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ORIGINAL RESEARCH

miRNA expression profiling in formalin-fixed paraffin-embedded endometriosis and ovarian cancer samples

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Abstract: Endometriosis is an inflammatory pathology associated with a negative effect on life quality. Recently, this pathology was connected to ovarian cancer, in particular with endometrioid ovarian cancer. microRNAs (miRNAs) are a class of RNA transcripts ~19–22 nucleotides in length, the altered miRNA pattern being connected to pathological status. miRNAs are highly stable transcripts, and these can be assessed from formalin-fixed paraffin-embedded (FFPE) samples leading to the identification of miRNAs that could be developed as diagnostic and prognostic biomarkers, in particular those involved in malignant transformation. The aim of our study was to evaluate miRNA expression pattern in FFPE samples from endometriosis and ovarian cancer patients using PCR-array technology and also to compare the differential expression pattern in ovarian cancer versus endometriosis. For the PCR-array study, we have used nine macrodissected FFPE samples from endometriosis tissue, eight samples of ovarian cancers and five normal ovarian tissues. Quantitative real-time PCR (qRT-PCR) was used for data validation in a new patient cohort of 17 normal samples, 33 endometriosis samples and 28 ovarian cancer macrodissected FFPE samples. Considering 1.5-fold expression difference as a cut-off level and a *P*-value <0.05, we have identified four miRNAs being overexpressed in endometrial tissue, while in ovarian cancer 15 were differentially expressed (nine overexpressed and six downregulated). The expression level was confirmed by qRT-PCR for miR-93, miR-141, miR-155, miR-429, miR-200c, miR-205 and miR-492. Using the interpretative program Ingenuity Pathway Analysis revealed several deregulated pathways due to abnormal miRNA expression in endometriosis and ovarian cancer, which in turn is responsible for pathogenesis; this differential expression of miRNAs can be exploited as a therapeutic target. A higher number of altered miRNAs were detected in endometriosis versus ovarian cancer tissue, most of them being linked with epithelial-to-mesenchymal transition.

Keywords: endometriosis, ovarian cancer, miRNA, formalin-fixed paraffin-embedded samples

Introduction

Endometriosis is presented as a benign gynecological condition that affects ~5%–10% of premenopausal women and is characterized by the presence of ectopic endometrial implants in pelvic area; however, these implants can also be extended to the abdomen and even to the central nervous system. In some cases, these implants can undergo malignant transformation and can lead to a condition called endometriosis-associated ovarian cancer (EAOC).[1] This progression is now supported by epidemiological studies and some molecular data; the development of cancer from endometriosis is a slowly progressing process that is difficult to be revealed by clinical evidences.[1,2] A recent study affirms that ~2.5% of women diagnosed with ovarian endometriosis undergo

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malignant conversion, but this percentage can be seriously underestimated due to unspecific molecular processes that intervene in the transit from benign to malignant status.[3] It is consequently anticipated to be a multifaceted process, based on the characteristic features of the disease, where coding and noncoding transcripts play an important role.[4]

The molecular characteristics of this carcinogenic event are still poorly understood, although there are a number of genes and molecular signaling pathways that are currently associated with the possible transition from endometriosis to ovarian cancer being regulated by an altered pattern of key for tumor suppressor genes or oncogenes,[5,6] microsatellite instability and noncoding genes, especially miRNA alteration pattern.

Ovarian cancer is the cause of greatest number of cancer-related deaths worldwide according to the latest statistical data, with high mortality rate due to the asymptomatic profile in early stages and nonsensitive diagnostic methods.[7–9] Ovarian cancer is frequently diagnosed in final stages when massive ascites and peritoneal spreading has already occurred, a stage that usually is nonresponsive to classic but to aggressive treatment methods,[10] and the survival rate remains low at 30%.[8,11] In this regard, there is an urgent need not only for more sensitive and precise diagnostic methods but also for methods that are able to discriminate this type of malignant pathology in the early stages of development, or more preferably, before the installation of this asymptomatic disease, in the form of prevention biomarkers.[9,12,13] The recently discovered miRNAs may lead to early diagnostic and therapeutic approaches, due to their gene expression regulation ability of the genes that play crucial roles in the installation and development of ovarian cancer, being actively involved in the etiology of this pathology.[14]

Ovarian epithelial tumors are classified as: type I tumors defined by a slow growth (comprising low-grade serous carcinoma, mucinous carcinoma, endometrioid carcinoma and clear-cell carcinomas) and type II tumors defined as a more aggressive form (including high-grade serous carcinoma, mixed mesodermal tumors and undifferentiated tumors).[15] The endometriomas or ovarian endometriosis cysts are retrieved in ~17%–44% of patients diagnosed with endometriosis, which are considered as the common precursor lesions to ovarian cancer. EAOC is especially noticeable and retrieved as endometrioid and clear cell ovarian tumor.[1]

Another important concern in ovarian cancer biology consists in a low amount of information about the primary site of origin, due to the absence of glandular epithelia in the ovary which acts like a direct precedent for cancer.[5] The most suitable candidate for the primary source of ovarian cancer nowadays is considered to be the ovarian surface epithelium, tissue formed of a monolayer of cells that covers the exterior of the ovaries.[5,10] However, this covering tissue is not the only origin for this malignant disease. Increased evidences show that endometriosis can be related to benign-malignant transition to ovarian cancer, particularly in the case of endometrioid and clear cell adenocarcinoma of the ovary (also named in the literature as EAOC); however, these conditions present discrete features which are difficult to be identifed.[16,17] The malignant transformation of endometriosis into ovarian cancer, particularly in endometrioid, clear cell and low-grade serous histotypes, is related to genetic and nongenetic factors or a combination of these factors.[15]

An increased number of studies incriminate miRNA in pathological status. miRNAs are presented today as key regulators involved in numerous processes in mammals and other multicellular organisms;[18–20] they control biological pathways involved in proliferation, apoptosis, migration, cell cycle control, differentiation and processes that under aberrant functioning can lead to pathological status, including malignant transformation; hence, there is an increasing interest to use these short transcripts as biomarkers for prevention,[21] detection and prognostic application and also as therapeutic targets.[20,22]

Formalin-fixed paraffin-embedded (FFPE) tissue is a key source of biological material for retrospective analysis, evaluation of molecular profiling and developing novel biomarkers for personalized health care.[23,24] Taking into consideration the crucial role of miRNA transcripts in the regulation of numerous signaling pathways,[19,20,25,26] there is a possibility to associate the expression of miRNAs in endometriosis and ovarian cancer samples with the risk for malignant transformation.[21,27,28] Identification of a specific panel of miRNAs that are differentially expressed in these diseases, in particular among these two pathologies, can enable the discovery of precise biomarkers for diagnostic and prognostic[21,25,28] purpose and in particular for assessing the risk involved in the transition from benign to malignant status.

