Expression of miR-146a in patients with ovarian cancer and its clinical significance

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Abstract. The aim of the present retrospective study was to compare microRNA (miR)-146a expression levels in primary tumors and omental metastases of 48 patients, who had undergone surgery for advanced ovarian serous cancer. Possible correlations between miR-146a expression level and clinicopathological features were investigated, including chemosensitivity and survival. miR-146a was evaluated in formalin-fixed, paraffin-embedded samples. miR-146a expression level in primary tumors was demonstrated to be increased in comparison with normal ovary tissues (P=0.02) and metastases (P=0.01). A negative correlation was demonstrated between miR-146a expression in primary tumors and serum levels of cancer antigen 125 (R=-0.37; P=0.03) and Risk of Malignancy Algorithm index (R=-0.79; P=0.0007). Overall survival positively correlated with miR-146a expression in primary tumor tissue samples (R=0.38; P=0.01). Probability of survival was decreased in patients with low miR-146a expression levels in primary tumor tissues (hazard ratio=0.21; P=0.003). Lower levels of miR-146a in primary tumor tissue samples were correlated with a shorter progression-free survival (P=0.04) and platinum-resistance of metastases (P=0.006). In conclusion, miR-146a may be a prognostic marker for serous ovarian cancer.

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Abbreviations: Tregs, T regulatory cells; NFκB, nuclear factor-κB; RANTES, regulated on activation, normal T-cell expressed and secreted; CXCR4-C-X-C; chemokine receptor type 4; FIGO, International Federation of Gynecology and Obstetrics; CA125, cancer antigen 125; HE4, serum human epidydimis antigen-4; FFPE, formalin-fixed, paraffin-embedded; PFS, progression-free survival; OS, overall survival; AUC, area under the curve

Key words: microRNA-146a, ovarian cancer, cancer antigen 125, risk of malignancy algorithm, survival, chemoresistance

Introduction

Ovarian cancer is one of the most fatal types of female neoplasms. Despite the availability of extensive management of the disease, the number of patients who survive ≥5 years following diagnosis remains low (1). Ultra-radical surgery, platinum- and taxane-based chemotherapy and immunotherapy are all improvements. However, the impact of these treatments on overall survival (OS) remains unsatisfactory (2). One of the main concerns is the low efficacy of chemotherapy due to the primary tumor platinum-refractoriness or acquired chemoresistance during the course of adjuvant treatment (3). It is not possible to predict which patients, subject to standard chemotherapeutic regimen, would respond to the therapy. Inefficient response to the treatment leads to tumor progression or recurrence. Therefore, identification of markers for potentially chemoinsensitive tumors may aid in modifying and individualizing treatments prior to the recognition of chemorefractoriness or chemoresistance. MicroRNAs (miRNA/miR), small non-coding RNAs involved in post-transcriptional gene regulation, may be candidates for potential markers (4).

miR-146a has been described as a modulator of differentiation and function of innate and adaptive immunity. In human T cells, miR-146a is expressed abundantly in memory T cells, and its expression is critical for function of T regulatory cells (Tregs). miR-146a was also demonstrated to upregulate the macrophage inflammatory response (5,6). The molecular function of miR-146a in the immune response involves negative regulation of the signal transduction pathway, which leads to activation of nuclear factor-κB (NFκB), disruption of downstream T lymphocyte receptor-4 signaling pathway, and modulation of chemokine interleukin-8, RANTES (regulated on activation, normal T-cell expressed and secreted) and CXC chemokine receptor type 4 expression (6). The association between miR-146a and the immune response may have a potential impact in solid tumors.

