Effect of the hsa-microRNA 200c-3p/ZEB1 loop in epithelial-mesenchymal transition of ovarian cancer cells

MERVE SENTURK[1], ECE GUMUSOGLU-ACAR[2], SAMET TOPUZ[3] and TUBA GUNEL[2]

[1]Molecular Biology and Genetics Programme, Institute of Graduate Studies in Sciences, Istanbul University, Istanbul 34134, Turkey;
[2]Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul 34134, Turkey;
[3]Department of Obstetrics and Gynecology, Istanbul Medical Faculty, Istanbul University, Istanbul 34104, Turkey

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Abstract. Ovarian cancer (OC) is a disease often diagnosed at advanced stages with distant metastasis, which is characterized by the epithelial-mesenchymal transition (EMT) of cells. The aim of the present study was to analyze the target genes of *Homo sapiens* microRNA 200c-3p (hsa-miR-200c-3p) and their associated proteins, which have a role in the EMT process. The expression level of hsa-miR-200c-3p was analyzed in ovarian tissues obtained from healthy individuals (n=15) and patients with advanced epithelial OC (EOC; n=15). Zinc finger enhancer-box binding homeobox 1 (ZEB1) is one of the target genes of hsa-miR-200c-3p and was identified using *in silico* methods. The expression level of ZEB1 was analyzed in both groups. ZEB1, vimentin and E-cadherin proteins were analyzed using immunohistochemistry (IHC) in healthy and OC tissues. The expression of hsa-miR-200c-3p was significantly increased in patients with OC (P<0.01). ZEB1 expression decreased significantly in the EOC group (P<0.01). The correlation analysis indicated a strong negative correlation between hsa-miR-200c-3p and the ZEB1 gene (ρ=-0.75; P<0.01). IHC staining revealed that the higher staining intensity of ZEB1 and E-cadherin proteins were found in cancerous tissues compared with healthy ones (P<0.05). However, the intensity and extent of vimentin staining was decreased in cancerous tissues (P<0.05). The decreased expression of ZEB1 is regulated by hsa-miR-200c-3p. The downregulation of ZEB1 may cause insufficient binding to the mRNA of E-cadherin, leading to increased E-cadherin expression. The hsa-miR-200c-3p/ZEB1 loop regulates vimentin and E-cadherin expression in OC. The increased expression level of hsa-miR-200c-3p may also cause mesenchymal-to-epithelial transition in OC cells.

Correspondence to: Professor Tuba Gunel, Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, 1 Bozdogankemeri Street, Vezneciler, Istanbul 34134, Turkey
E-mail: gunel@istanbul.edu.tr

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Introduction

Ovarian cancer (OC) is the most lethal gynecological cancer (1). The main reason for the high mortality rate of OC (70%) is it is asymptomatic in the early stages of the disease, therefore, ~70% of OC cases are diagnosed in advanced stages (2). The mortality rate also depends on therapy resistance occurrence (3), distant metastasis in advanced stages and epithelial-mesenchymal transition (EMT), which is the key molecular process in metastasis (4). It has been reported that 90% of primary ovarian tumors are of epithelial origin, described as the surface epithelium of the ovary or the surface epithelial inclusion cysts (5).

MicroRNAs (miRNAs/miR) are small [21-24 nucleotides (nt) long] non-coding RNAs and can regulate the translation via degradation of target mRNAs (6). Each miRNA has multiple targets and a specific sequence of 6-8 nt that binds to complementary sequences in the regulatory regions of target genes (7). miRNAs may serve a role as oncogenes (8) or tumor suppressors (9) depending on the target genes. Thus, they are marked regulators of complex processes such as tumor metastasis and differentiation.

Epithelial cells are tightly interconnected by various connecting structures such as tight junction complex, cell-cell adhesion complex and desmosomes. E-cadherin (epithelial cadherin) is responsible for cell-cell adhesion and binds to actin, which acts as a cytoskeletal element, through membrane proteins such as α- and β-catenin. The increased function of E-cadherin/catenin affects the EMT process and metastasis (4).

Factors that cause EMT are considered to initiate epithelial remodeling by impairing E-cadherin function or altering its expression (10,11). Vimentin belongs to the intermediate filament family that forms the cytoskeleton and is expressed in mesenchymal cells. Altered expression level of vimentin is associated with malignancy and causes poor prognosis in several cancer types, such as prostate, lung and breast cancer, melanoma and OC (12-15). Vimentin is a potential target in cancer therapy due to its upregulation in patients with tumors and the association with metastasis via its regulatory role in the EMT process (13,16).