Materials and methods

Sampling procedures

FFPE samples were collected from patients diagnosed at the Institute of Oncology "Prof Dr Ion Chiricuta" in Cluj-Napoca, Romania, with endometriosis and ovarian cancer between the years 2007 and 2014. Approval for the study was obtained from the ethical committee of the same institution (no 72/26). All the procedures for sample selection and processing were done in agreement with the Romanian and international legislation. All the clinical data obtained from the Pathology

Department were used anonymously, being in agreement with ethical and legal requirements. This study is in agreement with the institutional policy that imposes a signed informed consent, mentioning that the data can be used for additional future research focused on molecular profiling.

The patient cohort was selected based on clinical data prior to PCR-array or quantitative real-time PCR (qRT-PCR) evaluation. The control patient cohort was selected from a group of patients who had undergone benign surgical resection for uterine pathologies. PCR-array patient cohorts included two cases of endometrial polyps, one uterine fibroid and one leiomyoma. qRT-PCR control group included surgically resected tissue from three cases of ovarian or uterine fibroid, five cases of leiomyoma, two cases of salpingitis and four cases of benign endometrial polyps. The endometriosis patient cohort included cases with left/right adnexal endometriosis and bilateral endometriosis but showing no signs of malignity. The endometroid tissue represents over 90%.

Then the histopathological diagnosis was confirmed. The selection between endometriosis lesions and tumor area was done based on hematoxylin and eosin staining and macrodissected regions from serial unstained FFPE sections. All the cases were pathologically reviewed and the regions were isolated separately based on the tissue type. For ovarian cancer, only the endometriod carcinoma cases (Table S1) with hyperplasia more than 80% were selected, using four slides with macrodissected tissue.

For extraction, we used an average of eight 10 µm FFPE sections from the tissue block for miRNA profiling and for qRT-PCR data validation. None of the selected patients included in this study had undergone preoperative chemotherapy or radiation therapy.

miRNA isolation from FFPE samples

Total RNA was extracted from FFPE tissue using miRCURY RNA Isolation Kit–FFPE (Exicon cat no 300115) based on the instructions furnished by the manufacturer's protocol. Then the quantity of total RNA was evaluated using NanodropND-2000.

PCR array analysis

The cDNA synthesis was done using 100 ng of total RNA extracted from FFPE tissue, based on the recommended protocol, using the miScript HiSpec Buffer in a volume of 20 µL and the following program: 37°C for 60 minutes and then at 95°C for 5 minutes. The cDNA was then diluted and used for PCR array based on a SybrGreen protocol and Human Ovarian Cancer miScript miRNA PCR array plate (MIHS-110Z; Qiagen NV, Venlo, the Netherlands) following the protocol recommended by the manufacturer. These arrays contain probes for 84 miRNAs whose expression is known or expected to be altered in ovarian cancer, three wells for miRNA reverse transcription control assay and a positive PCR control. The qRT-PCR reaction was performed in Roche LightCycler480 instrument.

qRT-PCR measurements

For the validation of the most relevant altered miRNAs, we selected a subset of 17 normal samples, 33 endometriosis samples and 28 ovarian cancer samples. We used a two-step qRT-PCR. cDNA was generated using miScriptII RT Kit and then was diluted 1:5. The diluted cDNA sample was used as a template for performing qRT-PCR using QuantiTect (QuantiTect SYBR Green PCR kit, Qiagen) with specific miScript Primer (Qiagen). qRT-PCR assays were performed on ViiA 7 Real Time PCR System using the recommended amplification protocol (45 cycles of 30 seconds at 95°C, 30 seconds at 55°C and 30 seconds at 72°C) and a melting curve step.

Data analysis

The analysis of miRNA relative expression was done using the data analysis portal furnished by Qiagen, USA (http://sabiosciences.com/pcr/arrayanalysis.php). For the normalization of data, we used the average Ct value of the cel-miR-39, SNORD68, SNORD95, SNORD96A and RNU6-6P. The quantification for determining the relative expression level of miRNAs was done in endometriosis (nine samples) versus normal tissue (five samples) and ovarian cancer tissue (eight samples).

The altered miRNA pattern in endometriosis versus control, in ovarian cancer versus normal tissue and in ovarian cancer versus endometriosis was analyzed using Ingenuity Pathways Analysis (www.ingenity.com) for biological significance identification.

The qRT-PCR data analysis was done using ΔΔCt method, as previously described by Berindan-Neagoe et al,[29] statistical analysis was done using Graphpad Software (https://www.graphpad.com), the ROC (receiving-operating characteristic) curve was used for evaluating the AUC (area under the curve) offering information for the sensitivity of evaluated miRNA transcript.

Results

Evaluation of miRNA relative expression in endometriosis and ovarian cancer

This retrospective molecular profiling study was conducted on nine samples of endometriosis tissue, eight samples of ovarian cancer tissue and five samples of normal ovarian tissue using macrodissected specimens. The average age

of endometriosis patients was 40.3±5.57 years, for ovarian cancer patients was 59.33±10.36 years and for control group patients was 60±4.48 years.

First step in this study was to perform a comparative analysis of miRNA profile based on ΔΔCt method in endometriosis versus normal tissue, in ovarian cancer tissue versus normal tissue and then in endometriosis versus ovarian cancer tissue. Table 1 summarizes the differentially expressed miRNAs in endometriosis tissue versus normal tissue, ovarian cancer tissue versus normal tissue and ovarian cancer tissue versus endometriosis tissue. Of considerable interest were the miRNAs that had 1.5-fold expression difference as a cut-off level and a *P*-value of <0.05. Four miRNAs were identified to be overexpressed in endometriosis tissue, and in ovarian cancer tissue, 15 miRNAs were differentially expressed (nine overexpressed and six downregulated). A higher number of altered miRNAs (14 overexpressed and 23 downregulated) were observed when endometriosis versus ovarian cancer tissue was analyzed. Figure 1 presents Venn diagram for the main miRNAs expressed across the analyzed subgroups.

