SEER data submission, posted to the SEER web site, April 2018. Available at: https://seer.cancer.gov/archive/csr/1975_2015/. Accessed Dec 8, 2022.
3. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics. CA Cancer J Clin 2018;68:284–96. [CrossRef]
4. Robboy SJ. Robboy's pathology of the female reproductive tract. 2nd ed. London: Churchill Livingstone; 2008.
5. James NE, Chichester C, Ribeiro JR. Beyond the biomarker: understanding the diverse roles of human epididymis protein 4 in the pathogenesis of epithelial ovarian cancer. Front Oncol 2018;8:124. [CrossRef]
6. Dochez V, Caillon H, Vaucel E, Dimet J, Winer N, Ducarme G. Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. J Ovarian Res 2019;12:28. [CrossRef]
7. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 2006;94:776–80.
8. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 2006;6:259–69. [CrossRef]
9. Christodoulatos GS, Dalamaga M. Micro-RNAs as clinical biomarkers and therapeutic targets in breast cancer: Quo vadis? World J Clin Oncol 2014;5:71–81. [CrossRef]
10. Zhong X, Coukos G, Zhang L. miRNAs in human cancer. Methods Mol Biol 2012;822:295–306.
11. Wang X, Ivan M, Hawkins SM. The role of MicroRNA molecules and MicroRNA-regulating machinery in the pathogenesis and progression of epithelial ovarian cancer. Gynecol Oncol 2017;147:481–7.
12. Weidle UH, Birzele F, Kollmorgen G, Nopora A. Potential microRNA-related target for therapeutic intervention with ovarian cancer metastasis. Cancer Genomics Proteomics 2018;15:1–15 [CrossRef]
13. Deb B, Uddin A, Chakraborty S. miRNAs and ovarian cancer: An overview. J Cell Physiol 2018;233:3846–54. [CrossRef]
14. Yavuzsen HT, Aktas S, Saatli BH, Altun ZS. MicroRNA's in ovarian cancer. DEÜ Tıp Fakültesi Dergisi 2019;33:93–101.
15. Shi M, Mu Y, Zhang H, Liu M, Wan J, Qin X, et al. MicroRNA-200 and microRNA-30 family as prognostic molecular signatures in ovarian cancer: A meta-analysis. Medicine (Baltimore) 2018;97:e11505. [CrossRef]
16. Xing Z, Li D, Yang L, Xi Y, Su X. MicroRNAs and anticancer drugs. Acta Biochim Biophys Sin 2014;46:233–9.
17. Wiemer AC. The role of microRNAs in cancer: No small matter. Eur J Cancer 2007;43:1529–44. [CrossRef]
18. Duska LR, Park WP, Moore KN. Epithelial ovarian cancer in older women: defining the best managenment approach. Am Soc Clin Oncol Educ Book 2015;e311–21. [CrossRef]
19. Cavallari I, Ciccarese F, Sharova E, Urso L, Raimondi V, Silic-Benussi M, et al. The miR-200 Family of microRNAs: Fine tuners of epithelial-mesenchymal transition and circulating cancer biomarkers. Cancers (Basel) 2021;13:5874.
20. Zuberi M, Mir R, Das J, Ahmad I, Javid J, Yadav P, et al. Expression of serum miR-200a, miR-200b, and miR-200c as candidate biomarkers in epithelial ovarian cancer and their association with clinicopathological features. Clin Transl Oncol 2015;17:779–87.
21. Wang X, Seed B. A PCR primer bank for quantitative gene expression analysis. Nucleic Acids Res 2003;31:e154. [CrossRef]
22. Hulstaert E, Morlion A, Levanon K, Vandesompele J, Mestdagh P. Candidate RNA biomarkers in biofluids for early diagnosis of ovarian cancer: A systematic review. Gynecol Oncol 2021;160:633–42
23. Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, et al. MicroRNA signatures in human ovarian cancer. Cancer Res 2007;67:8699–707. [CrossRef]
24. Mateescu B, Batista L, Cardon M, Gruosso T, de Feraudy Y, Mariani O, et al. miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response, Nat Med 2011;17:1627–35. [CrossRef]
25. Suo HB, Zhang KC, Zhao J. MiR-200a promotes cell invasion and migration of ovarian carcinoma by targeting PTEN, Eur Rev Med Pharmacol Sci 2018;22:4080–89.
26. Guan W, Cui H, Huang P, Chun WJ, Lee JW, Kim H, et al. miR-200b/200a/429 cluster stimulates ovarian cancer development by targeting ING5. J Oncol 2020;2020:3404059.
27. Lu YM, Shang C, Ou YL, Yin D, Li YN, Li X, et al. miR-200c modulates ovarian cancer cell metastasis potential by targeting zinc finger E-box-binding homeobox 2 (ZEB2) expression. Med Oncol 2014;31:134. [CrossRef]
28. Sadlecki P, Jozwicki J, Antosik P, Grabiec M. Expression of selected epithelial–mesenchymal transition transcription factors in serous borderline ovarian tumors and type I ovarian cancers. Tumor Biology 2018;40:1010428318784807
29. Chen R, Zhang M, Liu W, Chen H, Cai T, Xiong H, et al. Estrogen affects the negative feedback loop of PTENP1-miR200c to inhibit PTEN expression in the development of endometrioid endometrial carcinoma. Cell Death Dis 2018;10:4. [CrossRef]
30. Liew PL, Hsu CS, Liu WM, Lee YC, Lee YC, Chen CL. Prognostic and predictive values of Nrf2, Keap1, p16 and E-cadherin expression in ovarian epithelial carcinoma. Int J Clin Exp Pathol 2015;8:5642–49.
31. Konstantinopoulos PA, Spentzos D, Fountzilas E, Francoeur N, Sanisetty S, Grammatikos AP, et al. Keap1 mutations and Nrf2 patthway activation in epithelial ovarian cancer. Cancer Res 2011;71:5081–89.
32. van Jaarsveld MTM, Helleman J, Boersma AWM, van Kuijk PF, Despierre E, Vergote I, et al. miR-141 regulates KEAP1 and modulates cisplatin sensitivity in ovarian cancer cells. Oncogene 2013;32:4284–93. [CrossRef]
33. Ouh YT, Cho HW, Lee JK, Choi SH, Choi HJ, Hong JH. CXC chemokine ligand 1 mediates adiponectin-induced angiogenesis in ovarian cancer. Tumour Biol 2019;42:1010428319842699.