Furthermore, it was demonstrated that miR-146a is involved in the regulation of various RNAs encoding a number of proteins involved in cell differentiation, proliferation and migration (7). Consequently, aberrant disturbed miR-146a expression level was observed in numerous types of malignancies, including thyroid, breast, gastric, prostate, pancreatic and ovarian cancer (8-10). miR-146a polymorphism may increase the risk of developing various types of cancer. For example, the G/C polymorphism in the pre-miR-146a sequence was associated with a decrease or an increase of miRNA-146a expression levels, depending on the cancer type. The change in miRNA-146a expression modified the risk of papillary thyroid, hepatocellular, gastric cancer and glioma (6,8,9). The G/C polymorphism (SNP no. rs2910164) may induce the onset of breast and ovarian cancers in breast cancer 1/2 (BRCA1/2) positive cases (11). The level of miR-146a expression may depend on the type of tumor and the aggressiveness of the tumor. Increased expression levels of miR-146a were reported in papillary thyroid and cervical cancer compared with normal tissues (12,13). However, in highly metastatic breast cancer cell lines, the expression of miR-146a was downregulated and exogenous miR-146a expression impaired the invasion and migratory capacity of cancer cells. Breast cancer metastasis suppressor-1, which affects multiple steps of the metastasizing process, may partially function by upregulating miR-146a expression in breast cancer cells (14). A study by Boldin *et al* investigating miR-146a-knock-out mice confirmed human studies and demonstrated that the lack of miR-146a expression favors development of hematologic neoplasms (15). Chang *et al* (16) suggested that downregulation of miR-146a may contribute to the Myc-mediated tumorigenesis. These observations strongly suggested that miR-146a may serve a role as a tumor suppressor.

Various miRs were revealed to be either upregulated or downregulated in patients with ovarian cancer (17,18). The expression of miR-30a-3p was increased in well-differentiated tumors compared with poorly differentiated tumors (19). Detection of high levels of plasma miR-205 and low Let-7f expression levels combined with high serum cancer antigen 125 (CA125) levels improved the accuracy of ovarian cancer detection (20). Let-7f was identified as a predictive factor for ovarian cancer prognosis (20). A predictive model based on the serum expression levels of miR-200b/miR-200c was able to discriminate between normal controls and age-matched patients with high-grade serous ovarian cancer (21). A number of other miRs were studied, and the levels of these miRs were correlated with the hazard ratio for patient survival or tumor recurrence (22). *In vitro* and *in vivo* studies have suggested that the pattern of miR expression may have an impact on the chemosensitivity of ovarian tumors (23-26). Vang *et al* (27) performed a study on a small group of patients with advanced ovarian serous cancer: The study revealed dysregulation of miR-146a and miR-150 in omental metastases and suggested their possible role in increased platinum tolerance (27).

The aim of this retrospective study was to compare expression levels of miR-146a in primary tumor tissues and omental metastases from patients who underwent surgery for advanced ovarian serous cancer. The second aim of the present study was to investigate an association between miR-146a expression levels and clinicopathological features, including chemosensitivity and survival.

Materials and methods

Patient collection. The present study was approved by the Ethics Committee of the Polish Mother's Memorial Hospital Research Institute (Lodz, Poland; grant no. 37/2014). Written informed consent was obtained from all patients prior to enrolment in the present study. A total of 48 patients with advanced ovarian cancer, who underwent cytoreductive abdominal surgery between March 2006 and December 2010, were included in the present study. The inclusion criteria were serous tumor histology and stage III/IV according to the International Federation of Gynecology and Obstetrics (FIGO) clinical staging system (28). Total hysterectomy with bilateral salpingo-oophorectomy, omentectomy and appendectomy was performed in all cases, supplemented with partial resection of infiltrated intestine or bowel, peritonectomy or splenectomy for the purposes of optimal cytoreduction. Systemic or sampling lymphadenectomy was performed only in cases when optimal cytoreduction was achieved or in the presence of bulky nodes. Adjuvant treatment with platinum-taxane regimen, six standard courses of carboplatin 5-7.5 area under the curve (AUC) and paclitaxel 175 mg/m^2 and modified according to the patient's general status, was introduced in all cases. Clinical information was acquired from medical records. Serum CA125 levels and Risk of Malignancy Algorithm (ROMA) index calculated based on the levels of serum CA125, serum human epidydimis antigen-4 (HE4) and pre-menopausal or menopausal status were acquired prior to cytoreductive surgery. Platinum-sensitive tumors where identified when there was no relapse ≥6 months following completion of the chemotherapy. Resistant patients were defined as patients with primary chemo-refractory tumors (progression despite treatment with a first-line chemotherapy). Platinum-resistance was also diagnosed when relapse occurred ≤6 months following completion of chemotherapy. A total of 27 patients were identified to be chemosensitive and 21 patients were recognized as chemoresistant. Clinical characterizations of chemosensitive and chemoresistant patients are presented in Table I.