The present study had identified a particular molecular signature based on the main oncogenic miRNAs overexpressed in endometriosis and ovarian pathology (Figure S1), and also we were able to identify an miRNA panel that was able to discriminate between ovarian cancer and endometriosis.

The differential expression of miRNA proved to be statistically significant, when analyzed using interpretative program Ingenuity Pathway Analysis (IPA), leading to summarization of their biological significance and to defining the molecular interaction between altered miRNAs and their related target gene (Table 2). The results are displayed as networks (Figures 2 and 3) and classified in terms of disease and cellular and molecular functions of the altered miRNAs (Table 3).

qRT-PCR data validation

qRT-PCR data validation comprises 17 control samples with an average age of 48.53±9.14 years, 33 endometriosis patients of 41.68±8.78 years, and 33 ovarian cancer patients of 55.75±10.98 years.

miR-93 was proved to be overexpressed in endometriosis and ovarian cancer patients, the expression level being much higher in ovarian cancer compared to endometriosis. Fold change (FC) for endometriosis was 2.42±1.63 and for ovarian cancer was 26.19±30.59, the results being statistically significant in agreement with the PCR-array data (Figure 4). ROC curves for miR-92 reveal a value of AUC for endometriosis group of 0.69 and for ovarian cancer patients of 0.82.

Table 1 Differentially expressed microRNAs considering as cut-off value a fold change ≤1.5 or ≥1.5 and *P*-value <0.05

Analysis	miRNA	FC	*P*-value
Endometriosis tissue versus normal tissue	miR-325	1.83	0.032
	miR-492	2.05	0.012
	miR-520e	1.55	0.028
	miR-203a-3p	−1.63	0.043
Ovarian cancer tissue versus normal tissue	miR-141-3p	26.54	0.027
	miR-182-5p	37.81	0.030
	miR-200a-3p	31.66	0.026
	miR-200b-3p	24.30	0.012
	miR-200c-3p	18.41	0.0002
	miR-325	2.54	0.014
	miR-429	22.40	0.048
	miR-492	5.63	0.028
	miR-93-5p	5.27	0.028
	let-7a-5p	−2.22	0.017
	let-7b-5p	−3.33	0.001
	let-7c-5p	−4.49	0.001
	miR-145-5p	−3.40	0.025
	miR-152-3p	−2.14	0.020
	miR-214-3p	−5.04	0.001
Ovarian cancer tissue versus endometriosis tissue	miR-103a-3p	2.47	0.002
	miR-106b-5p	4.95	0.002
	miR-141-3p	154.83	0.001
	miR-155-5p	1.76	0.045
	miR-15a-5p	4.89	0.004
	miR-16-5p	3.21	0.035
	miR-182-5p	27.58	0.0041
	miR-200a-3p	114.36	0.0025
	miR-200b-3p	67.07	0.0006
	miR-200c-3p	50.65	0.000001
	miR-205-5p	39.21	0.029
	miR-27a-3p	2.25	0.001
	miR-30a-5p	2.66	0.020
	miR-30e-5p	2.58	0.022
	miR-335-5p	6.95	0.009
	miR-346	2.33	0.045
	miR-370-3p	2.73	0.011
	miR-429	43.26	0.007
	miR-492	2.73	0.016
	miR-507	6.33	0.035
	miR-514a-3p	6.16	0.021
	miR-637	1.67	0.028
	miR-93-5p	7.01	0.001
	let-7a-5p	−2.38	0.0001
	let-7b-5p	−3.98	0.009
	let-7d-5p	−1.67	0.015
	miR-1-3p	−8.31	0.023
	miR-125b-5p	−3.41	0.002
	miR-134-5p	−1.85	0.023
	miR-143-3p	−2.51	0.007
	miR-145-5p	−3.79	0.00008
	miR-152-3p	−1.77	0.027
	miR-154-5p	−1.84	0.016
	miR-199b-3p	−2.98	0.043
	miR-199a-5p	−2.66	0.002
	miR-214-3p	−5.66	0.008
	miR-432-5p	−1.68	0.020

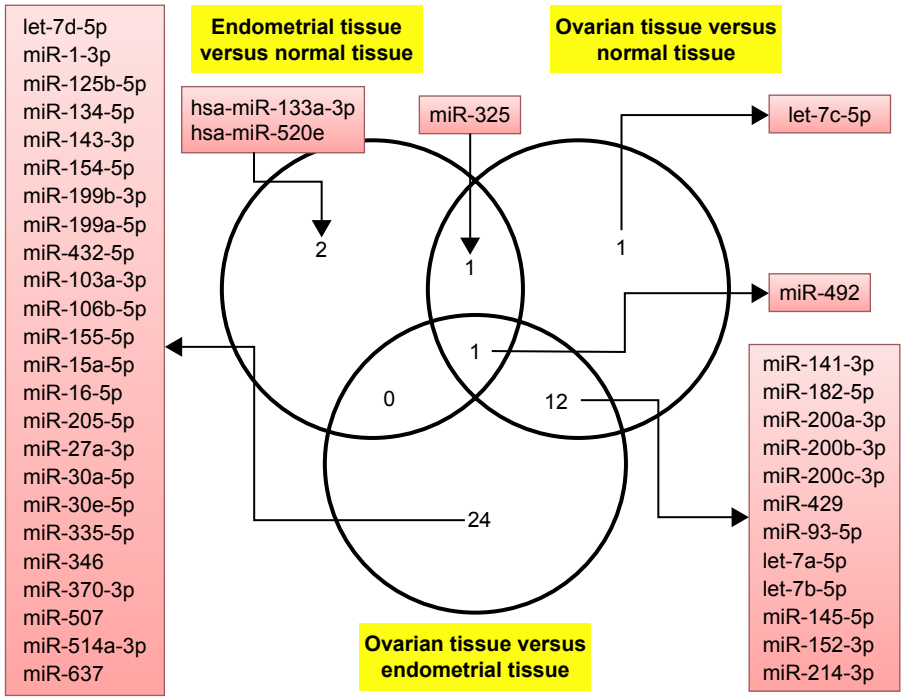

Figure 1 Venn diagram presenting the altered expression levels of common and differentially expressed miRNAs in the analyzed subgroups.

miR-200c was found to be overexpressed in ovarian cancer samples when they were analyzed versus control or versus endometriosis samples. The expression level for miR-200c in normal tissue was 1.63±1.53, in endometriosis 1.27±2.91 and in ovarian cancer 12.29±14.61. AUC values for miR-200c was 0.69 for endometriosis and 0.84 for ovarian cancer, displaying a good sensitivity and specificity for ovarian cancer. Similar expression level was observed for the other two representatives of the same family, namely miR-141 and miR-429, which have an expression level in endometriosis of 8.035±25.09 and 16.17±47.13 and in ovarian cancer of 203.1±275.6 and 104.6±172.6, respectively. A reduced sensitivity was observed for miR-141, whereas highest AUC values were obtained for miR-492 in both the analyses.

