RESEARCH ARTICLE

miR-200a Overexpression in Advanced Ovarian Carcinomas as a Prognostic Indicator

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Abstract

Background: miR-200a expression is frequently altered in numerous cancers. The aim of the present study was to determine the role of microRNA-200a in advanced ovarian carcinomas. Materials and Methods: We measured miR-200a expression in 72 matched normal ovarian tissues and advanced ovarian carcinomas, and also two ovarian carcinoma cell lines (SKOV3 and SKOV3.ip1 - the latter being more invasive and metastatic than the parental SKOV3) by stem-loop real-time RT-PCR based on TaqMan microRNA assay using U6 as a reference. Levels of miR-200a expression were compared by disease stage, tumor grade, histology, and lymph node involvement. To evaluate the role of microRNA-200a, cell proliferation and invasion of SKOV-3 and SKOV-3.ip1 were analyzed with miR-200a inhibitor/mimic transfected cells. Results: Of 72 paired samples, 65 cancer tissues overexpressed microRNA-200a greater than two fold in comparison with matched normal epithelium. Specifically, patients with lymph node metastasis showed significant elevation. The level correlated with clinicopathological features, including high tumor grade, late disease stage, most notably with lymph node metastasis, but not with tumor histology. In addition, SKOV-3.ip1 cells also overexpressed miR-200a compared with SKOV-3, and miR-200a inhibitor transfected SKOV-3.ip1 cells showed significant reduction in cellular proliferation and invasion, while a miR-200a mimic stimulated the opposite behavior. Conclusions: We provide definitive evidence that miR-200a is up-regulated in a significant proportion of advanced ovarian carcinomas, and that elevated miR-200a expression facilitates tumor progression. Our findings support the notion that miR-200a is an onco-microRNA for ovarian cancer, and elevation is a useful potential diagnostic indicator. This study also provides a solid basis for further functional analysis of miR-200a in advanced ovarian cancer.

Keywords: miR-200a - ovarian carcinoma - lymph node metastasis - overexpression - prognostic indicator

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Introduction

Epithelial ovarian cancer is the most important cause of death from gynecological cancers in the world (Wong et al., 2003). Stage of the disease, histological and molecular cell-biological characteristics have been shown to associate with prognosis or therapy outcome (Calin et al., 2005). For example, 20% of ovarian cancers are limited to the ovaries at time of diagnosis (stage I) and up to 90% of these patients can be cured using currently available therapies (Li et al., 2008). However, the cure rate decreases substantially when the disease has metastasized to the pelvic organs (stage II), the abdomen (stage III) or beyond the peritoneal cavity (stage IV) (http://seer.cancer.gov/statfacts/html /ovary.html). The management of ovarian cancer has improved over the last 20 years due to more effective surgery and optimized combinational chemotherapy, i.e., platinum-based drugs combined with taxanes (e.g. paclitaxel,docetaxel). however, the overall cure rate is only 30%.

Ovarian cancer is a very heterogeneous disease (Leskela et al., 2011). Epithelial ovarian cancers develop from flattened epithelial cells into four distinct histological types, i.e., serous (most frequent type), endometrioid (second most frequent type), mucinous (most frequent in stage I disease) and clear cell, while a mixture of these histology types can be present as well. Furthermore, the extent of differentiation of the tumor can vary from grade I (well),grade II (moderately) to grade III (poorly or undifferentiated). Clear-cell carcinomas, associated with worse prognosis in early stage disease, and mucinous carcinomas generally do not respond as well as serous and endometrioid tumors to platinum-and taxane-based chemotherapy. Landen et al. proposed a model for ovarian cancer pathogenesis, including a low-grade pathway and a more rapidly evolving high-grade pathway, which incorporates different molecular factors like tumor cell mutations and host micro-environmental factors (Landen et al., 2008). Given this heterogeneity, increasesin long-term survival may be achieved by integrating the recent insights at the pathological, molecular and cellular levels and translating it to personalized therapeutic strategies.

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At the molecular level a number of interesting genes and pathways have already been identified that may play essential roles in the development of ovarian cancer and could serve as molecular targets for therapy. The recently discovered microRNAs (miRNAs) constitute a novel regulatory layer of gene expression and have been implicated in the etiology of various kinds of human cancer (Lu et al., 2005; Calin et al., 2006). This provided a stimulus to investigate the involvement of miRNAs in ovarian cancer.