Sample collection. miR-146a expression was evaluated in tissues obtained from archival formalin-fixed (tissues were fixed with 10% formalin for 24-48 h at room temperature) paraffin-embedded (FFPE) serous ovarian cancer samples. Other histological types of ovarian cancer were excluded from the present study. All archival FFPE samples were re-evaluated by an experienced pathologist. Following confirmation of the cancer type, the areas of cancerous tissues were carefully selected and micro-dissected from the samples in order to avoid areas of extensive necrosis and to minimalize the risk of contamination with noncancerous tissues. From each patient, two samples were obtained, with one sample from primary ovarian tumor and another sample from omental metastasis. The reference group consisted of 48 normal ovarian tissue samples that were retrieved from peri-menopausal women during hysterectomy with bilateral salpingo-oophorectomy

Table I. Clinicopathological characteristics and outcomes of patients in the platinum-sensitive and platinum-resistant groups.

Parameter	Platinum-sensitive	Platinum-resistant
Case number (n)	27	21
Mean age, years (range)	48 (24-81)	54 (48-75)
FIGO stage		
III (n)	23	20
IV (n)	4	1
Tumor grade[a]		
1 (n)	2	2
2 (n)	8	8
3 (n)	17	11
Recurrence		
No (n)	5	6
Yes (n)	22	15
Median PFS, months (range)	25 (12-67)	4 (0-11)
Median OS, months (range)	33 (13-70)	16 (2-113)
Survival		
No (n)	3	9
Yes (n)	24	12

PFS, progression-free survival; OS, overall survival; FIGO, International Federation of Gynecology and Obstetrics. [a](55).

due to benign uterine disease (uterine leiomyoma). All hysterectomy procedures were performed between January 2014 and December 2014. The mean age of patients was 47 years (range, 39-56 years). Surgery was performed in the Polish Mother's Memorial Hospital Research Institute and informed consent was obtained from all of them.

Total RNA isolation and miRNA expression analysis. Total RNA was extracted from FFPE tissues using the Roche High Pure miR Isolation kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. In brief, the FFPE microsamples were processed in 2 ml Eppendorf tubes, deparaffinized with 100% xylene, washed in 100% ethanol and dried at 55˚C for ~10 min. The dried tissue was resuspended in 100 μl Paraffin Tissue Lysis Buffer (included in the kit) and digested with proteinase K at 55˚C overnight. Subsequent steps of RNA purification on columns were performed according to the manufacturer's protocol (Roche Diagnostics GmbH). Briefly, 325 μl of binding buffer and 325 μl of binding enhancer was added and the mixture applied on the columns, centrifuged for 30 s at 13,000 x g and washed twice with 500 μl and 300 μl of wash buffer. An additional step of centrifugation for 1 min at 13,000 x g was performed to dry the filter fleece completely and RNA was eluted with 50 μl of Elution Buffer. The yield and quality (260/280 optical density ratios) of the RNA products were determined using a PicoDrop spectrophotometer (Picodrop Ltd., Hinxton, UK). The purified total RNA was immediately used for cDNA synthesis or stored at -80˚C until use.

Quantification of differentially expressed miRNAs. Reverse transcription was carried out using the Universal cDNA Synthesis kit (Exiqon A/S, Vedbaek, Denmark), according to the manufacturer's protocol A for individual assays. A total of 10 ng total RNA were used. The RT reaction was diluted 80 times in nuclease-free water, and 4 μl aliquots were subsequently used for PCR amplification with 5 μl ExiLENT SYBR® Green Master mix (Exiqon A/S, Vedbaek, Denmark) and 1 μl commercially available primers (Exiqon A/S, Vedbaek, Denmark): hsa-miR-146a-5p LNA™ PCR primer set (cat. no. 204688; target sequence, UAGCAGCACAUAAUGGUUUGUG); SNORD48 (hsa) PCR primer set (cat. no. 203903; target sequence,AGUGAUGAUGACCCCAGGUAACUCUGAGUGUGUCGCUGAUGCCAUCACCGCAGCGCUCUGACC); and U6 snRNA (hsa, mmu, rno) PCR primer set (cat. no 203907; target sequence,GUGCUCGCUUCGGCAGCACAUAUACUAAAAUUGGAACGAUACAGAGAAGAUUAGCAUGGCCCCUGCGCAAGGAUGACACGCAAAUUCGUGAAGCGUUCCAUAUUUUU). U6 small nuclear RNA and small nuclear RNA, C/D box 48 were used as the internal controls. The reactions were incubated in a 96-well plate at 95˚C for 10 min, followed by 40 cycles of 95˚C for 15s and 60˚C for 1 min. All reactions were performed in duplicate using a 7900HT Fast Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Relative expression level was determined using to the $2^{-\Delta\Delta Cq}$ method (29).