Table 2 IPA analysis based on altered miRNA pattern in the analyzed subgroups, focusing on diseases

	Disorder	*P*-value	Number of molecules
Endometriosis versus normal tissue	Inflammatory response	2.35E-02–1.02E-03	2
	Cancer	3.15E-02–4.24E-03	3
	Organismal injury and abnormalities	3.56E-02–4.24E-03	3
Ovarian cancer versus normal tissue	Cancer	4.60E-02–3.76E-11	11
	Organismal injury and abnormalities	4.70E-02–3.76E-11	11
	Infectious diseases	6.38E-03–1.04E-10	4
	Reproductive system disease	3.88E-02–1.19E-10	10
	Endocrine system disorders	2.53E-02–2.11E-07	8
Ovarian cancer versus normal endometriosis	Cancer	4.60E-02–3.76E-11	11
	Organismal injury and abnormalities	4.70E-02–3.76E-11	11
	Infectious diseases	6.38E-03–1.04E-10	4
	Reproductive system disease	3.88E-02–1.19E-10	10
	Endocrine system disorders	2.53E-02–2.11E-07	8

Abbreviation: IPA, interpretative phenomenological analysis.

An increased level of miR-155 expression was observed in endometriosis and ovarian cancer tissues, but this difference was not statistically significant. For the endometriosis versus control group, FC was 1.416±0.7981 (*P*-value =0.0507) and for ovarian cancer group, FC was 2.703±2.830 (*P*-value =0.0552). miR-205 expression level was 7.235±15.49 (AUC =0.69) in endometriosis and 390.2±466.3 (AUC=0.79) in ovarian cancer. miR-492 was overexpressed in both pathologies, with a fold change of 5.40±6.48 for endometriosis and 10.17±10.29 for ovarian cancer. The AUC values for miR-492 revealed a good sensitivity for both pathologies.

A good level of reproducibility was observed among the two patient cohorts used for PCR-array evaluation and qRT-PCR, with an exception for miR-155 which showed the same expression level but the *P*-value was not statistically significant, emphasizing the potential prognostic significance for the validated miRNA.

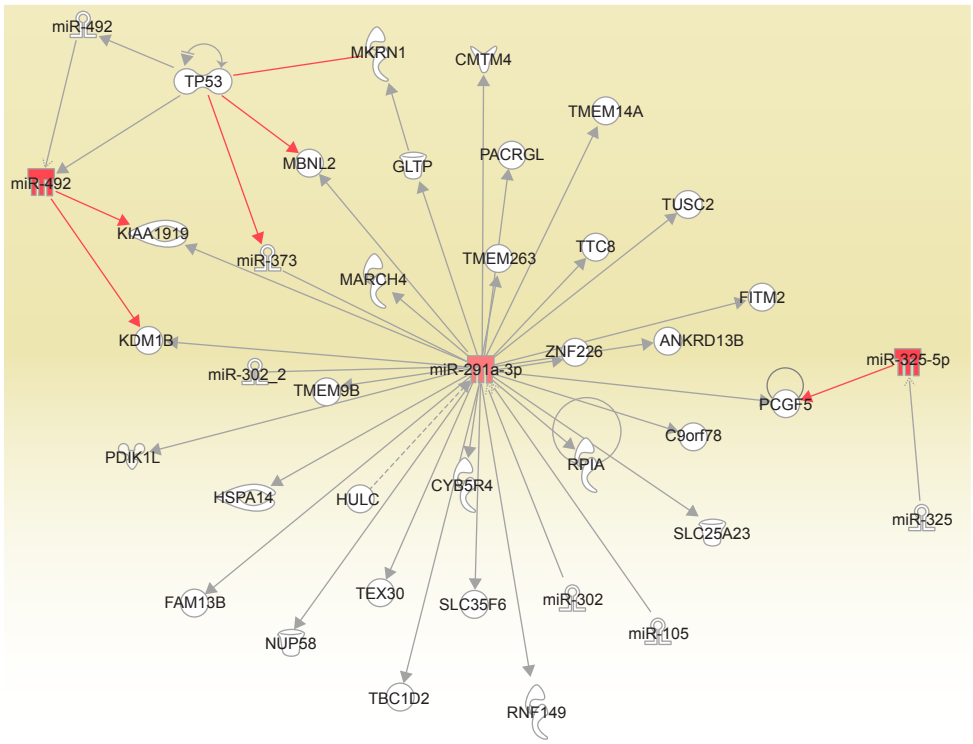

Figure 2 Network generated for the altered miRNAs in the analysis of endometriosis versus normal tissue. The upregulated miRNAs are displayed in red.