MicroRNAs are small (about 22 nucleotide) noncoding RNA gene products that post-transcriptional modulate gene expression by negatively regulating the stability or translational efficiency of their target mRNAs (He et al., 2004). Currently, more than several hundred unique mature human miRNAs are known (Yan et al., 2008). Aberrant expression of miRNAs has been linked to some cancers, leading to the use of miRNA profiles in the diagnosis/prognosis of specific cancers and to distinguish certain cancer types.

miRNAs have been reported to be involved in tumorigenesis (Hernando, 2007; Skaftnesmo et al., 2007), acting, as on might expect, variously as either tumor suppressors (e.g. miR-15a and miR-16-1) ,oncogenes (e.g.miR-155 and miR-21) and as promoters (e.g.miR-10b,miR-182 and miR-29a) or suppressors (e.g.miR-335 and miR-126) of metastasis. Several other reports have described altered expression of miRNAs in cancer tissues compared to normal tissues (Faragalla et al., 2012; Koberle et al., 2013), suggesting that these miRNAs could potentially represent novel clinical and prognostic markers.

The first study suggested that miRNAs also fulfill an important role in ovarian cancer was published in 2007 and showed that approximately 40% of the miRNA genes exhibit altered DNA copy number (Iorio et al., 2007). Later on, high expression levels of Dicer, Drosha and eIF6, proteins involved in miRNA maturation, were shown to be associated with a favorable prognosis of ovarian cancer patients (Flavin et al., 2008). Several studies have reported miRNAs that are aberrantly expressed in ovarian cancer and are associated with histological subtypes, tumor stage or grade, primary or recurrent tumors, BRCA mutated/epigenetically changed tumors and survival (Chen et al., 2013; Di Leva et al., 2013). Indeed, 39 miRNAs showed a differential expression in ovarian cancer compared to normal tissue or cell lines, including the miR-200 and let-7 families (Iorio et al., 2007; Yang et al., 2008). miR family frequently associated with ovarian cancer is the let-7 family, which in humans consists of 10 mature miRNAs (let-7a-g/i, miR-98 and miR-202) organized in several clusters throughout the genome. The let-7 family has been implicated in several types of cancer, and four let-7 family members are downregulated in ovarian cancer as well. In addition, low expression levels of let-7b are associated with poor prognosis in serous ovarian carcinomas (Tang et al., 2013).

The miR-200 family consists of five members localized on two genomic clusters (One cluster resides on human chromosome1 and encodes miR-200b,miR-200a, and miR-429; while the other cluster is located on human chromosome12, and encodes miR-200c and miR-141) (Korpal et al., 2008). Interestingly, high levels of all family members have been linked with ovarian cancer in multiple studies (Hu et al., 2009; Bendoraite et al., 2010; Chen et al., 2011; Mateescu et al., 2011). Moreover, high expression levels of the miR-200 family are associated with decreased progression-free survival and overall survival of ovarian cancer patients. Although the expression levels of miR-200a and miR-200b may be lower in late stage than in stage I tumors, a high level of miR-200a expression in advanced stage correlates with poor ovarian cancer outcome.

In this study, we report that miR-200a is overexpressed in advanced ovarian carcinomas tissues compared to the matched normal ovarian tissues. Furthermore, the expression level of miR-200a was correlated with clinicopathological features, ovarian carcinomas with metastasis tend to express high level of miR-200a than those without metastasis. Similarly, cell line assay also showed that miR-200a is also overexpressed in more invasive and metastatic epithelial ovarian cancer cell line SKOV3.ip1 than its parental line SKOV3. Notably, miR-200a inhibitor significantly reduces cellular proliferation, as well as invasiveness of SKOV-3.ip1 ovarian cancer cells.

Materials and Methods

Tissue samples and cell lines.

72 pairs of primary advanced ovarian carcinomas tissues and matched normal ovarian tissues were collected from patients in Zhong Nan hospital from 2008 to 2010, with informed consent and agreement. All tumors were staged according to the International Federation of Gynaecology and Obstetrics standards (FIGO). All tissue samples from untreated patients before undergoing surgery were snap frozen in liquid nitrogen and stored at -80°C until the extraction of RNA. clinicopathologic information (age, gender, pathology, differentiation, tumor-node-metastasis classification) of all samples was available. The SKOV-3 human ovarian carcinoma cell line was purchased from the American Type Culture Collection. The SKOV-3.ipl cell line was established from ascites that developed in a nu/nu mouse given an i.*p*. injection of SKOV-3 cells. Cells were grown in Dulbecco's modified Eagle's medium/F12 medium supplement (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Sigma-Aldrich), 2 mmol/L glutamine, 100 units of penicillin/mL, and 100μg of streptomycin/mL (Cambrex), and incubated at 37°C in a humidified chamber supplemented with 5% CO_2.

miRNA isolation

microRNA was extracted from the tissue using the mirVana™ miRNA Isolation Kit (AM1560, Ambion). Total RNA was isolated from 100 to 250 mg of frozen tissue or $1x10^6$ cultured cells according to the manufacturer's instructions. All preparation and handling steps of RNA were performed in a laminar flow hood under RNase-free conditions. The quality and quantity of the isolated RNA was analyzed using a Bioanalyzer 2100 system (Agilent).