EMT-mediated cancer progression and metastasis are regulated through several processes including miRNAs (17). miR-200 family can inhibit EMT and induce the mesenchymal-to-epithelial transition (MET) process (17). The EMT-inducing targets of the miR-200 family are the zinc finger enhancer (E)-box binding homeobox (ZEB) 1 and ZEB2 genes and elucidating their interaction mechanism could be a notable step in the EMT-associated treatment of cancer types such as breast, lung, colorectal, liver and ovarian cancer (17). A list of up- and downregulated-miRNAs in tumor tissues were previously identified with epithelial OC (EOC) compared with simple ovarian cyst tissues using a microarray, with miR-200c-3p reported to be markedly upregulated in EOC samples (2). In the present study, the function of miR-200c-3p was investigated with one of its target genes, ZEB1, to elucidate the underlying molecular mechanisms involved in the EMT process in high-grade serous OC (HGSOC). Furthermore, E-cadherin and vimentin proteins were evaluated immunohistochemically in terms of their roles in EMT and MET in the development and progression of ovarian tumors. The role of this miRNA and target genes in EMT will enhance current understanding of how cancer metastasis occurs and targeting these miRNAs will allow the potential development of novel ways to treat cancer by using them as therapeutic agents in the future.

Patients and methods

Sample collection. A two-sample independent t-test was used to assess statistical power to detect differences between tumor and healthy groups. Cohen's D was used to determine effect sizes based on the means and standard deviations of each group. Python (version 3.14.0; python.org) was used to perform power analysis, considering a sample size of n=15 per group. Power test values for sample size were visualized. A power level of 80% was used to assess whether the available sample size was sufficiently large to detect statistically significant differences.

The samples consist of healthy (n=15) and cancerous ovarian tissues (n=15). The present study was approved by the Istanbul Medical Faculty Clinical Research Ethics Committee (approval no. 2019/1206; Istanbul, Turkey) and written informed consent was obtained from each patient involved. All tissue samples had been collected at the Department of Obstetrics and Gynecology, Istanbul University Faculty of Medicine (Istanbul, Turkey) from December 2019 to April 2020. Cancerous ovarian tissues were resected from patients with HGSOC who did not undergo hormone therapy or chemotherapy prior to surgery. Healthy ovarian tissues were obtained from patients who underwent surgery for any reason other than cancer, such as total abdominal hysterectomy or uni/bilateral oophorectomy. All tissues were pathologically diagnosed and graded by Department of Pathology, Istanbul University Faculty of Medicine. All serous ovarian carcinoma tissue samples were staged according to the International Federation of Gynecology and Obstetrics staging system (18). After collection, tissue samples were transferred to 10% neutral buffered formalin to prevent tissue degradation for use in immunohistochemical studies and RNAlater solution (Invitrogen; Thermo Fisher Scientific, Inc.) to protect and stabilize RNAs for use in molecular genetic studies. Subsequently, 500 mg was extracted from all tissue samples and stored at -80˚C until total RNA isolation.

Table I. Primer sequences of ZEB1, hsa-miR-200c-3p, U6 snRNA and GAPDH.

Gene/miR	Primer sequence (5'-3')
ZEB1	F: AACTGCTGGGAGGATGACAC
	R: TCCTGCTTCATCTGCCTGA
Hsa-miR-200c-3p	F: GGCGTAATACTGCCGGGTA
	R: ATTGCGTGTCGTGGAGTCG
U6 snRNA	F: CTCGCTTCGGCAGCACA
	R: AACGCTTCACGAATTTGCGT
GAPDH	F: AGGGCTGCTTTTAACTCTGGT
	R: CCCCACTTGATTTTGGAGGGA

F, forward; R, reverse; miR, microRNA; snRNA, small nuclear RNA; ZEB1, zinc finger enhancer-box homeobox 1.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from tissues using the PureLink RNA Mini Kit (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The quantity and quality of the obtained total RNA were measured using NanoDrop 2000 (Thermo Scientific, Inc.). All samples were kept at -80˚C for further steps.