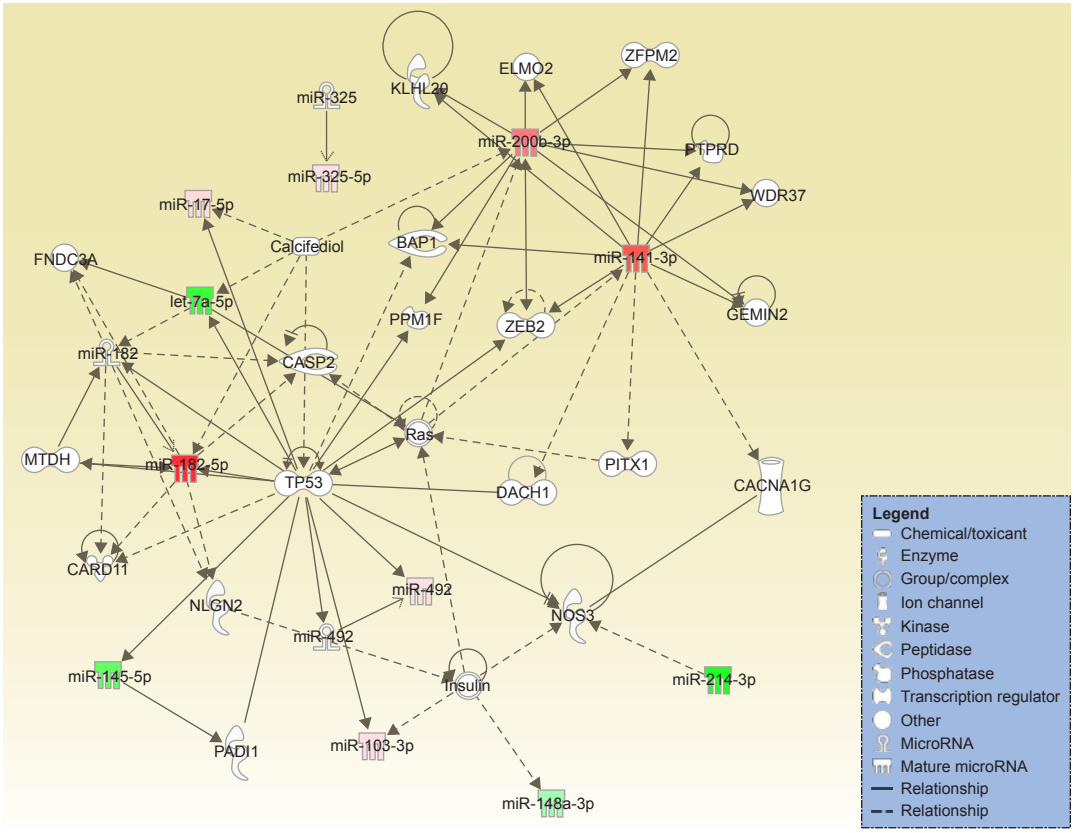

Figure 3 Network generated for the altered miRNAs in the analysis of ovarian cancer versus control. The upregulated miRNAs are displayed in red and the downregulated miRNAs in green.

Table 3 IPA analysis based on altered miRNA pattern in the analyzed subgroups, focusing on molecular and cellular functions

	Disorder	*P*-value	Number of molecules
Endometriosis versus normal tissue	Cell-to-cell signaling and interaction	2.35E-02–1.89E-03	1
	Cell cycle	3.56E-02–4.07E-03	1
	Cellular movement	4.07E-03–4.07E-03	1
Ovarian cancer versus normal tissue	Cellular movement	1.48E-02–1.46E-05	6
	Cellular development	4.65E-02–2.34E-05	7
	Cellular growth and proliferation	4.65E-02–1.08E-04	7
	Cell cycle	1.94E-02–1.27E-04	4
	Cell death and survival	3.26E-02–5.78E-04	6
Ovarian cancer versus normal endometriosis	Cellular movement	1.48E-02–1.46E-05	6
	Cellular development	4.65E-02–2.34E-05	7
	Cellular growth and proliferation	4.65E-02–1.08E-04	7
	Cell cycle	1.94E-02–1.27E-04	4
	Cell death and survival	3.26E-02–5.78E-04	6

Abbreviation: IPA, interpretative phenomenological analysis.

Discussion

In this study, we have demonstrated that miRNAs are aberrantly expressed in endometriosis versus normal tissue and ovarian cancer versus normal tissue, and we were also able to identify a panel of miRNAs that are differentially expressed in ovarian cancer versus endometriosis tissue, the details of which can be observed in Figures 1 and S1. Thus, miRNAs can be used as prognostic and diagnostic markers, emphasizing the functional differences that are able to regulate the key processes. The global miRNA expression pattern might undoubtedly discriminate endometriosis from normal tissue, ovarian cancer from normal tissue and also ovarian cancer from endometriosis tissue. The analysis has detected important number of miRNAs with an altered expression pattern being involved in malignant transformation. For qRT-PCR validated miRNAs, good sensitivity and specificity can be observed from ROC curves, in particular for ovarian cancer pathology. These panels of miRNAs can be taken into