Stem-loop real-time reverse transcription-PCR (TaqMan®MicroRNA Assays)

miRNAs was reverse transcribed in a 15 µl reaction using the TaqMan® MicroRNA Reverse Transcription Kit (4366596, Applied Biosystems) which contain a miR-200a-specific stem-looped primer for reverse transcription (made-to-order). qPCR was performed on a StepOnePlus Real-Time PCR System (ABI) using TaqMan® Universal PCR Master Mix II (4440040,Applied Biosystems) in a 20µl reaction and U6 as an endogenous control, result was determined using the $2^{-\Delta\Delta CT}$. The qPCR experiments were run triplely within each experiment run, relative expression values were normalized to standard deviations from the mean.

Transfection of miR-200a inhibitor/mimic

Cells were seeded in 0.5 ml serum containing medium in 24-well plates 24 h prior to transfection.To transfect, Dilute 5.5 pmoles of inhibitor/mimic (Ambion) in 50 μl of jet PRIME buffer (polyplus), Vortex 10 s and spin down, then add 2 µl of jetPRIME reagent (polyplus), followed with Vortex 10 s, spin down and incubate 10 min at RT. Next, add transfection mix to the cells-seeding plates. Random RNA duplex (Ambion) was used as negative control for the transfection experiments. GAPDH siRNA (Ambion) was used as positive control to monitor the transfection efficiency.

MTT assay for cell proliferation assay

MTT assay was performed in a 96-well plate using the Cell Proliferation Kit (I) (Roche) following the manufacturer's instructions. Four to six wells were done for each sample and experiments were repeated twice. The resulting colored solution was quantified using an Emax precision microplate reader (Molecular Devices) at 570 nm with a reference wave length of 650 nm.

Transwell invasion assay

SKOV-3 Cell invasion was assessed using the Matrigel Invasion Chamber (BD biosciences) in triplicate. SKOV-3 Cells were transfected with miR-200a mimic or inhinitor (Ambion) for 48 h, and then washed with PBS buffer, Fifty thousand cells in 100 µL serum-free media were added to the upper chamber precoated with Matrigel of each Transwell. Medium containing 10% fetal bovine serum in the lower chamber served as the chemoattractant. After the cells were incubated for 22h at 37°C in a humidified incubator with 5% CO_2, the noninvading cells were removed with cotton swabs .The invasive cells attached to the lower surface of the membrane insert were fixed in 100% methanol at room temperature for 30 min and stained with hematoxylin. The number of invasive cells on the lower surface of the membrane was then counted under a microscope.

Statistical analysis

Statistical analysis was performed using Prism 5.0 software (GraphPad), values are expressed as the Mean±SD. Differences between groups were Calculated with Student's t test, or ANOVA. $p < 0.05$ was defined as being significant.

Results

miR-200a is overexpressed in the human epithelial ovarian cancer tissues

The tissue characteristics of 72 patients are shown in Table 1. Using U6 as endogenous control, the miR-200a expression levels were determined by TaqMan microRNA qPCR. Of 72 matched normal and cancer tissues, 65 (90.3%) cancer tissues overexpressed miR-200a (fold > 2) in comparison with the matched normal tissues. As showed in Table 2, expression change of miR-200a in samples of ovarian carcinoma was calculated relative to matched normal tissues (miR-200a expression level in normal tissue was set to one). miR200a level was significantly higher in advanced ovarian carcinomas tissues than in normal tissues ($p<0.01$; Figure 1A).

miR-200a overexpression in relation to clinicopathological features

We next analyzed results from miR-200a expression level in advanced ovarian carcinoma to evaluate whether a correlation existed in various clinicopathological features with miR-200a expression. We analyzed advanced ovarian carcinomas of different clinical stages (stage I-II and III-IV), clinical tumor grade (0-2 and 3), lymph node

Table 1. Clinicopathologic Features of 72 Advanced Ovarian Cancer Patients

Characteristic		N	%
Median age(years)		56.7	
Tumor Histology	Serous	48	66.7
	Mucinous	12	16.7
	Clear cell	6	8.3
	Endomentrioid	6	8.3
FICO Stage	III	59	81.9
	IV	13	18.1
Grade	0-2	17	23.6
	3	55	76.4
Lymph node metastasis	absent	53	73.6
	present	19	26.4

Table 2. The Correlation of miR-200a Over Expression with Clinicpathologic Features of Advandced Ovarian Cancer Versus Normal Ovarian Tissues

