The importance of dysregulated miRNAs on ovarian cysts and epithelial ovarian cancer

Ece Gumusoglu[1], Tuba Gunel[1,*] [ID], Mohammad Kazem Hosseini[1], Nogayhan Seymen[2], Taylan Senol[3], Uğur Sezerman[2], Samet Topuz[4], Kılıç Aydınlı[5]

[1] Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, 34134, Istanbul, Turkey

[2] Acıbadem University, School of Medicine, Department of Basic Sciences, Biostatistics, 34684, Istanbul Turkey

[3] Istanbul Zeynep Kamil Women and Child Diseases Training and Research Hospital, 34668, Istanbul, Turkey

[4] Istanbul University, Istanbul Medical Faculty, Department of Obstetrics and Gynecology, 34093, Istanbul, Turkey

[5] Medicus Healthcare Centre, 34365, Istanbul, Turkey

*Correspondence: gunel@istanbul.edu.tr (Tuba Gunel)

Objective: Benign ovarian cysts (BOC) are the most common tumors in women of reproductive age. Usually, these cysts are harmless, but, a small number of them occasionally progress to malignancy. Among ovarian malignancies, epithelial ovarian cancer (EOC) comprises 90% and is the most important cause of gynecologic cancer-related deaths. We aimed to identify dysregulated miRNAs in patients with benign ovarian cysts (n = 11) compared to EOC (n = 10) and to healthy individuals (HI) (n = 15). *Methods*: The serum samples from EOC and BOC patients were collected before operation. We studied three different sample groups (serum of EOC (n = 8), HI (n = 8), and BOC (n = 8) patients) that contained the highest-quality of RNA. Microarray data were analyzed according to expression of miRNAs and target genes by bioinformatics tools. *Results*: When compared to EOC samples, 75 miRNAs were dysregulated in BOC samples. Sixty-six miRNAs from BOC were dysregulated when compared to HI samples. Bioinformatics analysis of BOC vs. EOC and BOC vs. HI showed that 46 miRNAs were congruent and their expression alterations were similar (up- or down-regulated). Further analysis showed that these 46 miRNAs are associated to one of three pathways involved in cancer pathogenesis. *Conclusion*: Several miRNAs might play a role in BOC formation and/or malignant transformation. These dysregulated miRNAs could potentially be a biomarker to distinguish between a completely BOC and one that is malignant or has potential for malignant transformation.

Keywords

Ovarian neoplasms; Ovarian cysts; MicroRNAs; Neoplastic transformation; Epithelial ovarian cancer

1. Introduction

An ovarian cyst is a type of structure encapsulated by a wall of epithelial cells, filled with fluid secreted from the lining epithelium [1]. Ovarian cysts can be divided into 2 groups: functional and non-functional. Functional cysts are frequently seen in reproductive aged women when the Graffian follicle cannot be properly hatched and the ovum fails to be released. They usually dissolve spontaneously after a few menstrual cycles [2]. However, most ovarian cysts are non-functional and asymptomatic [3, 4]. There are several subtypes of non-functional ovarian cysts originating from various cell lineage. In this group, polycystic ovaries, endometriomas, cystadenomas and dermoid cysts (mature teratoma) are the most common ovarian pathologies detected by gynecologists [5, 6]. Among these cysts, cystadenomas and mature teratomas, which are benign, may occasionally progress to malignancy [2, 7, 8]. Excluding malignancy or the possibility of malignant transformation without postoperative pathological evaluation is yet the most grueling task for a physician. In this study, we aim to identify miRNAs that might play a role in the transformation of BOC to malignancy. This in turn could help in distinguishing BOC from EOC prior to surgical removal.

Ovarian cancer (OC) is a heterogeneous histologic type and the leading cause of gynecological cancer-related deaths [9, 10]. Epithelial ovarian tumors are classified as benign, borderline and malignant and correspond to four major histologic subtypes based on morphologic criteria corresponding to the different types of epithelium in the Müllerian ducts, the embryological structures that eventually form vagina, cervix, uterus and fallopian tubes according to the World Health Organization criteria [11–15]. Due to lack of sensitive diagnostic tools, most of the ovarian cases are diagnosed in advanced stages; approximately 25% of ovarian cancer cases are detected at stage I or II. Besides, if 75% of ovarian cancer cases were to be diagnosed at an early stages, the survival rate would be increased by 50% [16]. Therefore, it is vital to distinguish lesions in early stages in terms of benign or malign characteristics. Thus, our study was designed to improve discrimination of benign and malignant lesions at the early stages using microRNA expressions.

MicroRNAs (miRNAs) are non-coding RNAs, about 22 nucleotides in length, involved in inhibition of translation,

mRNA degradation, and sequencing specific gene regulation. MicroRNAs regulate various biological processes such as cellular development, proliferation, differentiation, and apoptosis [10, 17]. Alles *et al.* discovered 2300 human mature miRNAs and 1115 of which are currently annotated in miRBase V22 [18]. The secretion and biological functions of extracellular miRNAs are still not fully understood [10, 19]. It is commonly known that miRNAs have a significant role in carcinogenesis via targeting crucial mRNAs, master regulators of implicit genes [15].