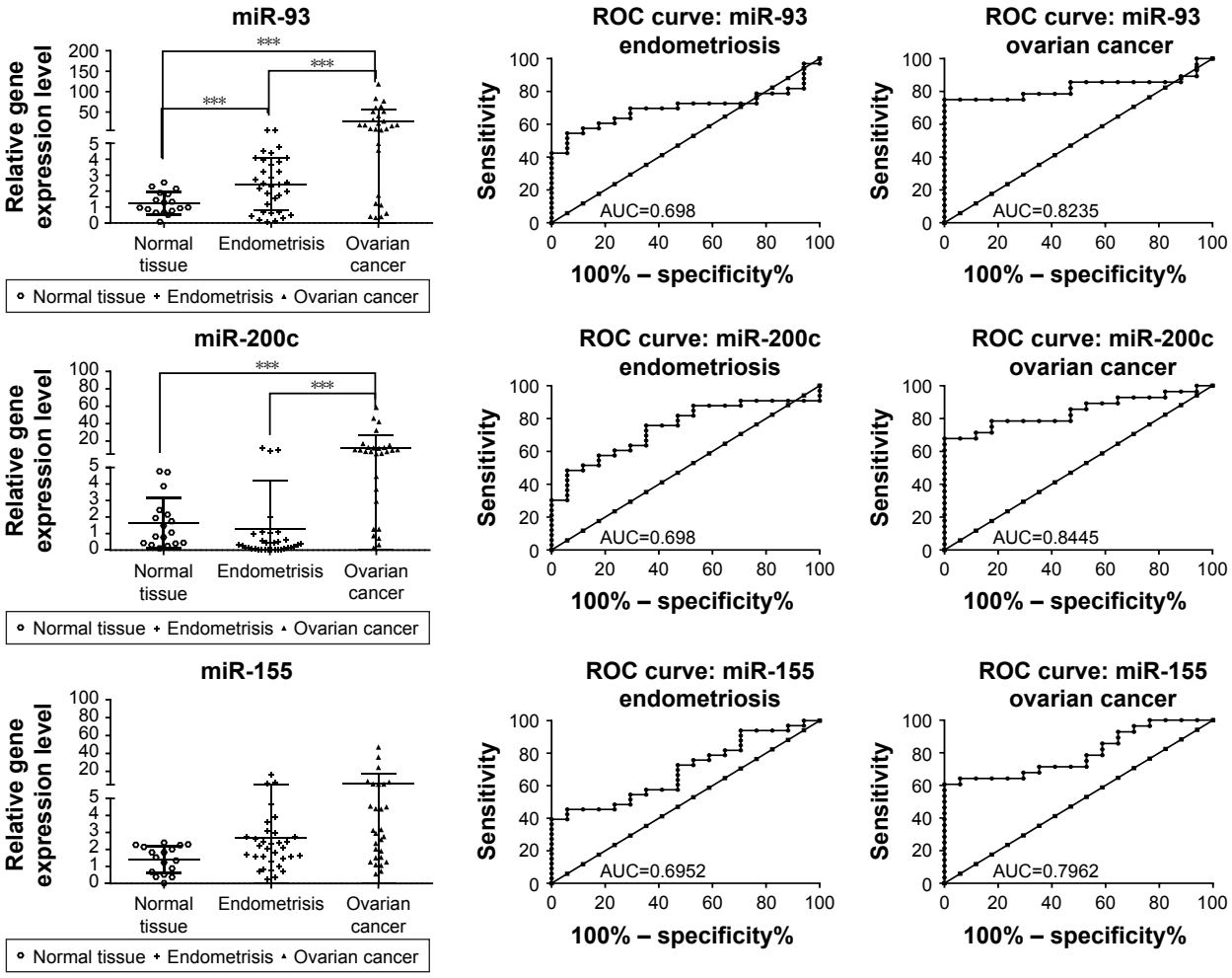

Figure 4 (*Continued*)

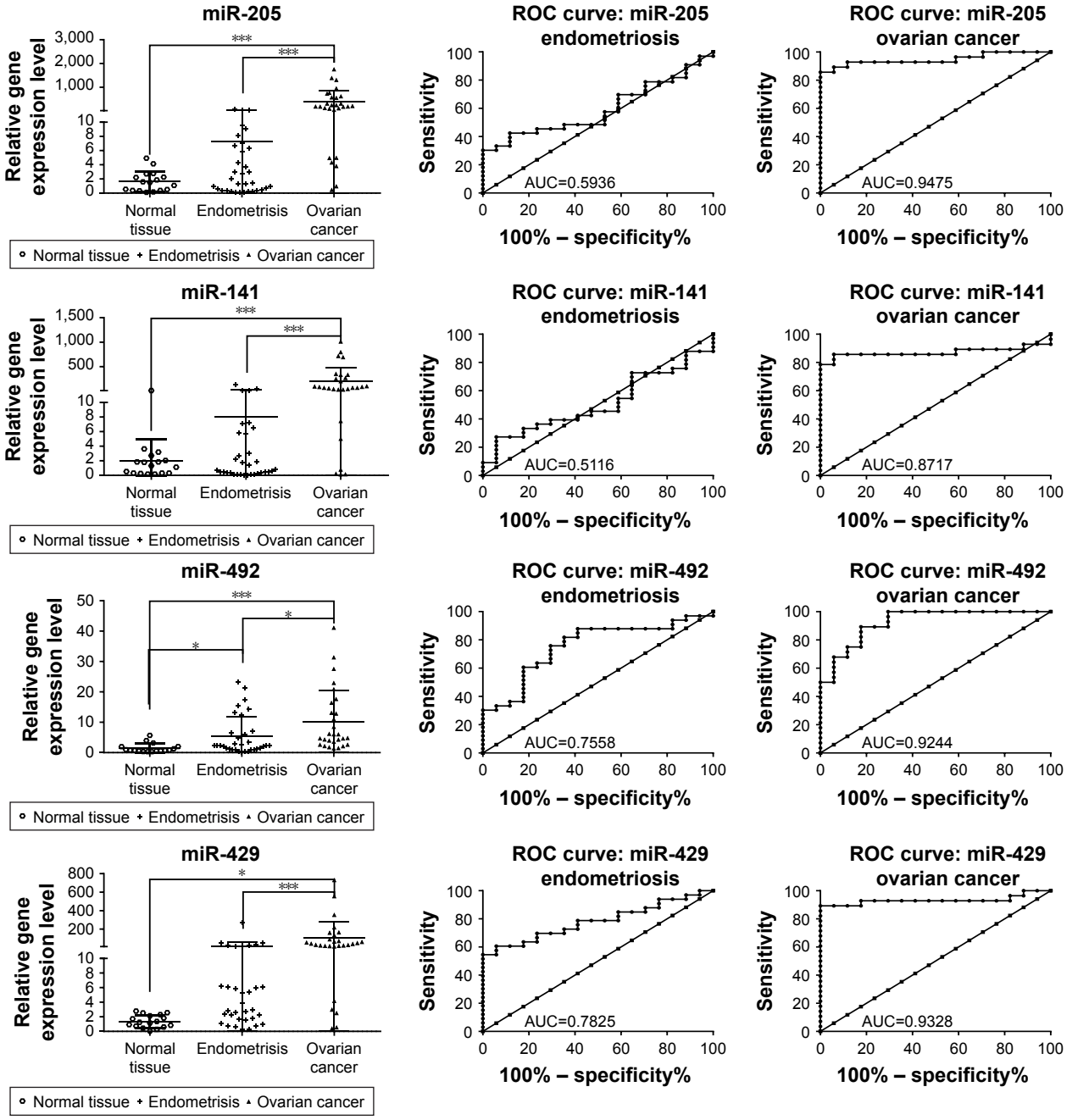

Figure 4 qRT-PCR validation in endometriosis and ovarian cancer patient cohort for miR-93, miR-141, miR-155, miR-429, miR-200c, miR-205 and miR-492. ROC curve analysis of expression levels of miRNAs for endometriosis and ovarian cancer group. The figure displays AUC for each evaluated miRNA, a parameter that indicates the precision in discriminating the endometrial tissue and ovarian cancer tissue from the normal tissue. *$P<0.05$; ***$P<0.001$.
Abbreviations: AUC, area under the curve; qRT-PCR, quantitative real-time PCR; ROC, receiver operating characteristic.

consideration for possible use as prognostic or diagnostic markers, especially for endometriosis patients undergoing surgical procedures.

One of the identified transcripts that was found to be over-expressed in endometriosis and ovarian cancer is miR-325, a transcript whose overexpression was confirmed even when comparing the degree of expression between the two pathologies using endometriosis samples as control group. The increased expression of miR-325 in ovarian cancer against endometriosis suggests a possible application for its use as a prognostic marker in regard to a possible transition from endometriosis to malignant pathologies. miR-325 has the potential of a tumor suppressor which is reflected by the promotion of tumor progression, increased angiogenesis, invasion and metastasis.[30] There is a lack of specific data regarding miR-325 expression in the context of ovarian

cancer or endometriosis, but there are several studies reporting its role in other malignant pathologies.[31,32] This way, the degree of expression of miR-325 can be utilized as a clinical parameter for detecting ovarian cancer invasion,[31] whose expression is inhibited in these tumors. miR-203, an inflammatory transcript,[33] was observed to be downregulated in the endometriosis group.