Characteristic	N	Relative overexpression (Mean+SD)
Stage		
I~II	13	2.27+0.75
III~IV	59	5.30+0.89
Tumor Histology		
Serous	48	4.73+1.61
Mucinous	12	5.27+0.97
Clear cell	6	3.52+1.51
Endomentrioid	6	5.14+0.50
Grade		
0-2	17	3.17+0.63
3	55	4.91+1.09
Lymph node metastasis		
absent	53	4.20+1.27
present	19	6.30+0.49

Using U6 as endogenous control, fold change of miR-200a expression was calculated relative to normal ovarian tissues, and their expression levels were set to one

metastasis, and tumor histology (Serous/ Mucinous/ Clear cell/ Endomentrioid). Statistically significant associations were observed between miR-200a expression and clinical stage (Figure 2B), high tumor grade (Figure 2A) or lymph node metastasis (Figure 1B), but was not showed with tumor histology (p=0.0959, Figure 2C). Specifically, expression of miR-200a was markedly higher in specimens with lymph node metastasis, those results suggest that miR-200a has the potential value to be a diagnostic indicator in ovarian carcinomas.

Overexpression of miR-200a in the human epithelial ovarian cancer cell line SKOV3.ip1

To examine the effects of miRNA in functional study, it is important to select a cell culture system that expresses the appropriate level of endogenous miRNAs. Here, we

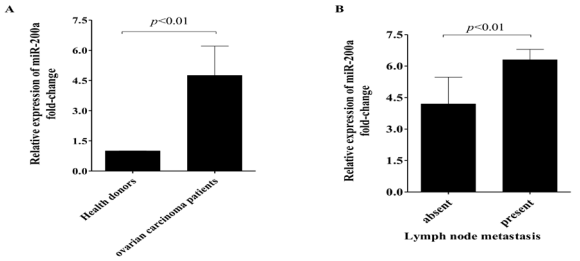

Figure 1. miR-200a Highly Expressed in Ovarian Carcinoma Tissues, Especially Which with Lymph Node Metastasis. miR-200a expression between patients and health controls were evaluated by matched-pair analysis. The level of miR-200a expression in normal tissues was set to one and then calculated miR-200a in matched ovarian carcinoma tissues, data showed as Mean±SD

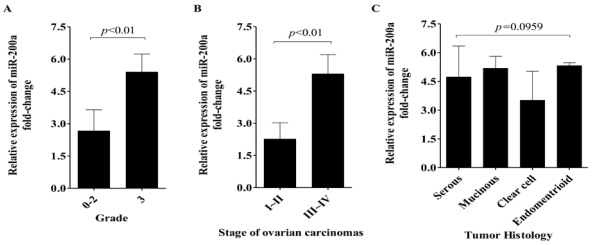

Figure 2. miR-200a Overexpression in Relation to Clinicopathological Features, Data Showed as Mean±SD. A, B and C corresponding to the difference of miR-200a expression in tumor grade, stage and history

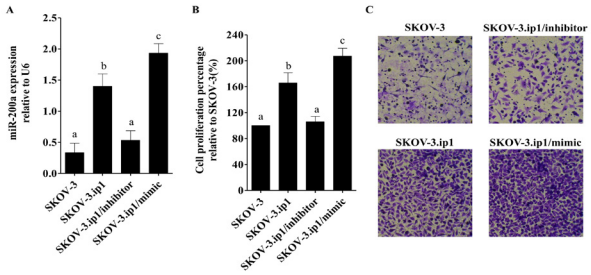

Figure 3. miR-200a Dysregulation Influenced Ovarian Carcinoma Cell Lines Invasion and Proliferation. A, miR-200a expression were identified in SKOV-3, SKOV-3.ip1 and its transfected cells. Proliferation and invasion of different cell groups were analyzed by MTT or transwell invasion assay (B and C). data showed as Mean±SD. Data were analysed by ANOVA and followed by posthoc Tukey's with the level of significance set at $p < 0.05$, letter indicate significant difference versus other letter labeled group

Table 3. Listed miRs with Potential Clinical Use in Ovarian Carcinoma. List of miRs with Potential Roles in Recurrence/Drug Resistance, Diagnosis/Detection, Prognosis of Ovarian Cancer

Potential Clinical Use	Up-Regulated miRs	Down-Regulated miRs	Reference
Prognosis	miR-200a	miR-429	Nam et al., 2008
	miR-200b	let-7	Yang et al., 2008
	miR-200c	miR-199a	Zhang et al., 2008
	miR-214	miR-377	
	miR-141	miR-368	
	miR-18a	miR-495	
	miR-93		
Diagnosis/detection	miR-146a	let-7	Shell et al., 2007;
	miR-21		Shen et al., 2008
	miR-92		
	miR-93		
	miR-126		
	miR-29a		
Recurrence/ drug resistance	miR-223	miR-9	Laios et al., 2008
	miR-214	let-7	Yang et al., 2008;
			Yang et al., 2008