Statistical analysis. Kaplan-Meier survival curves were used to evaluate the association between the expression levels of miR-146a and patient survival rate. The differences between the studied groups were determined by using the Mann-Whitney U test or Kruskal-Wallis. Spearman's rank correlation coefficient was used in order to determine the statistical dependence between two variables. Multivariate analysis was used to estimate correlations between ≥3 variables. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patients. Age, FIGO stage and histological grading distribution did not differ significantly between the groups of chemosensitive and chemoresistant patients. The number of patients who relapsed was similar in both groups. Progression-free survival (PFS) was longer for platinum-sensitive patients compared with platinum-resistant patients ($P<0.05$). OS was not significantly longer for platinum-sensitive patients compared with platinum-resistant patients ($P=0.07$; Table I).

miR-146a expression in primary tumor tissues, metastases and normal ovarian tissues. miR-146a expression in primary tumor samples was significantly increased in comparison with normal ovarian tissues ($P=0.02$) and metastases ($P=0.01$; Figs. 1 and 2). The range of relative quantification (RQ) values for primary tumors was heterogeneous, whereas the range of RQ values for metastases was homogeneous, suggesting stable and low level of miR-146a expression.

miR-146a expression and clinical parameters in ovarian cancer patients. For primary tumor tissues, a negative

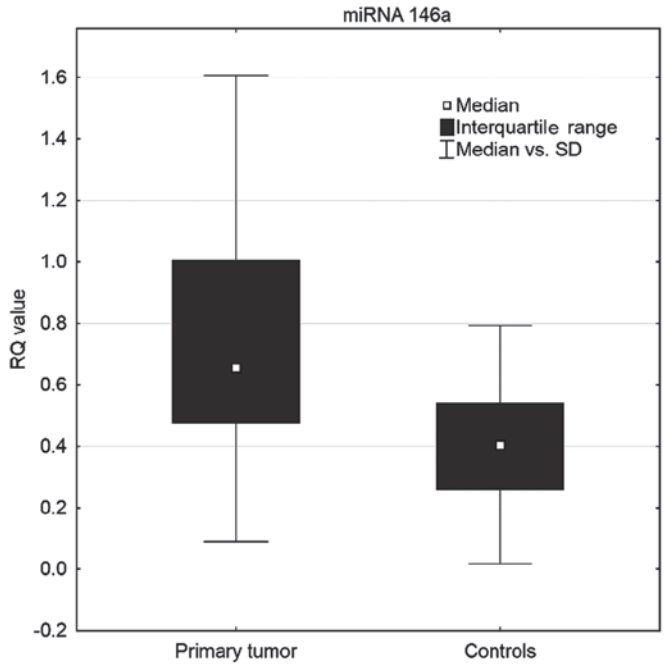

Figure 1. miR-146a expression level in primary tumor tissues and in normal ovarian tissues. P=0.02. miR, microRNA; RQ, relative quantification; SD, standard deviation.