To compare the expression of *Homo sapiens* (hsa)-miR-200c-3p in healthy ovarian and tumor tissues, total RNA samples were reverse transcribed into complementary DNA (cDNA) using the TaqMan™ MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc.). U6 small nuclear RNA was used for normalization. The primer sequences of hsa-miR-200c-3p and U6 are shown in Table I.

mRNA targets of hsa-miR-200c-3p were identified using the miRNA target prediction and functional annotation database (miRDB; mirdb.org/). The expression of ZEB1 gene was also analyzed using RT-qPCR. For cDNA synthesis, the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc.) was used according to the manufacturer's protocol. RT-qPCR was performed on the Stratagene™ Mx3005p thermal cycler (Agilent Technologies) using the iTaq Universal SYBR® Green Supermix kit (Bio-Rad Laboratories, Inc.). GAPDH was used as the reference gene. Primer sequences of ZEB1 and GAPDH genes are shown in Table I. TaqMan™ MicroRNA Master Mix (Thermo Fisher Scientific, Inc.) was used for TaqMan-based RT-qPCR assay.

Immunohistochemistry (IHC). Tissue samples were fixed in 10% neutral buffered formalin at room temperature for 24 h, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections of 3 μm thickness were cut from paraffin blocks and mounted on positively charged slides. Immunohistochemical staining was performed using the Ventana Benchmark Ultra fully automated staining system (Roche Tissue Diagnostics) according to the manufacturer's protocols. Antigen retrieval was performed using

Table II. Antibodies used in immunohistochemical staining and their properties.

Antibody name	Source	Clone	Cat. no.	Dilution	Positive control tissue
ZEB1	Invitrogen; Thermo Fisher Scientific, Inc.	Rabbit polyclonal	PA5 82982	1:3,000	Endometrium
E-cadherin	Roche Tissue Diagnostics; Roche Diagnostics, Ltd.	EP700Y/Rabbit monoclonal	05973872001	Ready to use	Breast carcinoma
Vimentin	Roche Tissue Diagnostics; Roche Diagnostics, Ltd.	V9/Mouse monoclonal	05278139001	Ready to use	Skin

ZEB1, zinc finger enhancer-box homeobox 1.

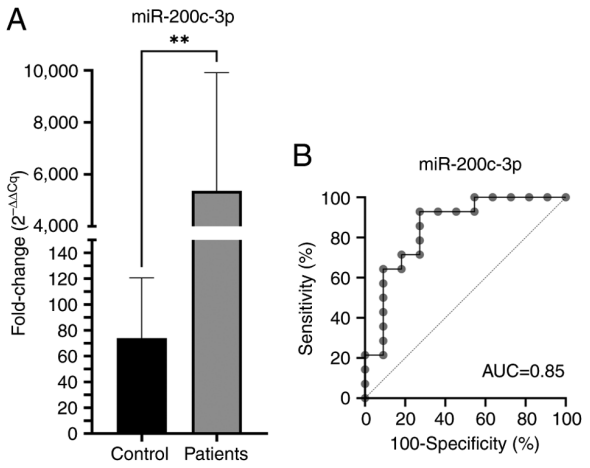

Figure 1. Expression and receiver operating characteristic curve analysis of hsa-miR-200c-3p. (A) Relative expression of hsa-miR-200c-3p in healthy and cancerous ovarian tissues. (B) Receiver operating characteristic curve analysis of hsa-miR-200c-3p in predicting ovarian cancer. **P<0.01. hsa-miR-200c-3p, *Homo sapiens* microRNA-200c-3p; AUC, area under the curve.

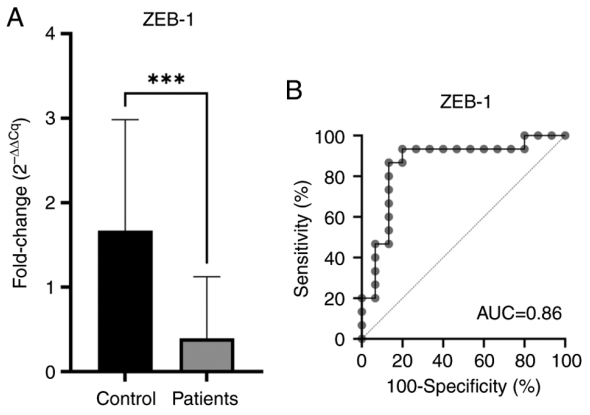

Figure 2. The expression and receiver operating characteristic curve analyses of ZEB1. (A) Relative expression of the ZEB1 gene in healthy and cancerous ovarian tissues. (B) Receiver operating characteristic curve analysis of the ZEB1 gene mRNAs in predicting ovarian cancer. ***P<0.001. ZEB1, zinc finger enhancer-box homeobox 1; AUC, area under the curve.