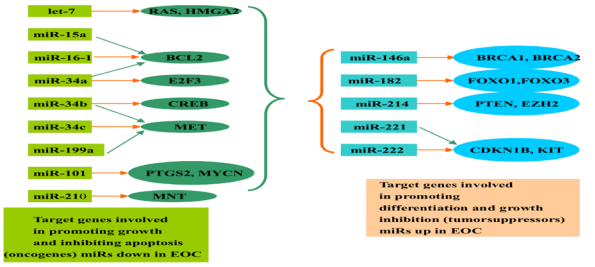

Figure 4. miR200a Up-Regulated in EOC and Could Serve as a New Diagnostic Marker. Schematic representation of selected known targets for miRs that are frequently altered in ovarian carcinoma

analyzed the expression level of miR-200a in paired high-metastatic human serous ovarian cancer cell SKOV-3.ip1 and low-metastatic human serous ovarian cell SKOV-3 by performing Real-time quantitative RT-PCR. We observed that miR-200a expression was relatively higher in the more invasive and metastatic SKOV3.ip1cell line (Figure 3A), suggesting that miR-200a expression may be associated with the ovarian caner metastasis. Based on this expression pattern, we therefore chose more invasive and metastatic epithelial ovarian cancer cell line SKOV3.ip1 for the following dyregulation-function studies.

miR-200a dysregulation influences proliferation and invasion ability of ovarian carcinomas cell line SKOV3.ip1

To validate the involvement of miR-200a dysregulation in advanced ovarian carcinomas tumorigenesis and metastasis, functional analysis were performed to test the effects of miR-200a on ovarian cancer cells invasion and proliferation. The effect of miR-200a inhibitor or mimic on ovarian carcinoma cell proliferation and invasion were examined in vitro. More invasive and metastatic epithelial ovarian cancer cell line SKOV3.ip1 was used for the proliferation assay and the invasion assay. SKOV3.ip1 cell was suitable for the invasion assay because it showed well invasion to the Matrigelmembranes. We transfected SKOV3.ip1 cell with miR-200a inhibitor or mimic. 48 h later, miR-200a expression in SKOV3.ip1 cells was

evaluated, transfection of miR-200a inhibitor could significantly decrease the expression level of miR-200a in SKOV3.ip1 cell, as compared with normal or miR-200a mimic-treated groups (Figure 3A). Subsequently, MTT assay showed that miR-200a inhibitor markedly reduced the cellular proliferation of SKOV-3.ip1 (Figure 3B). Matrigel invasion assay showed that knock-down of miR-200a in SKOV3.ip1 cell reduced invasion (Figure 3C). These results indicate that miR-200a act as an onco-microRNA promotes the proliferation and invasion of ovarian cancer cells in vitro.

Discussion

Since numerous investigations demonstrated that miRNAs are involved in cancer development and metastasis (Tavazoie et al., 2008; Mateescu et al., 2011), miRNAs are now being explored to have great cancer diagnostic and prognostic potentials. Emerging evidence revealed that pattern of miRNAs expression correlated well with clinicopathological characteristics and disease outcome. In a previous study of chronic lymphocytic leukemia (Calin et al., 2005), a unique signature of 13 miRNAs was associated with prognostic factors and disease progression. Another study reported that expression signatures of several miRNAs could accurately discriminate acute myeloid leukemia with common translocation (Li et al., 2008). Furthermore, high expression of miR-155 and low expression of let-7a-2 were associated with poor prognosis in human lung cancer (Yanaihara et al., 2006).

In human ovarian cancer, By using multiple molecular techniques, which include Northern blot analysis, real-time PCR, miRNA microarray, up- or down-expression of specific miRNAs, Several miRNA profiling studies have identified changes in miRNA patterns occurring during Epithelial ovarian cancer development and progression, opening a new field for the understanding of this disease and providing improved diagnostic and prognostic approaches (Table 3).

Croce and colleagues found that the miR-200 family (miR-200a, miR-200b, miR-200c and miR-141) were up regulated in human ovarian cancers compared to normal ovarian tissue (Iorio et al., 2007). This up-regulation of miR-200 family members was particularly pronounced in serous and endometroid histotypes. A subsequent study confirmed this result in serous ovarian cancers (Nam et al., 2008). Moreover, over-expression of the miR-200 family significantly correlated with decreased survival. Patients with ovarian tumors with high miR-200a expression had an approximately 50% decrease in median survival time compared to those lacking significant miR-200a expression (27.5 months *vs* 61 months, respectively) (Nam et al., 2008). In addition to the difference in expression levels between normal and cancerous tissues, it has been shown that miRNAs can also be histotype-specific. In endometrioid tumors, miR-21, miR-182, and miR-205 are considerably overexpressed while miR-144, miR-222, and miR-302a have reduced expression compared to normal. However, in serous and clear cell tumors, these miRNAs are normally expressed (Iorio et al., 2007).