Regarding the expression of miR-200 family, our study has highlighted a significant overexpression value for miR-200a, miR-200b, miR-200c, miR-429, miR-141 and members of the ovarian tumor samples, these members topping the list for positive aberrant expression. Of the five members, miR-200a, miR-200b and miR-200c are currently proposed for use as biomarkers in ovarian cancer.[34–38] The ROC data being in agreement with our study[34] encourage their application in clinical practice. Furthermore, the expression level of miR-200a and miR-200c has been identified to be associated with the degree of progress for ovarian pathology, whereas the upregulation of miR-200a was found to be correlated with the histology and tumor stage; patients with metastasis in lymph nodes presented a significant elevation of mirR-200c.[38] Also, miR-200b has been characterized as a possible therapeutic target related with drug resistance.[35] With regard to the expression of miR-141, an increased resistance for cisplatin was found to be proportional to the grade of positive aberrancy of this transcript.[39] Members of the miR-200 family (miR-141 and miR-200a) have also been identified in the endometriosis samples but with an expression opposite to that found in ovarian cancer.[40] These two transcripts are currently proposed as potential noninvasive biomarkers, their expression from tissue samples being correlated with the values from blood samples.[41] Given the role of miR-200 family members in numerous biologic and pathologic processes, especially in epithelial–mesenchymal transition (EMT), we can assume that these transcripts may be involved in the transition from benign endometriosis to malignant pathologies such as ovarian cancer.

Another overexpressed transcript found with an altered expression level in endometriosis samples is miR-520e. This transcript has been studied in hepatocarcinoma[42] and breast cancer,[43] where its role as a tumor suppressor has been proven.[44] This feature places the transcript in a list of potential therapeutic elements. Given the similarity of endometriosis and malignant pathologies in terms of high levels of cell proliferation and also the presence of miR-520 in samples from endometriosis patients, there is a possibility of using this transcript as a therapeutic target for inhibiting cellular expansion.

miR-492 has been identified as overexpressed in both pathologies in the present study, with a more noticeable level in ovarian cancer; however, information on its exact role in progression of endometriosis and ovarian cancer is absent in literatures. miR-492 promotes the progression of hepatic cancer by targeting the *PTEN* gene and increasing the level of AKT activation in cancer cells, thus having an oncogenic role.[45] Patients with an overexpression of miR-492 and an inhibition of the *PTEN* gene show a lower rate of survival,[46] thus sustaining metastatic features.[47] Considering the different expression levels of miR-492 in endometriosis and ovarian cancers, with a higher level in the latter, follow-up studies could confirm the quality of miR-492 as a prognostic biomarker in terms of transition from benign to malignant; given its role in abnormal cellular proliferation, this transcript could be placed on the list of potential therapeutic targets for inhibiting aberrant cell growth.

miR-182 was proved to be overexpressed in ovarian cancer, sustaining the present findings. This transcript is presented as a prognostic marker for ovarian cancer,[48] as it plays a role in malignant transformation by sustaining cell proliferation, invasion and migration processes, as was emphasized by Liu et al.[49,50]

The let-7 family is composed of 13 distinct members found on nine different chromosomes.[51] Of these 13 members, our study has identified three of them (let-7a, let-7b and let-7c) as being overexpressed in ovarian cancer samples.[52] Because of their inhibiting activity on oncogenes associated with ovarian cancer, such as KRAS,[52] HRAS,[53,54] c-MYC[54] and HMGA-2,[55] downregulation of the let-7 family members correlates with advanced tumor stages and a lower survival rate.[56] Together with the miR-200 family, the let-7 group represents an important potential prognostic and diagnostic biomarker for ovarian cancer as well as a therapeutic element involved in the regulation of key oncogenes.[54] An interesting study on miR-30a reported this to be related to decidualization and to endometriosis tissue transformation,[57] sustaining our finding related to malignant transformation and therapeutic target for ovarian serous adenocarcinoma.[58] miR-30a was found to have an oncogenic role and to modulate BCL2A1, IER3 and cyclin D2 expression via FOXL2,[59] the expression of which is related to drug resistance mechanisms.[60]

miR-145 was found to be downregulated in ovarian cancer and thus has a prognostic role.[61] A functional study on ovarian cancer cell lines reveals that treatment with agomiR-145 caused inhibition of TRIM2 and leads to the inhibition of Bim, a proapoptotic Bcl-2 family member.[62] miR-145 is related to ovarian cancer chemoresistance by regulation of

cell cycle-related proteins: Cdk6 and Sp1.[61] This transcript was not found to be statistically significant in endometriosis group but was downregulated in ovarian cancer versus control or ovarian cancer versus endometriosis analysis. Therefore, miR-145 can be involved in malignant transformation, due to the fact that miR-145 was related to an increased cell proliferation, invasiveness and stem cells features in endometrioid primary cell cultures and stabilized cell lines.[63]

miR-205 is a highly controversial miRNA that was retrieved statistically significantly only in ovarian cancer versus endometriosis group. There are no data available in literature with regard to miR-205 implication in endometriosis, but in ovarian cancer, it is presented to be connected to the regulation of cell proliferation, EMT and invasion mechanism and metastasis processes.[64] miR-205 directly targets VEGF-A[65] or promotes cell motility via ZEB1,[66] which is related with the suppression of SMAD4 and PTEN, an event that favors ovarian cancer progression and[67] therefore sustaining malignant transition as showed in our data. miR-27a regulates cancer cell growth, EMT and migration in ovarian cancer.[68] miR-27a is an important transcript responsible for drug resistance by targeting MDR1/P-gp and HIPK2 in ovarian cancer cells.[69]

The EMT-related miRNA has an important role in modulation and favoring proangiogenic mechanism; therefore, this miRNA can be considered as both a prognostic and a therapeutic target in the selected pathologies. These EMT-related miRNAs are prone for activation by angiogenic mechanims[70,71] and, in particular, can influence the implantation of endometrial cells on ectopic sites.[40]

Conclusion

Our data have demonstrated that differentially expressed miRNAs might contribute significantly to regulating the functions of pathological processes sustaining cell proliferation. Endometriosis still remains an enigmatic disease, but now it is clear that miRNAs play an important role in this pathology and can be related to malignant transition. miRNAs that are related to inflammation (miR-325) and those regulating the EMT (miR-200a, miR-200b, miR-200c, miR-141, miR-429, miR-30a, miR-145 and miR-205) can contribute to malignant transformation.