Numerous miRNAs have been found to be significant for ovarian cancer pathogenesis in diagnosis, treatment, prognosis, and therapy response [20]. In our previous studies, we published several important miRNAs, such as hsa-miR-1273g-3p and has-let-7d-3p, specific to ovarian cancer [21, 22]. However, there is still a substantive requirement for discrimination of an ovarian lesions for malignant features as early as possible [23]. Hopefully, our study will contribute to distinguish ovarian lesions using differentially expressed miRNAs.

In brief, we mainly aimed to predict malignant or benign characteristics of ovarian tumors in early stages. We planned to detect circulating miRNAs to distinguish ovarian lesions via analyzing expression profiles. Besides, in this way, we can illuminate the physiopathology of benign ovarian cysts (BOC) by comparison of malignant ovarian cancers with healthy individuals (HI). Our other goal was to gain a new perspective to literature about malignant transformation potentials of BOCs, and surveillance of disease after treatment, using miRNAs and their target genes and pathways.

2. Material method

This study was approved by the Istanbul University Faculty of Medicine Clinical Research Ethics Committee (Permission no: 2014/1175) on 08/08/2014. Two main steps followed in this study were serum miRNA profiling by microarray approaches and bioinformatics analysis of target genes and pathways.

2.1 Sample collection

10 epithelial ovarian cancer (EOC) (high-grade serous carcinoma) serum samples, 11 benign ovarian cyst (BOC) serum samples and 15 serum samples from healthy individuals (HI) were collected to compare miRNA expression levels by microarray. The samples were collected by members of the Department of Obstetrics and Gynecology, Istanbul Medical Faculty at Istanbul University. All volunteers opted to confirm and sign the informed consent approved by the ethics committee. The pathology diagnosis was confirmed by examination of the surgically removed specimens. Peripheral blood samples were collected from each group, before cyst or tumor removal surgery, in clot activator tube (10 ml) and centrifuged immediately at 3500 rpm for 15 min at 4 °C. The supernatant serum, was transferred to RNase-free tubes after centrifugation. Following this step, serum samples were immediately stored at -80 °C until study.

2.2 Total RNA extraction

Total RNA was extracted with the mirVana™ miRNA Isolation kit (Ambion, Life Technology, USA) for microarray analysis following the manufacturer's protocols. In the final step of extraction, total RNAs were eluted into 35 μl mirVana elution solution.

2.3 Quality control of extracted RNAs

The quantity control step was performed on all extracted RNAs by NanoDrop ND-2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Eight samples with the highest quality were selected from each group (EOC, BOC and HI) for microarray study. All total RNA samples were stored in -80 °C until laboratory preparation.

2.4 MiRNA expression analysis with microarray

The miRNA expressions from all sample types were analyzed by Agilent miRNA microarray chips (Agilent SurePrint G3 Human miRNA r21 8x60K). The miRNA Labeling and Hybridization Kit (Agilent Technologies) was used according to the manufacturer's instructions. Cyanine 3-cytidine bisphosphate (pCp-Cy3) was used for each total RNA (which included small RNAs) labelling. Following this step, hybridization was performed for 24 hours on Human miRNA Microarray Version 16 (Agilent Technologies) slides which include 1368 miRNAs encoded by genes located across all chromosomes. These slides were scanned by an Agilent SureScan Microarray Scanner (Model G2600D) and "Agilent Feature Extraction" software (v.12.0) was used for image analysis. Tissue and serum-derived RNAs from each group (EOC and BOC) were analyzed in a single microarray chip, followed by comparison. Before progressing any further in the study, a one-way ANOVA power analysis was done in R [24] (version 3.6.1) using the pwr package [25] with true positive rate (power) set to 0.95 and significance level set to 0.05 as per Cohen's suggestions [26]. Prior miRNA analyses show a fold change of 2 (log FC = 1) which is considered a substantial effect [27]. Given these parameters, n = 2.6 is the smallest required sample size. Our proposed sample size of 8 is therefore more than adequate for the purposes of the study.

Differentially expressed miRNAs were obtained using the limma package [28] (version 3.40.6) to determine important miRNAs amongst 'BOC vs. EOC' and 'BOC vs. HI' samples. Median intensity values were read into R and background-corrected using the 'normexp' method. Quantile normalization was performed and Bayes moderated *P*-values were generated to identify differentially expressed miRNAs [29] The final processed data was obtained after removing control probes and averaging over replicate probes.

2.5 Target gene and pathway analysis

Further functional analysis was performed using DIANA tools' miRPath (v3) [30] web server, a commonly used tool when performing miRNA pathway analysis [29, 31]. Stringent filtering was done where miRNAs with |log 2 (FC, fold change)| $\geq$ 1 and adjusted *P*-value $\leq$ 0.05 were selected in order to reduce the number of miRNAs [29, 32] . The miR-

Table 1. Demographic and clinical characteristics of BOC and EOC.