Cell Conditioning 1 (CC1) solution (cat. no. 950-224; Roche Tissue Diagnostics) at 95˚C for 30 min. Permeabilization, blocking of nonspecific binding, and quenching of endogenous peroxidase activity were performed automatically by the Benchmark Ultra system using proprietary reagents included in the detection kit. Immunodetection was carried out using the UltraView Universal DAB Detection Kit (cat. no. 760-500; Roche Tissue Diagnostics). Primary and secondary antibody incubation was performed at 37˚C, with incubation times controlled automatically by the instrument according to the manufacturer's recommendations. Slides were counterstained with hematoxylin at room temperature using the automated protocol and subsequently mounted. Immunohistochemical staining and evaluation were performed using the Ventana BenchMark Ultra system and Ventana System Software (version 12.1; Roche Tissue Diagnostics). The primary antibodies are provided in Table II.

Immunohistochemically stained sections were observed and evaluated with a Nikon Eclipse E200 (Nikon Corporation) light microscope. Microscopic images were obtained from the digital imaging system Aperio ScanScope XD (Leica Biosystems Nussloch GmbH). Two different criteria, which are the percentage of staining area and staining intensity, were evaluated using a semi-quantitative scoring system for ZEB1, E-cadherin and vimentin (10,11,13). The percentage of the staining area was obtained with the formula: (Total number of positive cells/total number of tumor cells) x100. Data were scored as 0 (0%), 1 (1-10%), 2 (10-50%), 3 (<51%). Staining intensity was determined according to the brown shade formed in DAB chromogen^{+} cells. Scoring was evaluated as 0 (negative, -), 1 (weak, +), 2 (medium, ++) and 3 (strong, +++). The sum of staining intensity and percent staining area scores was used as the final staining score.

Statistical analysis. The RT-qPCR results of ZEB1 mRNA and hsa-miR-200c-3p were analyzed according to Cq values. ΔΔCq values are calculated using Microsoft Excel (Microsoft Corporation, Version 16.103.3) and $2^{-\Delta\Delta Cq}$ demonstrated the fold-changes between the test and control groups.

GraphPad Prism (version 9.3.0; Dotmatics) was used for statistical analysis of RT-qPCR results. Data distribution was evaluated for normality using the Shapiro-Wilk test RT-qPCR data are expressed as mean ± SD and an independent sample t-test was used for comparison. Since the IHC staining results were categorical data, they are expressed as median (minimum-maximum) and a Mann-Whitney U test was used for comparison. Correlation analysis was performed using Spearman's rank correlation analysis for all data. Receiver

Table III. Semi-quantitative scoring of immunohistochemical staining of ZEB1, E-cadherin and vimentin antibodies.

Sample no.	ZEB1						E-cadherin						Vimentin					
	HOT			OCT			HOT			OCT			HOT			OCT		
	PSA	SI	FS	PSA	SI	FS	PSA	SI	FS	PSA	SI	FS	PSA	SI	FS	PSA	SI	FS
1	2	1	3	2	1	3	1	1	2	3	3	6	3	3	6	2	3	5
2	2	1	3	3	3	6	3	2	5	3	3	6	3	3	6	2	2	4
3	0	0	0	3	2	5	3	1	4	3	3	6	3	3	6	2	3	5
4	1	1	2	3	3	6	2	2	4	3	3	6	3	3	6	2	2	4
5	0	0	0	3	3	6	1	1	2	2	3	5	3	3	6	2	2	4
6	2	2	4	3	3	6	1	1	2	2	3	5	3	3	6	2	3	5
7	1	1	2	3	3	6	1	1	2	3	3	6	2	3	5	3	2	5
8	1	1	2	3	3	6	2	2	4	2	3	5	3	3	6	2	2	4
9	2	2	4	2	2	4	1	1	2	2	3	5	2	3	5	2	2	4
10	2	2	4	3	2	5	2	1	3	2	3	5	2	3	5	3	2	5
11	2	2	4	3	3	6	2	2	4	2	3	5	2	3	5	2	2	4
12	2	1	3	3	2	5	2	2	4	2	3	5	3	3	6	2	2	4
13	2	1	3	3	2	5	1	2	3	2	3	5	2	3	5	2	2	4
14	2	2	4	3	3	6	2	2	4	2	3	5	2	3	5	2	2	4
15	2	2	4	2	3	5	1	2	3	2	3	5	2	3	5	3	2	5

HOT, healthy ovarian tissue; OCT, ovarian cancer tissue; PSA, percentage of staining area; SI, staining intensity; FS, final score; ZEB1, zinc finger enhancer-box homeobox 1.