In our study, of 72 paired samples , 65 cancer tissues overexpressed microRNA-200a in comparison with matched normal epitheliums. Specifically, patients with lymph node metastasis showed significantly high expression of microRNA-200a. Similarly, miR-200a is also overexpressed in more invasive and metastatic epithelial ovarian cancer cell line SKOV3.ip1 than its parental line SKOV3. Consistent with previous several studies, the overexpression of miR-200a was correlated with clinicopathological features. High miR-200a expression was associated with features of aggressive disease, including high tumor grade, late disease stage, lymph node metastasis. However, correlation of miR-200a with tumor histology was not observed.

Our findings seem to be inconsistent with the observations of several earlier studies on miR-200a in advanced ovarian cancer. The miRNAs that were identified in various studies show only partial overlap. This may be due to differences in type of specimens (cell lines vs. tumor tissue), the inclusion of different histological subtypes, heterogeneity of the tumor, RNA isolation protocols and detection platforms. Another complicating factor is the choice and availability of the appropriate controls. Healthy ovarian tissue only contains a minor amount of epithelia cells. Therefore, some studies used cultured primary (immortalized) ovarian epithelial cells (HOSE) as reference tissue, despite in vitro culture artifacts. It also suggests that different miRNAs are involved in different stages of ovarian carcinogenesis. Nevertheless, miRNAs that are identified in multiple studies are more likely to be truly associated with ovarian cancer.

Although miRNAs have been identified as biomarkers either in cancer cell lines or in biopsy specimens, the invasive nature of a biopsy makes it unsuitable for cancer screening in high-risk populations. It would be desirable to have a method that could accurately detect cancer without resorting to an invasive procedure. Recently, several reports suggest that cell-free circulating miRNAs are detectable in serum/plasma and the levels of tumor-derived miRNAs elevated in the patients with tongue cancer, lung cancer, prostate cancer, ovarian cancer, and colorectal cancer (Mostert et al., 2011; Mo et al., 2012). In the serum of OVCA patients, miRNAs21, 29a, 92, 93, and 126 were overexpressed while miRNAs 127, 155, and 99b were under expressed as compared to controls (Resnick et al., 2009). Another new non-invasive method with the potential for diagnosing OVCA involves isolating tumor-derived exosomes, miRNA 21, 141, 200a, 200b, 200c, 203, 205, and 214 are all overexpressed in tumor-derived exosomes (Taylor et al., 2008). These findings suggest that blood-based miRNAs could emerge a revolutionary sources of biomarker for ovarian cancer diagnosis. It would be of interest to extend this study in a larger number of paired fresh frozen advanced ovarian cancer samples, further investigation is required for evaluating this miR-200a as specific and blood-based biomarkers in ovarian tumors. With increasing knowledge about miRNAs associated with molecular subtypes and clinicopathological characteristics of ovarian cancer, we believe that miRNAs may prove useful as diagnostic and prognostic tools in the future.

As emerging evidences highlight the importance of miRNAs in diagnosis and prognosis of ovarian cancer, the usefulness of miRNA-based ovarian cancer therapy is now being explored. Recent evidence indicate that miRNAs can function both as tumor suppressors or oncogenes and promoters or suppressors of invasion and metastasis. Usually, miRNAs are dysregulated in cancers. Some miRNAs are temporally overexpressed in the early stage of cancer progression and they act like oncogenes by promoting proliferation and/or repressing apoptosis. Conversely, some miRNAs with tumor-repressor functions are downregulated in cancers. The association of aberrant miRNA expression with ovarian cancer, and functional analyses of specific miRNAs which has established their roles as tumor suppressors or oncogenes, illustrates the feasibility of manipulating miRNA expression as a therapeutic strategy for ovarian cancer. MicroRNA-based therapeutic strategies can be formulated by either antagonizing or restoring the functions of miRNAs.

Besides a putative (additive) role in ovarian cancer screening, miRNAs associated with ovarian cancer biology could also serve as targets for therapy. Figure 4 showed schematic representation of selected known targets of microRNAs that are frequently altered in ovarian carcinoma. The microRNAs frequently down-regulated in ovarian carcinoma typically target genes that have growth promoting functions, while microRNAs that are up-regulated target genes that have negative effects on cell growth. These microRNAs may represent targets for therapeutic interventions.