	BOC	EOC	HI
Number of Samples	11	10	15
The median age	40	47	44
The mean of CA-125 (U/mL)	85.3	1520.2	N
Alcohol	N	N	N
Cigarette	N	N	N
Histological types and stages	4 cases are endometrioid cysts, 2 cases are dermoid cysts, 2 cases are mucinous cystadenoma and 3 cases are serous cystadenoma (No valid staging system for cyst patients)	5 cases are stage 3C grade 3 serous cancers, 2 cases are stage 3B grade 3 serous cancers, 1 case is 2B grade 1 serous cancer, 1 case 2A endometrioid and 1 case is 1A grade 3 serous cancers	N
The number of patients with metastasis or other diseases occurred after sample collection	1 patient developed borderline ovarian cancer	9 patients developed metastasis	N
Survival	All still alive	8 patients alive (followed up for 5 years), two patients died (23 and 32 months)	N

*N: No usage

Path analysis was performed using the KEGG (Kyoto Encyclopedia of Genes and Genomes) [33–35]. Human database, aiming to achieve a higher diversity while checking common miRNAs and genes for stricter filtering. miRPath's microT-CDS (v. 5.0) algorithm was used with P-value ≤ 0.01 and 'pathways union' option selected for the KEGG database [31].

3. Results

3.1 Sample characterization

The median age of EOC and BOC, the mean CA-125 (cancer antigen-125) levels, alcohol and tobacco consumption, histological types and stages, the number of patients with metastasis or other diseases and their survival situations are shown in Table 1. None of the patients in our study had received neoadjuvant chemotherapy, or had assisted reproduction, and all patients had undergone primary cytoreductive surgery.

3.2 Microarray results

MiRNA expression profiling results have shown that there were several significantly altered miRNAs in serum samples. All dysregulated miRNAs were selected based on P values (< 0.05) and fold changes (> 2). When compared to the EOC samples 75 miRNAs were dysregulated in BOC samples. Similarly, 66 miRNAs from BOC were dysregulated when compared to HI samples. After bioinformatics analysis of these results (BOC vs. EOC and BOC vs. HI), we found that 46 miRNAs were congruent in both groups and their expression alterations were also similar (up- or down-regulation) (Table 2).

3.3 Target gene and pathway analysis of miRNAs

According to the target gene and pathway analysis of these 46 miRNAs, the altered miRNAs were analysed in the KEGG database and showed 3 significant pathways *Glycosphingolipid biosynthesis - lacto and neolacto series, Mucin type O-Glycan biosynthesis* and *ECM-receptor interaction* which are related to cancer pathogenesis (Table 3). Glycosphingolipids

are part of a complex network affecting N-glycosylation in ovarian cancer cells which have major role in ovarian cancer pathogenesis [36]. Other important pathway is mucin type O-Glycan biosynthesis, since it produces mucin type O-glycans which are elevated in ovarian cancer patients [37]. Extracellular matrix-receptor interaction is another detected pathway, according to our results and it is one of the leading biological pathways playing a role on cancer development [38, 39]. Therefore, the 3 pathways mentioned above support our results for the gravity of dysregulated miRNAs. The target genes of altered miRNAs, which take action in these 3 pathways, are listed in Table 3.

4. Discussion

Epithelial ovarian cancer is the leading cause of gynecologic mortality in women [40]. According to traditional theory, ovarian cancer originates from surface epithelium of ovaries and differentiates into histological types of OC. Another well debated theory is that OC originates from Müllerian cysts located in paratubal and paraovarian regions. A more recent theory is the advanced-stage, high-grade serous ovarian carcinoma originates from the fallopian tubes, and not from ovaries [41–46]. The origin of ovarian cancer is still debatable. Enlightening the physiopathology of malignant transformation and clinical detection is certainly the key to reduce mortality for ovarian cancer. Therefore, we aimed to come up with specific markers in serum feasible enough to detect and/or predict the disease in early stages.

Benign ovarian cysts (BOC) are not life threatening, however, may occasionally (0.17% to 3%) [47–51] progress to malignancy depending on the histopathology [2, 7, 8]. Studies showed that some borderline tumors were derived from cystadenoma and cystadenofibroma [52]. The mechanism for malignant transformation of ovarian cyst is not entirely known and we still need markers or tools to assess which cysts have malignant potential. Our study aimed to understand the biology of malignant transformation of ovarian tu-

Table 2. Dysregulated common miRNAs in serum samples in BOC vs. EOC and BOC vs. HI comparison.