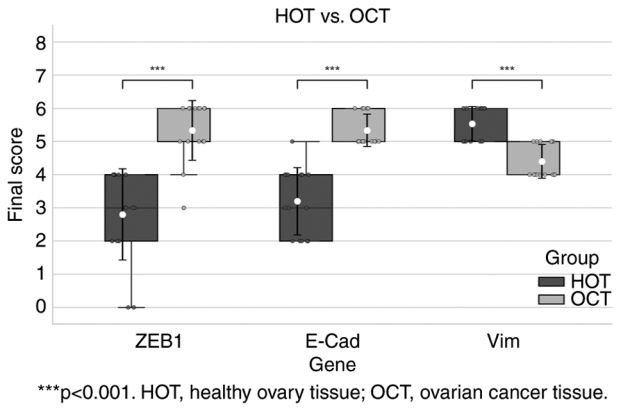

Figure 3. Semi-quantitative scoring results of immunohistochemical staining of ZEB1, E-Cad and Vim antibodies. ***P<0.001. HOT, healthy ovarian tissue; OCT, ovarian cancer tissue; E-Cad, E-cadherin; Vim, vimentin; ZEB1, zinc finger enhancer-box homeobox 1.

operating characteristic (ROC) curve analysis was applied to continuous numerical data, and cut-off values were determined by selecting the point on the ROC curve closest to the upper-left corner (0,1). P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. In the present study, 15 healthy and cancerous ovarian tissues [13 were serous (86.6%), 1 was mucinous (6.6%) and 1 was heterologous carcinoma (6.6%)] were included. All serous adenocarcinoma samples were in stage III-IV, except one stage III sample. The age range of all patients in both groups was 43-74 years. The median age was 59 years (interquartile range, 52-67 years).

miRNA expression analysis. Concentrations of total RNAs isolated from healthy and tumor ovarian tissues were measured with NanoDrop. The concentration values were between 25.5-1,054.2 ng/μl. A260/280 purity values were between 1.93 and 2.13. According to the RT-qPCR results, hsa-miR-200c-3p expression was significantly increased in OC (P<0.01) compared with that in healthy tissues (Fig. 1A). ROC curve analysis results revealed that hsa-miR-200c-3p has 90% sensitivity and 100% specificity for OC (Fig. 1B).

Gene expression analysis. mRNA targets of hsa-miR-200c-3p were identified using the miRNA target prediction and functional annotation database (miRDB; mirdb.org/) and ZEB1 is identified as one of the target genes (Table SI). RT-qPCR of healthy and tumor cDNA samples demonstrated that the expression level of the ZEB1 was significantly decreased in OC tissues compared with healthy tissues (P<0.001). The obtained relative expression chart is displayed in Fig. 2A.

ROC curve analysis results demonstrated that the ZEB1 gene had 93% sensitivity and 100% specificity for OC (Fig. 2B). ROC curve analysis of mRNAs of the ZEB1 gene in predicting OC is shown in Fig. 2B.

IHC analysis. Semi-quantitative staining scores of antibodies are displayed in Fig. 3 and Table III. Certain IHC staining images of ZEB1, E-cadherin and vimentin proteins in healthy and cancerous ovarian tissues are displayed in Fig. 4.