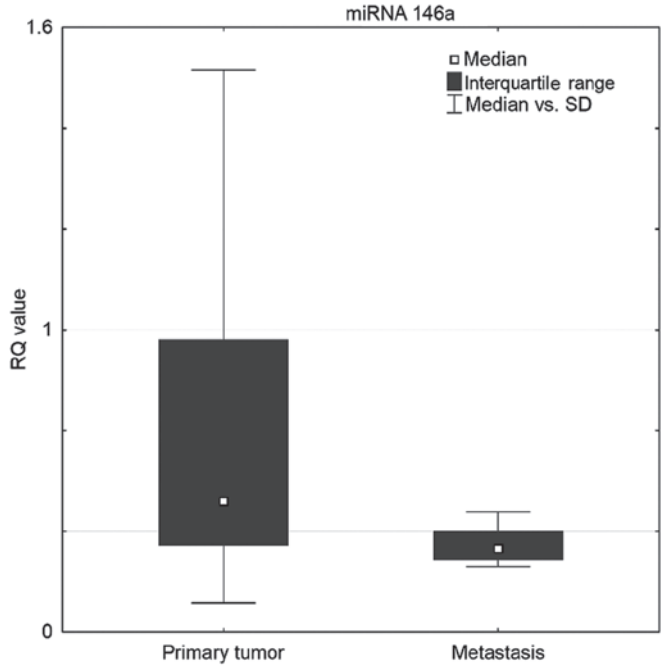

Figure 2. miR-146a expression level in primary tumor tissues and in metastases (P=0.01). miR, microRNA; RQ, relative quantification; SD, standard deviation.

Spearman's correlation was identified between miR-146a expression and serum levels of CA125 (R=-0.37; P=0.03) and ROMA index (R=-0.79; P=0.0007; Figs. 3 and 4). Serum CA125 levels and ROMA index were determined prior to cytoreductive surgery. The expression level of miR-146a was not correlated with FIGO stage or histological grading. The OS of the group of patients with ovarian cancer was

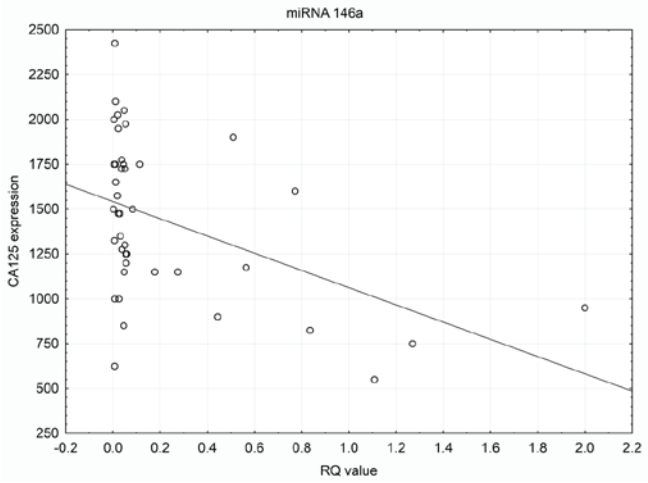

Figure 3. Correlation between miR-146a expression in primary tumor tissues and preoperative CA125 serum levels. P=0.03. miR, microRNA; RQ, relative quantification; CA125, cancer antigen 125.

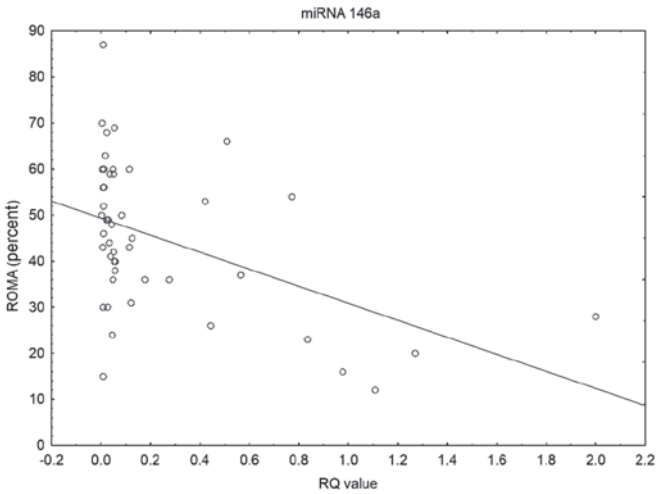

Figure 4. Correlation between miR-146a expression in primary tumor tissues and preoperative ROMA index values. P=0.0007. miR, microRNA; RQ, relative quantification; ROMA, Risk of Malignancy Algorithm.