		BOC vs. EOC		BOC vs. HI				BOC vs. EOC		BOC vs. HI	
miRNA	Regulation	log FC	adj.P.Val	log FC	adj.P.Val	miRNA	Regulation	log FC	adj.P.Val	log FC	adj.P.Val
hsa-miR-1207-5p	Down	-1.362	0.0130798	-1.864	0.003935	hsa-miR-6125	Down	-2.39	0.0001107	-2.218	0.000796
hsa-miR-1227-5p	Down	-2.742	2.43E-05	-2.484	0.000244	hsa-miR-638	Down	-1.564	1.63E-03	-1.175	0.03977
hsa-miR-1915-3p	Down	-3.134	2.29E-06	-2.374	0.000282	hsa-miR-6724-5p	Down	-1.957	2.98E-04	-2.356	0.000122
hsa-miR-2861	Down	-2.127	0.0001048	-1.659	0.003935	hsa-miR-6791-5p	Down	-3.662	1.16E-08	-3.163	5.42E-07
hsa-miR-328-5p	Down	-1.331	0.0479035	-2.06	0.010635	hsa-miR-6800-5p	Down	-2.58	4.77E-06	-1.977	0.000454
hsa-miR-3656	Down	-1.851	8.38E-05	-2.133	2.92E-05	hsa-miR-6821-5p	Down	-2.452	5.80E-06	-2.08	1.85E-04
hsa-miR-3663-3p	Down	-1.452	0.027134	-1.621	0.040485	hsa-miR-6850-5p	Down	-2.207	0.0056294	-2.264	0.014502
hsa-miR-3665	Down	-2.544	4.85E-08	-1.792	4.21E-05	hsa-miR-7108-5p	Down	-2.25	1.18E-04	-2.478	1.22E-04
hsa-miR-371b-5p	Down	-3.304	1.79E-06	-3.006	1.15E-05	hsa-miR-7704	Down	-1.668	3.86E-03	-1.687	1.13E-02
hsa-miR-3940-5p	Down	-1.748	5.35E-06	-1.978	1.76E-06	hsa-miR-3162-3p	Up	1.479	4.76E-03	1.515	1.29E-02
hsa-miR-3960	Down	-1.402	0.0055253	-1.508	0.010635	hsa-miR-34b-3p	Up	2.099	0.0000123	1.27	0.008384
hsa-miR-4281	Down	-2.097	0.0001107	-2.498	2.92E-05	hsa-miR-3591-3p	Up	1.884	0.0001365	1.313	1.34E-02
hsa-miR-4433a-3p	Down	-1.074	0.0186955	-1.717	0.001791	hsa-miR-4254	Up	1.594	0.0000058	1.119	0.001554
hsa-miR-4466	Down	-2.632	2.29E-06	-2.357	2.85E-05	hsa-miR-4446-5p	Up	1.379	1.50E-04	1.314	8.51E-04
hsa-miR-4530	Down	-1.21	0.0311594	-1.639	0.013973	hsa-miR-451a	Up	2.521	0.016838	2.95	0.018417
hsa-miR-4763-3p	Down	-2.552	4.94E-05	-2.447	0.000228	hsa-miR-451b	Up	2.183	2.09E-03	2.283	0.004671
hsa-miR-4787-5p	Down	-2.245	0.0001291	-2.117	0.000805	hsa-miR-466	Up	1.627	0.0000134	1.006	0.007591
hsa-miR-5001-5p	Down	-3.01	1.54E-05	-2.602	0.000282	hsa-miR-4730	Up	2.335	5.01E-03	2.411	0.012906
hsa-miR-572	Down	-1.297	0.0111494	-1.377	0.022998	hsa-miR-5010-3p	Up	1.729	0.0002176	1.771	0.000639
hsa-miR-6068	Down	-2.639	0.0001094	-2.249	0.00202	hsa-miR-6514-3p	Up	2.233	0.0001094	1.564	0.010302
hsa-miR-6087	Down	-1.782	0.0039709	-1.823	0.010952	hsa-miR-6716-3p	Up	1.986	0.0098549	2.357	0.009626
hsa-miR-6088	Down	-2.439	3.78E-06	-2.488	5.61E-06	hsa-miR-6834-3p	Up	1.704	8.04E-04	1.292	2.33E-02
hsa-miR-6090	Down	-1.296	0.0196903	-2.38	0.000421	hsa-miR-8485	Up	2.08	0.0010381	1.65	0.021096

mors by studying malignant tumor specific pathways, which are targets of our dysregulated miRNAs, as discussed below.

In both benign and malignant lesion formation, genetic and epigenetic factors play a key role by regulating the biological processes such as development, cell proliferation, differentiation, and apoptosis. MicroRNAs are one form of epigenetic regulation and the physiological and pathological roles of altered miRNAs have been demonstrated in many tumor types [53]. In our study, we aimed to find a new perspective to illuminate the pathogenesis of ovarian cyst and cancer. Additionally, we tried to find miRNAs specific to cyst formation and to detect potential biomarkers for non-invasive distinction of benign from malignant ovarian lesions. Therefore, we focused on miRNA expression in benign and malignant ovarian cyst groups comparing them to healthy controls.

According to our results, among 13 miRNAs which were found in KEGG database for pathway analysis, 5 miRNAs (hsa-miR-34b-3p, hsa-miR-1915-3p, hsa-miR-4530, hsa-miR-4466, hsa-miR-7704) were found oppositely regulated comparing with ovarian cancer cases investigated by previous studies [54–58]. For instance, Li *et al.* found that has-miR-34b-3p is down-regulated in ovarian cancer cases when compared to tumor tissue samples from healthy individuals [56]. Provided that this miRNA is a well-known tumor suppressor miRNA [56]. Likewise, in our study, hsa-miR-34b-3p is up-regulated in benign ovarian cyst samples, so we concluded that upregulated hsa-miR-34b-3p in circulation can be a sign for a benign lesion. Hsa-miR-6088 was the only miRNA similarly regulated in our BOC samples and in EOC

cases from the literature [57]. This miRNA may have a role in malignant transformation. We believe that with further investigation, this miRNA can be a marker for the malignant potential of ambiguous lesions. The rest of 13 dysregulated miRNAs (hsa-miR-3665, hsa-miR-6514-3p, hsa-miR-5010-3p, hsa-miR-6090, hsa-miR-6724-5p, hsa-miR-3663-3p, hsa-miR-4254) have not been associated with ovarian cancer and ovarian cyst cases in the literature. Thus, they may have a high potential as novel biomarkers of BOC lesions. We suggest further validations to evaluate their discrimination capacity for BOC from EOC.