The members of the let-7 family, which are potential negative regulators of the EMT (which is associated with tumor aggressiveness, invasion and chemoresistance) and repressors of oncogenes such as K-Ras, would be interesting targets for therapy. Recently, numerous reports have demonstrated that delivery of let-7 or miR-26 family miRNAs could suppress tumor growth in a mouse model of lung or liver cancer (Kumar et al., 2008; Kota et al., 2009; Trang et al., 2010). In the case of liver cancer, trials with viral mediated delivery of miR-26 in mice showed great potency. These studies suggest that targeting of miR-200a in ovarian cancer is also feasible and might lead to a new therapeutic strategy.

Since miR-200a upregulation was significantly associated with advance clinicopathological data and could be detected in ovarian cancer cell line as well as ovarian cancer tissue, we focused further studies on this miRNA and conducted functional experiments in vitro in one ovarian cancer cell line (SKOV-3.ip1) which showes high expression of miR-200a. In cell line with high miR-200a expression downregulation of miR-200a resulted in decreased tumor cell proliferation as well as invasive ability. However, unlike the published reports that the miR-200 family was previously shown to promote epithelial characteristics by inhibiting the transcriptional repressor Zeb2 and thereby enhancing E-cadherin expression (Korpal et al., 2008), our results showed that down-regulation of miR-200a expression can suppress cell proliferation and slow down tumor invasion. The role of the miR-200 family as inhibitor of the EMT seems difficult to reconcile with the fact that miR-200 family members are found up-regulated in ovarian tumors. It is possible that the increased expression of the miR-200 family is the result of comparing epithelial-derived tumors with control ovarian tissue containing only a low amount of epithelial cells. However, as high expression levels of miR-200 family members correlate with poor outcome, another possibility is that an additional, yet unidentified function of the miR-200 family is responsible for this observation. More studies should investigate functional effects of miR-200a in other tumor cell lines, The exact nature of the miR-200a regulatory mechanism awaits further investigation

In summary, miR-200a is overexpressed in advanced ovarian carcinoma tissues, and miR-200a inhibitor inhibits cellular proliferation and invasion in vitro. These findings raise the possibility that miR-200a can be used as biomarker and a powerful diagnostic tool for detecting ovarian cancers; and also, miR-200a inhibitor may have potential therapeutic value in ovarian carcinoma patients. Therefore, microRNAs, in particular miR-200a, may serve as potentially useful targets for ovarian cancer diagnosis and therapy. Further research will advancedly explore the function of miR-200a in tumor generation and development. Taking into account the great importance of miR-200 family in cancer, and their promising potential as new specific diagnostic biomarkers as well as molecular targets for the development of novel cancer therapeutics, we strongly believe that our results will be of importance for both clinical researchers and those who design miR-200 based novel cancer therapeutics. It would be of interest to extend this study in a larger number of paired fresh frozen samples, and to design more prospective studies to verify these important findings.

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References

Bendoraite A, Knouf EC, Garg KS, et al (2010). Regulation of miR-200 family microRNAs and ZEB transcription factors in ovarian cancer: evidence supporting a mesothelial-to-epithelial transition. *Gynecol Oncol*, **116**, 117-25.

Calin GA, Ferracin M, Cimmino A, et al (2005). A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med*, **353**, 1793-801.

Calin GA and Croce CM (2006). MicroRNA signatures in human cancers. *Nat Rev Cancer*, **6**, 857-66.

Chen J, Wang L, Matyunina LV, et al (2011). Overexpression of miR-429 induces mesenchymal-to-epithelial transition (MET) in metastatic ovarian cancer cells. *Gynecol Oncol*, **121**, 200-5.

Chen Y, Zhang L and Hao Q (2013). Candidate microRNA biomarkers in human epithelial ovarian cancer: systematic review profiling studies and experimental validation. *Cancer Cell Int*, **13**, 86.