positively correlated with the level of miR-146a expression in the primary tumor tissues (R=0.38; P=0.01; Fig. 5). The Kaplan-Meier analysis revealed that the probability of survival was significantly decreased for patients with lower levels of miR-146a expression (HR=0.21; P=0.003) in the primary tumor tissues (Fig. 6). In multivariate analysis, lower levels of miR-146a expression in primary tumor tissues were associated with shorter PFS (P=0.04). Multivariate analysis indicated that lower expression levels of miR-146a in metastases correlated with platinum-resistance (P=0.006). This finding was not demonstrated for primary tumor tissues.

Discussion

The present retrospective study demonstrated that there is a difference in miR-146a expression in primary tumor tissues and omental metastases from patients with advanced serous ovarian cancer, who were subject to routine cytoreductive surgery and standard chemotherapy. Furthermore, an

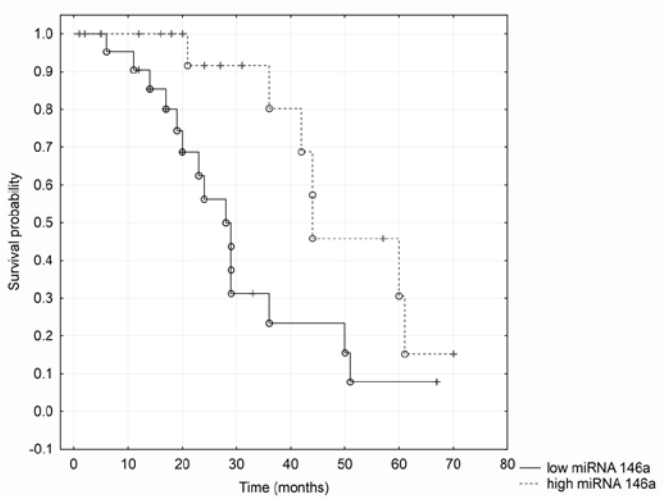

Figure 5. Correlation between miR-146a expression level in primary tumor tissues and overall survival (P=0.01). miR, microRNA.

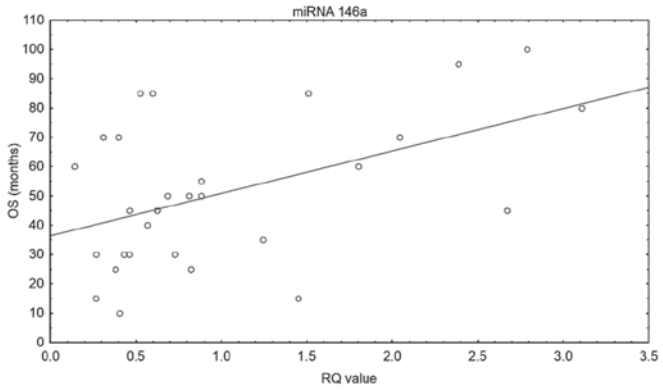

Figure 6. Kaplan-Meier analysis of survival patients with ovarian cancer and miR-146a expression levels in primary tumor tissues. (P=0.003). OS, overall survival; miR, microRNA; RQ, relative quantification.

association was identified between level of miR-146a expression and clinicopathological factors, including platinum resistance, survival rate, CA125 serum levels and ROMA index values.

miR-146a expression was observed to be increased and downregulated in various human cancer types compared with expression in normal tissues (6). Aberrant participation of miR-146a in the regulation of the immune response may result in a local inflammatory environment typical for many solid tumors, including ovarian cancer (18). A number of data studies have demonstrated expression of various miRs in serum or peripheral blood exosomes (17). However, the data concerning the levels of miR-146a expression in ovarian tumors are limited. Wyman *et al* (30) observed decreased expression of miR-146a in ovarian tumor tissue samples compared with normal ovarian cells. However, the tissue samples used in the study contained various histological types of cancer (including clear cell and endometrial cancer) and the reference samples used were cultured human ovarian surface epithelial (HOSE) cells. Similarly, Cui *et al* (31) detected downregulation of miR-146a in OVCAR3, CAOV3 and HEY ovarian cancer epithelial cells compared with HOSE cells. To the best of our knowledge, the only previous published study investigating the differences between ovarian primary tumor and metastases in the context of miR expression levels, was performed by Vang *et al* (27) on 9 paired tissue specimens of primary lesions and omental metastases in serous cancer: Vang *et al* (27) demonstrated that miR-146a expression level was increased in metastases compared with in primary lesion tissues. It was demonstrated that high miR-146a expression was able to induce the formation of spheroids from cancer cells *in vitro*. This process mimics the *in vivo* intraperitoneal dissemination of cancer cell conglomerates over peritoneal cavities to form metastases (27).