After the analysis of dysregulated miRNA's target genes and pathways, we found 3 significant pathways relevant to BOC pathogenesis. The first one is *Glycosphingolipid biosynthesis - lacto and neolacto series*. Glycosphingolipids (GSLs) are important types of glycolipids and with a hydrophobic ceramide backbone and a hydrophilic carbohydrate residue [59]. All animal cells express GSLs in their surface membranes to maintain plasma membrane stability [59]. They are involved in several biological processes such as embryonic development, signal transduction, cell signaling, apoptosis, receptor modulation, cell adhesion, growth, cell differentiation and carcinogenesis [60]. Malignant tumors often express high levels of GSLs, which interfere with immunological defensive mechanisms [61]. Jacob *et al.* demonstrated that neolacto-series GSL was highly expressed in ovarian cancer cells, and appears to be a novel tumor-associated antigen associated with cellular migration in ovarian cancer [60]. In another study, GSL levels were compared among EOC SKOV3

Table 3. The pathways and target genes of common 46 miRNAs from the KEGG database.

Pathways	miRNAs	Regulation	P-value	Target Genes
	hsa-miR-1915-3p	Down	2.46E-18	FUT3, FUT6
	hsa-miR-3665	Down	9.08E-27	FUT3, FUT6
Glycosphingolipid biosynthesis - lacto and neolacto series	hsa-miR-4530	Down	8.07E-08	FUT3, B4GALT3
	hsa-miR-34b-3p	Up	0.0020494	FUT9
	hsa-miR-6514-3p	Up	1.14E-08	B3GALT5, FUT9
	hsa-miR-4466	Down	8.44E-12	GALNT14
	hsa-miR-6088	Down	0.0042684	GALNT7, WBSCR17
	hsa-miR-6090	Down	5.53E-07	GALNT8
Mucin type O-Glycan biosynthesis	hsa-miR-6724-5p	Down	6.17E-11	GALNT6, GALNT8
	hsa-miR-34b-3p	Up	1.02E-07	GALNT13, GALNT1, C1GALT1
	hsa-miR-5010-3p	Up	0.000405	POC1B-GALNT4, B4GALT5, GALNT4, GALNT1, GCNT1
	hsa-miR-3663-3p	Down	1.34E-13	COL27A1, COL3A1, COL1A1, COL4A4, COL11A1
ECM-receptor interaction	hsa-miR-6088	Down	0.0049984	LAMA3, COL24A1, COL1A1
	hsa-miR-7704	Down	3.77E-06	COL27A1
	hsa-miR-4254	Up	1.18E-11	COL24A1, COL27A1, COL6A3, COL4A6

cells and benign ovarian T29 cells [62]. The researchers found differentially expressed sialylated GSLs and 5 neutral GSLs only in SKOV3 [62]. GSLs also interact with important proteins such as VEGFR4, TGFβ1R3, and EGFR2 in various malignancies [36]. Alam *et al.* showed that GSLs are part of a complex network affecting N-glycosylation in ovarian cancer cells, having an important role in ovarian cancer pathogenesis [36]. Therefore, we consider that our dysregulated miRNAs which target several genes involved in Glycosphingolipid biosynthesis may have an effect on BOC pathogenesis. Also, difference in expression levels of dysregulated miRNAs between BOC and EOC may be adapted to the diagnosis of these diseases.

Second significant pathway, *Mucin Type O-Glycan Biosynthesis,* is an evolutionarily conserved protein modification present on membrane-bound and secreted proteins [63]. Aberrations in O-glycosylation cause some human diseases and are related to disease risk factors [63]. There are studies about this pathway and its relation with cancer pathogenesis. During carcinogenesis, neo-glycan structures emerge on various glycoproteins including mucins [64]. One such mucin, MUC1, is known to play a key role in ovarian cancer [65]. Enhanced fucosylation, truncated O-glycans, and increased sialylation are a well-recognized trace of malignant cell transformation [37]. Brockhausen indicated that O-Glycan structures are often unusual or abnormal in cancer, and greatly contribute to the phenotype and biology of cancer cells [66]. Some of the mechanisms of changes in O-glycosylation pathways have been determined in cancer model systems. Therefore, this pathway can be effective in cyst formation.

The last pathway is the *extracellular matrix (ECM)-receptor interaction.* ECM has a complex mixture of structural and functional macromolecules [38]. ECM plays an important role in tissue and organ morphogenesis and in the maintenance of cell and tissue structure and function [38]. Transmembrane molecules act as mediators to specific interactions

between cells and ECM [38]. Thus, the ECM responsible for intercellular communication, adhesion, apoptosis, migration, and proliferation [38, 39]. General knowledge is that cancer cells change the ECM to control cancer-initiating processes like apoptosis, proliferation and migration [39]. Any aberration in pathways involved in ECM structure and function can directly cause the cancer progression and malignant transformation [67]. Dysregulation, abnormal accumulation or loss of ECM components play an important role in the progression to ovarian cancer [68, 69]. Therefore, we advocate that our pathway analysis findings are innovative in ovarian cancer research. The differentially dysregulated miRNAs imprinting the ECM pathways are recommended for validations to prove their biomarker potential in malignant transformation and cyst formation.