Di Leva G and Croce CM (2013). The role of microRNAs in

the tumorigenesis of ovarian cancer. *Front Oncol*, **3**, 153.
Faragalla H, Youssef YM, Scorilas A, et al (2012). The clinical utility of miR-21 as a diagnostic and prognostic marker for renal cell carcinoma. *J Mol Diagn*, **14**, 385-92.
Flavin RJ, Smyth PC, Finn SP, et al (2008). Altered eIF6 and Dicer expression is associated with clinicopathological features in ovarian serous carcinoma patients. *Mod Pathol*, **21**, 676-84.
He L, Hannon GJ (2004). MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet*, **5**, 522-31.
Hernando E (2007). microRNAs and cancer: role in tumorigenesis, patient classification and therapy. *Clin Transl Oncol*, **9**, 155-60.
Hu X, Macdonald DM, Huettner PC, et al (2009). A miR-200 microRNA cluster as prognostic marker in advanced ovarian cancer. *Gynecol Oncol*, **114**, 457-64.
Iorio MV, Visone R, Di Leva G, et al (2007). MicroRNA signatures in human ovarian cancer. *Cancer Res*, **67**, 8699-707.
Koberle V, Kronenberger B, Pleli T, et al (2013). Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. *Eur J Cancer*, **49**, 3442-9.
Korpal M, Lee ES, Hu G and Kang Y (2008). The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *J Biol Chem*, **283**, 14910-4.
Kota J, Chivukula RR, O'Donnell KA, et al (2009). Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell*, **137**, 1005-17.
Kumar MS, Erkeland SJ, Pester RE, et al (2008). Suppression of non-small cell lung tumor development by the let-7 microRNA family. *Proc Natl Acad Sci USA*, **105**, 3903-8.
Laios A, O'Toole S, Flavin R, et al (2008). Potential role of miR-9 and miR-223 in recurrent ovarian cancer. *Mol Cancer*, **7**, 35.
Landen CN, Jr., Birrer MJ and Sood AK (2008). Early events in the pathogenesis of epithelial ovarian cancer. *J Clin Oncol*, **26**, 995-1005.
Leskela S, Leandro-Garcia LJ, Mendiola M, et al (2011). The miR-200 family controls beta-tubulin III expression and is associated with paclitaxel-based treatment response and progression-free survival in ovarian cancer patients. *Endocr Relat Cancer*, **18**, 85-95.
Li Z, Lu J, Sun M, et al (2008). Distinct microRNA expression profiles in acute myeloid leukemia with common translocations. *Proc Natl Acad Sci USA*, **105**, 15535-40.
Lu J, Getz G, Miska EA, et al (2005). MicroRNA expression profiles classify human cancers. *Nature*, **435**, 834-8.
Mateescu B, Batista L, Cardon M, et al (2011). miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response. *Nat Med*, **17**, 1627-35.
Mo MH, Chen L, Fu Y, et al (2012). Cell-free circulating miRNA biomarkers in cancer. *J Cancer*, **3**, 432-48.
Mostert B, Sieuwerts AM, Martens JW and Sleijfer S (2011). Diagnostic applications of cell-free and circulating tumor cell-associated miRNAs in cancer patients. *Expert Rev Mol Diagn*, **11**, 259-75.
Nam EJ, Yoon H, Kim SW, et al (2008). MicroRNA expression profiles in serous ovarian carcinoma. *Clin Cancer Res*, **14**, 2690-5.
Resnick KE, Alder H, Hagan JP, et al (2009). The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. *Gynecol Oncol*, **112**, 55-9.
Shell S, Park SM, Radjabi AR, et al (2007). Let-7 expression defines two differentiation stages of cancer. *Proc Natl Acad Sci USA*, **104**, 11400-5.
Shen J, Ambrosone CB, DiCioccio RA, et al (2008). A functional polymorphism in the miR-146a gene and age of familial breast/ovarian cancer diagnosis. *Carcinogenesis*, **29**, 1963-6.
Skaftnesmo KO, Prestegarden L, Micklem DR and Lorens JB (2007). MicroRNAs in tumorigenesis. *Curr Pharm Biotechnol*, **8**, 320-5.
Tang Z, Ow GS, Thiery JP, et al (2013). Meta-analysis of transcriptome reveals let-7b as an unfavorable prognostic biomarker and predicts molecular and clinical subclasses in high-grade serous ovarian carcinoma. *Int J Cancer*, **134,** 306-18.
Tavazoie SF, Alarcon C, Oskarsson T, et al (2008). Endogenous human microRNAs that suppress breast cancer metastasis. *Nature*, **451**, 147-52.
Taylor DD, Gercel-Taylor C (2008). MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol Oncol*, **110**, 13-21.
Trang P, Medina PP, Wiggins JF, et al (2010). Regression of murine lung tumors by the let-7 microRNA. *Oncogene*, **29**, 1580-7.
Wong AS and Auersperg N (2003). Ovarian surface epithelium: family history and early events in ovarian cancer. *Reprod Biol Endocrinol*, **1**, 70.
Yan LX, Huang XF, Shao Q, et al (2008). MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. *RNA*, **14**, 2348-60.
Yanaihara N, Caplen N, Bowman E, et al (2006). Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell*, **9**, 189-98.
Yang H, Kong W, He L, et al (2008). MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. *Cancer Res*, **68**, 425-33.
Yang N, Kaur S, Volinia S, et al (2008). MicroRNA microarray identifies Let-7i as a novel biomarker and therapeutic target in human epithelial ovarian cancer. *Cancer Res*, **68**, 10307-14.
Zhang L, Volinia S, Bonome T, et al (2008). Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. *Proc Natl Acad Sci USA*, **105**, 7004-9.