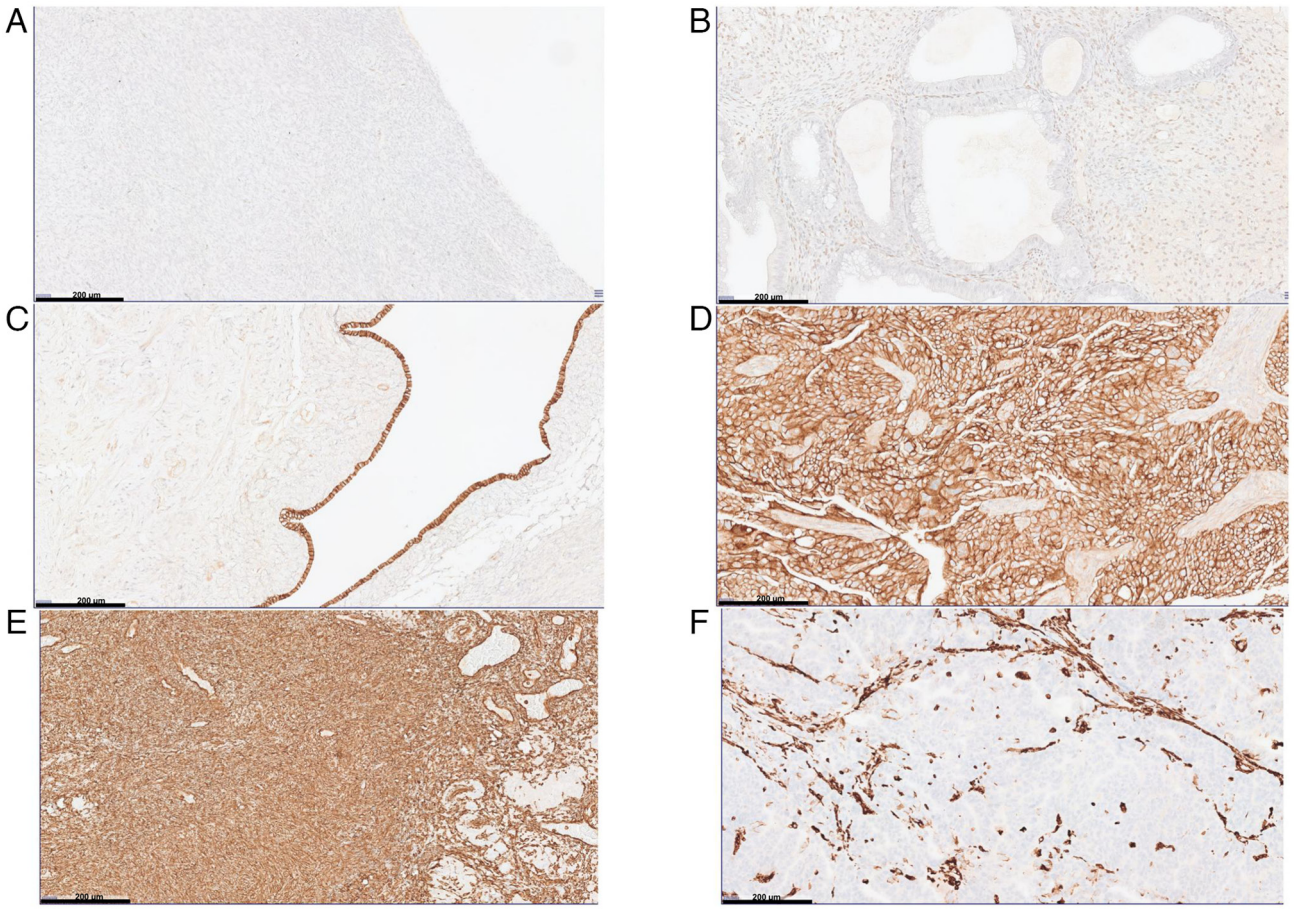

Figure 4. IHC staining samples of ZEB1 protein from (A) healthy ovarian tissues and (B) cancerous ovarian tissues. A sample of IHC staining of E-cadherin of (C) healthy ovarian tissues and (D) cancerous ovarian tissues. A sample of IHC staining of vimentin from (E) healthy ovarian tissues and (F) cancerous ovarian tissues. Magnification, x20. IHC, immunohistochemical; ZEB1, zinc finger enhancer-box homeobox 1.