5. Conclusions

Depending on their related pathways, the dysregulated miRNAs might play a role in cyst formation malignant transformation. Therefore, we suggest that these genes and pathways should be further studied to confirm their role in cyst and cancer development. The dysregulated miRNAs can be considered as potential non-invasive biomarkers to distinguish a lesion as benign or malignant. Moreover, for the BOC patients, altered miRNA levels may indicate malignant transformation (from BOC to borderline-ovarian cancer) which might enable us to diagnose preclinical ovarian cancer in early stages having a huge impact on survival. The main limitations of our study were working with different histologic types of ovarian cysts. Since the pathophysiology of different histologic origins may have different pathways, hence different miRNA. To move our research one step further, we plan to study miRNA profiles for every histological type of BOC. Nevertheless, our miRNA findings contribute to the understanding of ovarian cyst and cancer mechanism.

Ethics approval and consent to participate

Author contributions

TG, EG, ST and KA designed the research study. ST and TS provided samples. EG and MKH performed the research. NS and US analyzed the data. EG, TG and MKH wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Onur MR, Bakal U, Kocakoc E, Tartar T, Kazez A. Cystic abdominal masses in children: a pictorial essay. Clinical and Translational Imaging. 2013; 37: 18-27.

[2] Wang Y, Sundfeldt K, Mateoiu C, Shih Ie M, Kurman RJ, Schaefer J, *et al.* Diagnostic potential of tumor DNA from ovarian cyst fluid. Elife. 2016; e15175.

[3] Lee HJ, Woo SK, Kim JS, Suh SJ. "Daughter cyst" sign: a sonographic finding of ovarian cyst in neonates, infants, and young children. American Journal of Roentgenology. 2000; 174: 1013-1015.

[4] Woo YM, Park JH. MicroRNA biomarkers in cystic diseases. BMB Reports. 2013; 46: 338-345.

[5] Patel S. Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy. The Journal of Steroid Biochemistry and Molecular Biology. 2018; 182: 27-36.

[6] Prefumo F, Rossi AC. Endometriosis, endometrioma, and ART results: current understanding and recommended practices. Best Practice & Research Clinical Obstetrics & Gynaecology. 2018; 51: 34-40.

[7] Cheng EJ, Kurman RJ, Wang M, Oldt R, Wang BG, Berman DM, *et al.* Molecular genetic analysis of ovarian serous cystadenomas. Laboratory Investigation. 2004; 84: 778-784.

[8] Levine D, Brown DL, Andreotti RF, Benacerraf B, Benson CB, Brewster WR, *et al.* Management of asymptomatic ovarian and other adnexal cysts imaged at US: Society of Radiologists in Ultrasound Consensus Conference Statement. Radiology. 2010; 256: 943-954.

[9] Chu CS, Rubin SC. Screening for ovarian cancer in the general population. Best Practice & Research Clinical Obstetrics & Gynaecology. 2006; 20: 307-320.

[10] Gumusoglu E, Gunel T. The role of circulating biomarkers in the early diagnosis of ovarian cancer. 2018.

[11] Dubeau L. The cell of origin of ovarian epithelial tumours. The Lancet Oncology. 2008; 9: 1191-1197.

[12] Rodriguez M, Dubeau L. Ovarian tumor development: insights from ovarian embryogenesis. European Journal of Gynaecological Oncology. 2001; 22: 175-183.

[13] Serov SF, Scully RE, Sobin LH, World Health O. Histological typing of ovarian tumours / S. F. Serov, R. E. Scully, in collaboration with L. H. Sobin and pathologists in ten countries. World Health Organization. 1973.

[14] Thor AD, Young RH, Clement PB. Pathology of the fallopiantube, broad ligament, peritoneum, and pelvic soft-tissues. Human Pathology. 1991; 22: 856-867.

[15] Yang J, Zhou Y, Ng SK, Huang KC, Ni X, Choi PW, *et al.* Characterization of MicroRNA-200 pathway in ovarian cancer and serous intraepithelial carcinoma of fallopian tube. BMC Cancer. 2017; 17: 422.

[16] Retamales-Ortega R, Orostica L, Vera C, Cuevas P, Hernandez A, Hurtado I, *et al.* Role of Nerve Growth Factor (NGF) and miRNAs in epithelial ovarian cancer. International Journal of Molecular Sciences. 2017; 18: 507.

[17] Zhang B, Cai FF, Zhong XY. An overview of biomarkers for the ovarian cancer diagnosis. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2011; 158: 119-123.

[18] Alles J, Fehlmann T, Fischer U, Backes C, Galata V, Minet M, *et al.* An estimate of the total number of true human miRNAs. Nucleic Acids Research. 2019; 47: 3353-3364.

[19] Gold B, Cankovic M, Furtado LV, Meier F, Gocke CD. Do circulating tumor cells, exosomes, and circulating tumor nucleic acids have clinical utility? A report of the association for molecular pathology. Journal of Molecular Diagnostics. 2015; 17: 209-224.

[20] Chen SN, Chang R, Lin LT, Chern CU, Tsai HW, Wen ZH, *et al.* MicroRNA in ovarian cancer: biology, pathogenesis, and therapeutic opportunities. International Journal of Environmental Research and Public Health. 2019; 16: 1510.