The association between endometriosis and ovarian carcinoma remains debated but is sustained by the common miRNAs. At the same time, we have identified a particular miRNA signature that is capable of discriminating between endometriosis and ovarian cancer, this finding being the ground for additional functional studies needed to clarify the prospective role of miRNAs in endometriosis and its related malignancies.

This panel of identified miRNAs can enable the discovery of dysregulated miRNAs that can act as precise biomarkers for diagnostic and prognostic applications and in particular for assessing the risk involved in the transition from benign to malignant status.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Patient characteristics for PCR-array and qRT-PCR patient cohort

Pateint cohort	Case	Age (years)	Diagnostic	Differentiation stage	Pathological diagnostic
PCR array	1	58	Endometrioid carcinoma	G1	pT1aNxMxL0V0
	2	67	Endometrioid carcinoma	G2	pT2aNxM1L1V0
	3	62	Endometrioid carcinoma	G2	pT1cN0MxL1V0
	4	54	Endometrioid carcinoma	G3	pT2N1M0LoVo
	5	59	Endometrioid carcinoma	G2	pT1aNxMxLoVo
	6	65	Endometrioid carcinoma	G3	pT2cN1bMxL1Vo
	7	45	Endometrioid carcinoma	G3	pT2N1M0LoVo
	8	78	Endometrioid carcinoma	G2	pT3bNxMxLoVo
	9	46	Endometrioid carcinoma	G1	pT2aNxMxLoVo
qRT-PCR	1	49	Endometrioid carcinoma	G2	pT1aNxMxLoVo
	2	62	Endometrioid carcinoma	G3	pT1aNxMxLoVo
	3	50	Endometrioid carcinoma	G3	pT3bNxMxLoVo
	4	67	Endometrioid carcinoma	G3	pT3cN1MxL1V1
	5	51	Endometrioid carcinoma	G2	pT2bN0MxLoVo
	6	66	Endometrioid carcinoma	G3	pT1aNxMxLoVo
	7	56	Endometrioid carcinoma	G3	pT3cNxMxL1V1
	8	42	Endometrioid carcinoma	G3	pT2aN0MxLoVo
	9	54	Endometrioid carcinoma	G2	pT3cN1M1LoVo
	10	51	Endometrioid carcinoma	G3	pT1aNxMxLoVo
	11	64	Endometrioid carcinoma	G1	pT1aNxMxLoVo
	12	45	Endometrioid carcinoma	G1	pT1cNxMxLoVo
	13	60	Endometrioid carcinoma	G2	pT1aNxMxLoVo
	14	66	Endometrioid carcinoma	G2	pT2aN1MxLoVo
	15	34	Endometrioid carcinoma	G2	pT2bN0MxLoVo
	16	41	Endometrioid carcinoma	G2	pT3cN1MxL1Vo
	17	63	Endometrioid carcinoma	G1	pT2aN1MxLoVo
	18	62	Endometrioid carcinoma	G3	pT2bN0MxLoVo
	19	44	Endometrioid carcinoma	G3	pT1bNxMxLoVo
	20	48	Endometrioid carcinoma	G1	pT2bN0MxLoVo
	21	66	Endometrioid carcinoma	G2	pT2bN0MxLoVo
	22	64	Endometrioid carcinoma	G1	pT1aNxMxLoVo
	23	76	Endometrioid carcinoma	G2	pT1aNxMxLoVo
	24	53	Endometrioid carcinoma	G1	pT1aNxMxLoVo
	25	68	Endometrioid carcinoma	G1	pT1aNxMxLoVo
	26	73	Endometrioid carcinoma	G3	p3bN0MxLoVo
	27	46	Endometrioid carcinoma	G1	pT1aNxMxLoVo
	28	40	Endometrioid carcinoma	G2	pT2cNoMxLoVo

Abbreviation: qRT-PCR, quantitative real-time PCR.

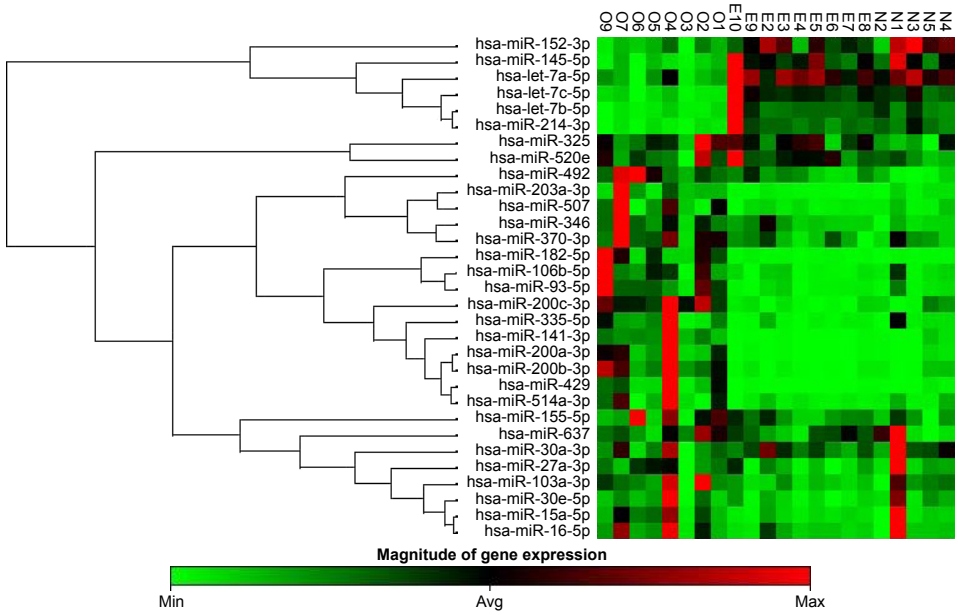

Figure S1 Cluster analysis of endometriosis, ovarian cancer and normal tissues (statistically significant miRNAs were selected).
Abbreviations: E, endometriosis tissue; N, normal tissue; O, ovarian cancer tissue.

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