IHC statistical analysis. Immunohistochemical staining results in healthy and cancerous ovarian tissues (Fig. 3) were compared using a Mann-Whitney U test. Expression of ZEB1 protein in patients with OC was significantly increased compared with the in healthy individuals (U=11; P<0.001). The results demonstrated that the levels of E-cadherin protein in patients with OC were significantly increased compared with healthy individuals (U=5; P<0.001). In addition, the levels of vimentin protein in patients with OC were demonstrated to be significantly reduced compared with healthy individuals (U=21; P<0.001). The correlation between five different parameters (hsa-miR-200c-3p, ZEB1 mRNA, ZEB1, E-cadherin and vimentin proteins) in OC was evaluated and correlation coefficients were calculated (Fig. S1). In the correlation analysis, the relationship with the correlation coefficient was accepted as a medium linear relationship ±0.3-0.7 and the relationship with the correlation coefficient was considered as a strong linear relationship ±0.7-1. Strong negative correlations were detected between hsa-miR-200c-3p and ZEB1 mRNA (P=-0.75) and between ZEB1 and vimentin proteins (P=-0.78; both P<0.05). Correlations between other parameters were moderately correlated. Parameters exhibiting moderate positive correlations were ZEB1 mRNA with vimentin protein (P=0.47), hsa-miR-200c-3p with E-cadherin protein (P=0.58), hsa-miR-200c-3p with ZEB1 protein (P=0.53) and E-cadherin with ZEB1 protein (P=0.66; all P<0.05). Moderately negatively correlated parameters were ZEB1 mRNA and E-cadherin protein (P=-0.66, P=0.00007), ZEB1 mRNA and ZEB1 protein (P=-0.51, P=0.004), hsa-miR-200c-3p with vimentin (P=-0.53, P=0.01), and vimentin and E-cadherin proteins (P=-0.56, P=0.001).

Discussion

OC is the most lethal gynecological malignancies worldwide with an estimated global incidence of 6-7 cases/100,000 women per year (5). The incidence of OC increases with age particularly after the mid-40s and because of the lack of specific symptoms, the early diagnosis of OC remains difficult in patients with OC (19). The absence of a highly sensitive biomarker for early diagnosis of disease has led to research into to the identification of gene-based biomarkers to be used in the clinic. miRNAs are non-coding small RNAs (~22 nt) which regulate gene expression by suppressing the translation of targeted mRNAs or triggering the degradation of mRNA (6). They function as both oncogene and tumor suppressors in the pathogenesis of cancer. miRNAs serve a role in cell pathways such as cell invasion, carcinogenesis and metastasis (20). The use of miRNA-based biomarkers in the early diagnosis and prognosis of OC provides more specific and sensitive results compared with other biomarkers such as members of the miR-200 family, miR-21, and miR-125b (20).

In our previous study (2), hsa-miR-200c-3p was significantly increased (P=0.0000965; log FC=7.873392) in tumor tissues with EOC compared with simple ovarian cyst tissues by microarray and was validated using qPCR (2). The present study aimed to determine differences in hsa-miR-200c-3p expression in OC tissues and healthy ovarian tissues and to analyze the target gene of candidate mRNAs. The present study demonstrated a marked upregulation of hsa-miR-200c-3p in OC tissues, supporting its potential role as a diagnostic biomarker consistent with previous reports (21-23). Therefore, these findings suggest a potential association with ovarian cancer biology, indicating that hsa-miR-200c-3p may have value as a candidate biomarker, although its role in tumor progression and early diagnosis requires further validation.

Using bioinformatic tool, miRDB, the present study determined that hsa-miR-200c-3p serves a role in the EMT pathway and one of its target genes in this pathway was ZEB1. Investigation of the EMT pathway may help elucidate the metastasis mechanism of OC. Thus, the expression level of the ZEB1 gene was assessed and demonstrated to be downregulated in OC tissues.

EMT is a key mechanism facilitating tumor invasion and metastasis (4,10,11). Among the transcription factors regulating EMT, ZEB1 has been identified as a direct target of hsa-miR-200c-3p (24). It regulates EMT in cancer development and progression by suppressing the adhesion molecule E-cadherin (24), expressed by the cadherin-1 (CDH1) gene, which controls the activity of genes involved in chemical signal transduction, cell maturation and regulating cellular movement in the cell. ZEB1 binds to E-boxes within the E-cadherin promoter and stops expression of E-cadherin (25). These results suggest that decreased suppression of CDH1 expression, potentially driven by hsa-miR-200c-3p-mediated regulation of its target ZEB1, may contribute to the enhancement of EMT. According to the staining results, the levels of ZEB1 protein were increased in OC tissues compared with healthy tissues and were demonstrated to be negatively correlated with hsa-miR-200c-3p expression. The observed inverse correlation between ZEB1 mRNA and protein levels suggests the involvement of post-transcriptional regulatory mechanisms (26).