[21] Gunel T, Dogan B, Gumusoglu E, Hosseini M, Topuz S, Aydinli K. Regulation of HMGA2 and KRAS genes in epithelial ovarian cancer by miRNA hsa-let-7d-3p. Journal of Cancer Research and Therapeutics. 2019; 15: 1321-1327.

[22] Günel T, Gumusoglu E, Dogan B, Ertem FB, Hosseini MK, Cevik N, *et al.* Potential biomarker of circulating hsa-miR-1273g-3p level for detection of recurrent epithelial ovarian cancer. Archives of Gynecology and Obstetrics. 2018; 298: 1173-1180.

[23] Yokoi A, Matsuzaki J, Yamamoto Y, Yoneoka Y, Takahashi K, Shimizu H, *et al.* Integrated extracellular microRNA profiling for ovarian cancer screening. Nature Communications. 2018; 9: 4319.

[24] R core team. R: a language and environment for statistical computing, r foundation for statistical computing. Vienne, Austria. 2019.

[25] Champely S. Pwr: basic functions for power analysis. 2020.

[26] Kok MGM, de Ronde MWJ, Moerland PD, Ruijter JM, Creemers EE, Pinto-Sietsma SJ. Small sample sizes in high-throughput miRNA screens: a common pitfall for the identification of miRNA biomarkers. Biomolecular Detection and Quantification. 2018; 15: 1-5.

[27] Cohen J. Statistical power analysis. Current Directions in Psychological Science. 1992; 1: 98-101.

[28] Ritchie ME, Phipson B, Wu D, Hu YF, Law CW, Shi W, *et al.* Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research. 2015; 43: e47.

[29] Cavalcanti E, Galleggiante V, Coletta S, Stasi E, Chieppa M, Armentano R, *et al.* Altered miRNAs expression correlates with gastroenteropancreatic neuroendocrine tumors grades. Frontiers in Oncology. 2020; 10: 1187.

[30] Vlachos IS, Zagganas K, Paraskevopoulou MD, Georgakilas G, Karagkouni D, Vergoulis T, *et al.* DIANA-miRPath v3.0: deciphering microRNA function with experimental support. Nucleic Acids Research. 2015; 43: W460-W466.

[31] Eneh S, Heikkinen S, Hartikainen JM, Kuopio T, Mecklin JP, Kosma VM, *et al.* MicroRNAs associated with biological pathways of left- and right-sided colorectal cancer. Anticancer Research. 2020; 40: 3713-3722.

[32] Tang S, Wang X, Deng T, Ge H, Xiao X. Identification of C3 as a therapeutic target for diabetic nephropathy by bioinformatics analysis. Scientific Reports. 2020; 10: 13468.

[33] Kanehisa M. Toward understanding the origin and evolution of cellular organisms. Protein Science. 2019; 28: 1947-1951.

[34] Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Research. 2000; 28: 27-30.

[35] Kanehisa M, Sato Y, Furumichi M, Morishima K, Tanabe M. New approach for understanding genome variations in KEGG. Nucleic Acids Research. 2019; 47: D590-D595.

[36] Alam S, Anugraham M, Huang YL, Kohler RS, Hettich T, Winkelbach K, et al. Altered (neo-) lacto series glycolipid biosynthesis impairs α2-6 sialylation on N-glycoproteins in ovarian cancer cells. Scientific Reports. 2017; 7: 45367.

[37] Blanas A, Sahasrabudhe NM, Rodríguez E, van Kooyk Y, van Vliet SJ. Fucosylated antigens in cancer: an alliance toward tumor progression, metastasis, and resistance to chemotherapy. Frontiers in Oncology. 2018; 8: 39.

[38] KEGG. Kegg: kyoto encyclopedia of genes and genomes. Japan: Kanehisa Laboratories. 2019.

[39] Walker C, Mojares E, Hernandez AD. Role of extracellular matrix in development and cancer progression. International Journal of Molecular Sciences. 2018; 19: 3028.

[40] Srivastava SK, Ahmad A, Zubair H, Miree O, Singh S, Rocconi RP, et al. MicroRNAs in gynecological cancers: small molecules with big implications. Cancer Letters. 2017; 407: 123-138.

[41] Crum CP, Drapkin R, Miron A, Ince TA, Muto M, Kindelberger DW, et al. The distal fallopian tube: a new model for pelvic serous carcinogenesis. Current Opinion in Obstetrics & Gynecology. 2007; 19: 3-9.

[42] Eckert MA, Pan S, Hernandez KM, Loth RM, Andrade J, Volchenboum SL, et al. Genomics of ovarian cancer progression reveals diverse metastatic trajectories including intraepithelial metastasis to the fallopian tube. Cancer Discovery. 2016; 6: 1342-1351.

[43] Kurman RJ, Shih IM. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. American Journal of Surgical Pathology. 2010; 34: 433-443.

[44] Medeiros F, Muto MG, Lee Y, Elvin JA, Callahan MJ, Feltmate C, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. American Journal of Surgical Pathology. 2006; 30: 230-236.

[45] Singh N, Gilks CB, Hirschowitz L, Kehoe S, McNeish IA, Miller D, et al. Primary site assignment in tubo-ovarian high-grade serous carcinoma: consensus statement on unifying practice worldwide. Gynecologic Oncology. 2016; 141: 195-198.