By contrast to the previously mentioned results, the present study revealed that miR-146a expression was increased in primary tumor tissues compared with normal ovarian tissues, and that miR-146a expression level in omental metastases was reduced compared with primary ovarian tumor tissues. Similar inconsistencies in miRNA expression have been also observed by another study (32).

There are several factors that may account for the differences in miR-146a expression identified in the present study when compared with previous studies. First, only tumors of serous histology were included in the present study and tumors of different origin may exhibit different patterns of miRNA expression. Previous profiling studies that included different histological types of ovarian cancer support this observation and reported different miRNA expression in serous, mucinous, endometrial and clear-cell types of cancer (33), as well as in borderline and invasive tumors (19). It has also been demonstrated that miR-146a expression was downregulated in highly metastatic breast cancer. However, in breast tumors originating from BRCA1/2 mutation, miR-146a was overexpressed (34). The cancer tissues samples used in the present study were micro-dissected from the paraffin-embedded specimens. The specimens not only contained cancerous epithelium but also infiltrated stroma. Similarly, the reference samples also comprised normal surface epithelium and ovarian stroma. Heterogeneity of tissue samples may have had an impact on the expression level of miR-146a observed in the present study.

Furthermore, tissue samples extracted *in vivo* from the primary tumor were a heterogeneous population of cells compared with cultured cancer cell lines. An extensive range of miR-146a expression levels observed in primary tumor tissues in the present study may support this hypothesis. Primary tumors are composed of cell clones with different invasive potentials (35). It was demonstrated that the pattern of miRNA expression varied between cultured SKOV-3 and OVCAR-3 cell lines and was associated with invasiveness (36). The present study, which compared primary lesions and metastases, indicated that miR-146a expression levels in metastases were reduced compared with primary lesions. RQ values for metastases were more reproducible and demonstrated a more homogeneous range compared with the primary tumor tissues. Peritoneal metastases in ovarian cancer grow from small cell conglomerates, which originate from primary lesions that spread via lymphatic vessels or ascitic fluid (37). Metastatic cells create a less heterogeneous population and usually represent a more aggressive phenotype (38). Differences in the expression of miRNAs and other

small RNAs may regulate the type of tumor spread (milliary vs. bulky) in serous ovarian cancer (39). In the majority of cancer cases, miR-146a acts as a tumor suppressor, and its expression level was demonstrated to be downregulated in highly metastatic types of cancer (40). This finding suggested that more aggressive metastases may exhibit lower expressions of miR-146a, and the results of the present study supported this finding.