IHC results demonstrated increased levels of E-cadherin in patients with OC compared with that in healthy women. This result is contrary to the previously reported loss of E-cadherin in the EMT process in OC (27). This may suggest that there is a transition process from mesenchymal to epithelium in ovarian carcinogenesis. In addition, the ovarian surface epithelium may have trigger abnormal epithelial differentiation. In epithelial tumors, E-cadherin expression decreases as cancer progresses so that cells can become more mobile. Loss of E-cadherin in cells is a key marker of EMT (28). It is hypothesized that this process is different in OC, indicating that E-cadherin may be a biomarker that can potentially identify OC at an early stage and be a key target in the treatment of the disease in the future.

Vimentin is a type III filament protein that is highly expressed in mesenchymal cells, such as fibroblasts and smooth muscle cells (29), and is a mesenchymal biomarker of EMT. The present study showed that vimentin protein had a weaker staining pattern in tumorous ovarian tissues compared with healthy subjects. Huo *et al* (30) reported that vimentin expression is decreased in drug-resistant ovarian cancer cells compared with control cells. The authors stated that the downregulation of vimentin provides stem cell properties to the cells and causes them to develop drug resistance by cell cycle arrest in the G_2 phase (30). Vimentin may be a potential therapeutic target used to treat drug-resistant OC in the future.

The present study demonstrated an increased level of epithelial proteins and miR-200c, but a decreased level of mesenchymal proteins. These results support the MET process instead of EMT in ovarian carcinogenesis (31). The histological structure of the ovarian surface contains cells having both epithelial and mesenchymal phenotypes. The ovarian surface cells contain epithelial markers such as mucin and E-cadherin and also mesenchymal markers such as N-cadherin and vimentin (32). The hypothesis that OC originates from the ovarian surface epithelium may be supported by the transition of surface epithelial cells from mesenchymal to epithelial structure. Tumor cells can become invasive or metastatic without losing their epithelial properties or molecular markers and without triggering the expression levels of genes of mesenchymal cells. Therefore, it may not always be appropriate to describe the terms EMT or MET with metastasis (33). Collectively, the present study results indicated a MET-like phenotype in OC, rather than a classical EMT pattern. This finding supports the hypothesis that OC may originate from the ovarian surface epithelium, which exhibits both epithelial and mesenchymal characteristics. However, the limited sample size and the fact that 87% of the patients had advanced-stage OC may have limited the ability to detect differences in gene expression between disease stages. In the future, analysis of a large number of patient samples including all stages of OC may help to elucidate the ovarian carcinogenesis process.

The present study has some limitations that should be acknowledged. First, the relatively small sample size may limit the generalizability of the findings. Moreover, the analysis was conducted on a single ovarian cancer cohort, and no subgroup analyses were performed according to disease stage, histological subtype, or tissue of origin, which may have obscured potential stage- or origin-specific differences. Finally, the lack of validation in independent cohorts limits the strength of the conclusions.

In conclusion, the present study results support the hypothesis that hsa-miR-200c-3p negatively regulates ZEB1 expression by binding to the promoter region of the ZEB1 gene. Burk U *et al* showed that miR-200c and its families bind to at least two highly conserved sites in their putative promoter to the ZEB1. Our hypothesis is based on this finding (34). The ZEB1 protein, whose levels are then decreased in the cell, cannot sufficiently bind to the promoter of the CDH1 gene expressing E-cadherin. Therefore, the levels of E-cadherin protein increase. The findings suggest that hsa-miR-200c-3p promotes epithelial characteristics in OC by repressing ZEB1, leading to increased E-cadherin expression. This regulatory axis may represent a potential diagnostic biomarker and therapeutic target for OC in the future.

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Availability of data and materials

The RT-qPCR data generated in the present study may be found in Mendeley database at the following URL: https://data.mendeley.com/datasets/9zbnzv8dh8/1; doi: 10.17632/9zbnzv8dh8.1. The remaining data generated in the present study may be requested from the corresponding author.

Authors' contributions

MS and TG designed and supervised the present study. EA, MS and ST contributed to sample and data collection, devised the methodology, validation of data and statistical analysis. EA, MS and TG contributed to the formal analysis, writing and editing of the manuscript. ST performed sample handling and medical assistance. TG and ST confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was performed in line with the principles of the Declaration of Helsinki. The present study was approved by the Istanbul Medical Faculty Clinical Research Ethics Committee (approval no. 2019/1206; Istanbul, Turkey). All patients and volunteers provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

ORCID: MS, 0000-0002-1941-7135; EA, 0000-0003-3807-0330; ST, 0000-0002-9069-0185; TG, 0000-0003-3514-5210.

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