[46] Zong XY, Nephew KP. Ovarian cancer stem cells: role in metastasis and opportunity for therapeutic targeting. Cancers. 2019; 11: 934.

[47] Disaia P CW. Germ cell stromal and other ovarian tumours. Clinical Gynaecological Oncology. 1997; 351-371.

[48] Sagae S, Kudo R. Surgery for germ cell tumors. Annals of Surgical Oncology. 2000; 19: 76-81.

[49] Curling OM, Potsides PN, Hudson CN. Malignant change in benign cystic teratoma of the ovary. British Journal of Obstetrics and Gynaecology. 1979; 86: 399-402.

[50] Caspi B, Lerner-Geva L, Dahan M, Chetrit A, Modan B, Hagay Z, et al. A possible genetic factor in the pathogenesis of ovarian dermoid cysts. Gynecologic and Obstetric Investigation. 2003; 56: 203-206.

[51] Park CH, Jung MH, Ji YI. Risk factors for malignant transformation of mature cystic teratoma. Obstetrics and Gynecology Science. 2015; 58: 475-480.

[52] Tavassoli FA DP. Pathology and genetics: tumours of the breast and female genital organs. classification of tumours working group on gynecologic tumours. tumors of the ovary and peritoneum. IARC Press. 2003; 124-127.

[53] Wan WN, Zhang YQ, Wang XM, Liu YJ, Zhang YX, Que YH, et al. Down-regulated miR-22 as predictive biomarkers for prognosis of epithelial ovarian cancer. Diagnostic Pathology. 2014; 9: 178.

[54] Cha SY, Choi YH, Hwang S, Jeong JY, An HJ. Clinical impact of micrornas associated with cancer stem cells as a prognostic factor in ovarian carcinoma. Journal of Cancer. 2017; 8: 3538-3547.

[55] Chong GO, Jeon HS, Han HS, Son JW, Lee YH, Hong DG, et al. Differential MicroRNA expression profiles in primary and recurrent epithelial ovarian cancer. Anticancer Research. 2015; 35: 2611-2617.

[56] Li Y, Yao L, Liu F, Hong J, Chen L, Zhang B, et al. Characterization of microRNA expression in serous ovarian carcinoma. International Journal of Molecular Medicine. 2014; 34: 491-498.

[57] Pandey R, Woo HH, Varghese F, Zhou M, Chambers SK. Circulating miRNA profiling of women at high risk for ovarian cancer. Translational Oncology. 2019; 12: 714-725.

[58] Reza AM, Choi YJ, Yasuda H, Kim JH. Human adipose mesenchymal stem cell-derived exosomal-miRNAs are critical factors for inducing anti-proliferation signalling to A2780 and SKOV-3 ovarian cancer cells. Scientific Reports. 2016; 6: 38498.

[59] Zhuo DH, Li X, Guan F. Biological roles of aberrantly expressed glycosphingolipids and related enzymes in human cancer development and progression. Frontiers in Physiology. 2018; 9: 466.

[60] Jacob F, Anugraham M, Pochechueva T, Tse BWC, Alam S, Guertler R, et al. The glycosphingolipid P-1 is an ovarian cancer-associated carbohydrate antigen involved in migration. British Journal of Cancer. 2014; 111: 1634-1645.

[61] Zhang T, de Waard AA, Wuhrer M, Spaapen RM. The role of glycosphingolipids in immune cell functions. Frontiers in Immunology. 2019; 10: 90.

[62] Rajanayake KK, Taylor WR, Isailovic D. The comparison of glycosphingolipids isolated from an epithelial ovarian cancer cell line and a nontumorigenic epithelial ovarian cell line using MALDI-MS and MALDI-MS/MS. Carbohydrate Research. 2016; 431: 6-14.

[63] Tran DT, Ten Hagen KG. Mucin-type O-Glycosylation during development. Journal of Biological Chemistry. 2013; 288: 6921-6929.

[64] Chugh S, Gnanapragassam VS, Jain M, Rachagani S, Ponnusamy MP, Batra SK. Pathobiological implications of mucin glycans in cancer: Sweet poison and novel targets. Biochimica et Biophysica Acta. 2015; 1856: 211-225.

[65] Akita K, Yoshida S, Ikehara Y, Shirakawa S, Toda M, Inoue M, et al. Different levels of sialyl-Tn antigen expressed on MUC16 in patients with endometriosis and ovarian cancer. International Journal of Gynecological Cancer. 2012; 22: 531-538.

[66] Brockhausen I. Mucin-type O-glycans in human colon and breast cancer: glycodynamics and functions. EMBO Reports. 2006; 7: 599-604.

[67] Eble JA, Niland S. The extracellular matrix in tumor progression and metastasis. Clinical & Experimental Metastasis. 2019; 36: 171-198.

[68] Cho A, Howell VM, Colvin EK. The extracellular matrix in epithelial ovarian cancer-a piece of a puzzle. Frontiers in Oncology. 2015; 5: 245.

[69] Januchowski R, Zawierucha P, Ruciński M, Zabel M. Microarray-based detection and expression analysis of extracellular matrix proteins in drug-resistant ovarian cancer cell lines. Oncology Reports. 2014; 32: 1981-1990.