miRNA expression may be associated with clinical outcomes of ovarian cancer, and this has been reported by a number of studies. The level of miR-370 expression was increased in FIGO stage I/II samples compared with FIGO stage III/IV tissue samples. Additionally, upregulated expression of miR-181d, miR-30c, miR-30d and miR-30e-3p significantly improved disease-free survival or OS (19). To the best of our knowledge, miR-146a expression has not been associated with clinical outcomes of ovarian cancer. The results of the present study demonstrated that the levels of miR-146a expression in primary serous ovarian tumor tissues may be negatively correlated with serum levels of CA125 and ROMA index values. Assessment of the serum level of CA125 has been widely used in the diagnosis of ovarian cancer (41). High serum CA125 levels have been associated with FIGO stage, but predominantly with serous histology, low-grade cancer cases and the presence of ascites (42-45). Patients with CA125 levels >100 U/l were demonstrated to have shorter OS compared with patients with CA125 levels ≤100 U/l (46). The CA125 AUC value was also associated with FIGO stage, residual disease, response to chemotherapy and final outcome (47). ROMA index, originally described by Moore *et al* (48), is an algorithm, which takes into consideration the level of HE4 and CA125 together with menopausal status in order to classify patients with adnexal mass into groups of high or low risk of ovarian cancer. In patients with ovarian cancer, increased ROMA values were associated with advanced FIGO stage, undifferentiated tumors, ascites and lymph node involvement, as well as shorter overall, disease-free and progression-free survival rates (49,50). In the present study, high CA125 serum levels and increased ROMA values (reflecting the advancement of ovarian cancer) correlated with low expression of miR-146a in primary tumor tissues. OS was longer for patients with increased miR-146a expression in the primary tumor, and the probability of survival was significantly decreased for patients with low levels of miR-146a expression. In multivariate analysis, lower miR-146a expression levels in primary tumor tissues were associated with shorter PFS. These observations are consistent with previous studies performed in gastric cancer, which demonstrated that patients with high-expression profiles of miR-146a exhibited reduced lymph node metastases and exhibited longer OS compared with patients with low miR-146a expression profiles (9). These observations further supports the hypothesis that miR-146a may act as a tumor suppressor.

In the context of ovarian cancer chemosensitivity, various miRNAs have been studied. For example, the upregulation of miR-125b and miR-106a was previously reported *in vitro* in platinum-resistant ovarian cancer cells (23,51,52). miR-31 was also demonstrated to be downregulated in taxane-resistant ovarian cancer cell lines (24). Similarly, downregulated expression of miR-9, -22, -129-5p, -155, -320a and -640 was observed in paclitaxel-resistant compared with paclitaxel-sensitive serous ovarian adenocarcinoma tissue samples (26). Validation of miRNA profiles in tumor tissue samples revealed that it was possible to predict chemo-sensitivity of ovarian tumors by detecting expression of miR-484, miR-642 and miR-217 (25). In the present study, a correlation was identified between lower miR-146a expression in metastatic tissue samples and platinum-resistance, but this correlation was not observed in primary tumor tissues. There are a number of potential miRNA-dependent mechanisms underlying chemoresistance in ovarian cancer. Analysis of miR-484 revealed that the chemosensitive tumor phenotype was induced by modulation of vascular endothelial growth factor (VEGF) B and VEGFR2 signaling pathways (25). Another possible mechanism underlying chemoresistance may be dependent on miR-146a and miR-150 (27). The expression levels of miR-146a and miR-150 were studied in SKOV-3, OVCAR-8 and IGROV-1 cell lines, and it was demonstrated that increased platinum tolerance was associated with spheroid formation, a model of peritoneal spread of cancer cells (27). Additionally, it was revealed that spheroid formation correlated with elevated miR-146a expression in metastases (27), contrary to the results of the present study. §By contrast, another study reported that miR-146a is able to downregulate the expression of superoxide dysmutase-2 and increase reactive oxygen species generation, which leads to increased apoptosis, inhibition of proliferation and increased sensitivity to chemotherapy (20). miR-146a may also mediate its effect via upregulating epidermal growth factor receptor, NFκB, interleukin-1 receptor-associated kinase 1 and metastasis-associated protein-2 (MTA2) (40). MTA2 is a transcription factor that regulates metastasis and may be triggered by a low expression of miR-146a, as it was demonstrated in pancreatic cancer cultured cells (40). Similarly in breast cancer cell lines, downregulation of miR-146a induced NFκB activation and augmented metastatic potential (53). It was revealed that the upregulation of transcription factor NFκB in numerous solid tumors prevents apoptosis induced by stress signals, including chemotherapeutic agents (54).

In conclusion, the results of the present study indicated differences in miR-146a expression levels between primary tumor tissues and in metastases of ovarian cancer. A correlation between the expression of miR-146a and clinicopathological features characterizing cancer advancement and chemoresistance was demonstrated. Low miR-146a expression level was also revealed to be a prognostic factor for an unfavorable outcome in patients with cancer. Further studies are required in order to investigate the precise pattern of miR-146a expression in various types of ovarian cancer, its role as a disease marker and as a potential target for novel therapeutic regimens.

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