Modern Pathology (2012) 25, 1644–1653

Expression analysis of *MIR182* and its associated target genes in advanced ovarian carcinoma

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***BRCA1/BRCA2* mutations are common and the hallmarks of high-grade serous ovarian carcinoma. We found that *MIR182*, a negative *BRCA1* regulator, is significantly overexpressed in high-grade serous ovarian carcinoma. To examine whether overexpression of *MIR182* and its target genes, including *BRCA1*, *HMGA2* (high-mobility group A2), *FOXO3* and *MTSS1*, are associated with high-grade serous ovarian carcinoma tumor types and clinical outcome, we studied *MIR182* by *in situ* hybridization and its target gene expression by immunohistochemistry in 117 cases of advanced ovarian cancer. We found that high-grade serous ovarian carcinoma had significantly higher *MIR182* ($P=0.0003$) and *HMGA2* ($P=0.04$) expression, and significantly lower *BRCA1* ($P<0.0001$) and *FOXO3* ($P<0.001$) expression than normal controls. *MIR182* is significantly correlated with *MTSS1* expression ($r=0.31$; $P<0.001$), whereas other target genes did not show a significant correlation with *MIR182*, indicating a complicated regulatory mechanisms of these genes in high-grade serous ovarian carcinoma. Among the examined *MIR182* target genes, only *HMGA2* was significantly associated with serous type carcinomas ($P<0.01$), ascites ($P<0.01$) and high death rate ($P=0.02$). *FOXO3* expression was associated with lower-stage disease ($P=0.04$) and solid growth pattern ($P=0.03$). *MIR182* expression is significantly higher in high-grade serous ovarian carcinoma than in fallopian tubes.**
Modern Pathology (2012) **25,** 1644–1653; doi:10.1038/modpathol.2012.118; published online 13 July 2012

Keywords: miR-182; ovarian cancer; *BRCA1*; *HMGA2*; *MTSS1*

High-grade serous ovarian carcinoma is an aggressive and deadly form of ovarian cancer, yet its pathogenesis is poorly understood. Despite significant efforts of clinical researchers, the survival rate of women with high-grade serous ovarian carcinoma has not changed in the past 50 years.[1] These tumors are often high-grade and aggressive at presentation, with a poor prognosis. Recent recognition of the existence of high-grade serous ovarian carcinoma precursor lesions, serous tubal intraepithelial carcinoma,[2,3] in the distal (fimbriated) ends of the fallopian tubes has renewed hope that we will be able to identify early tumorigenic events leading to high-grade serous ovarian carcinoma and reveal new opportunities for early detection and treatment that may decrease the mortality rate among women with this cancer.

BRCA1 and *BRCA2* mutations are a hallmark of high-grade serous ovarian carcinoma tumorigenesis.[4] Women with germline *BRCA1/2* mutations have a 30–70% chance of developing high-grade serous ovarian carcinoma by age 70.[5] Germline *BRCA* mutations,[6] somatic mutations and epigenetic inactivation of *BRCA1/2* (via methylation) can be found in nearly 30% of high-grade serous ovarian carcinoma cases.[7] *BRCA1* has a broader role upstream of *BRCA2*, participating in various cellular

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Received 25 March 2012; revised 3 May 2012; accepted 14 May 2012; published online 13 July 2012

processes in response to DNA damage repair[8] and transcriptional regulation.[9] If inactivation of *BRCA1/2* is critical in high-grade serous ovarian carcinoma cases, other regulation mechanism for BRCA1/2 expression may exist. In their study of cell response to DNA damage, Moskwa *et al*[10] found that *MIR182* can specifically repress *BRCA1* expression, suggesting a role for microRNA in the regulation of *BRCA1* expression. In a recent study of miRNA profiling analysis in normal fallopian tube and serous tubal intraepithelial carcinoma, we found that *MIR182* is one of a few miRNAs with significant overexpression in serous tubal intraepithelial carcinoma.[11]

MIR182 is an oncogene (onco-miR) and its oncogenic properties are characterized by its negative regulation of several tumor suppressor genes including *BRCA1*,[10] *FOXO1*,[12,13] *FOXO3* and *MITF*.[14] We recently found that *HMGA2* (high-mobility group A2) and *MTSS1* are the direct and indirect target genes of *MIR182*.[11] To better understand the role of *MIR182* overexpression in the tumorigenesis of high-grade serous ovarian carcinoma, it is important to examine the expression of *MIR182* and its target genes in ovarian cancer cases. It is also important to test whether *MIR182*-mediated target gene dysregulation is associated with different histological subtypes of ovarian cancer and outcome of the disease. In this study, we examined the expression patterns of *MIR182* and its five target genes in a large cohort of advanced ovarian cancer patients, and determined if expression levels correlated with survival or prognostic factors.

Materials and methods

Case Selection

Archived tumor tissues were collected retrospectively from patients who underwent surgery for ovarian cancer at the Northwestern Memorial Hospital between 2002 and 2007 and that were treated at our institution by reviewing electronic medical records and paper charts. We selected all patients diagnosed with FIGO stage III or IV epithelial ovarian cancer that had undergone initial surgery at the Northwestern University, including 117 cases (100 high-grade serous ovarian carcinoma, 3 low-grade serous ovarian carcinoma 14 nonserous carcinoma) and 30 normal fallopian tubes were collected as normal tissue controls. Patients' clinical biodemography, pathological and clinical outcomes were collected by retrospective chart review and are summarized in Table 1. Approval of the Institutional Review Board from Northwestern University was obtained.

Table 1 Clinical characteristics of 117 ovarian carcinomas

Parameters	*Scale and range*
Age, years	
Range	34–90
Mean ± s.d.	58.8 ± 10.7
Median	59
Tumor type	
Serous ovarian carcinoma—high grade	100
Serous ovarian carcinoma—low grade	3
Nonserous ovarian carcinoma	14
Tumor stage	
I	0
II	0
IIIA	3
IIIB	5
IIIC	91
IV	18
Lymph node metastases	
None	13
Pelvic	86
Periaortic	3
Both	15
Follow-up (14–82 months)	
Alive with disease	24
Death	71
Disease free	22

Tissue Preparation, Antibodies and Immunohistochemistry

All cases were reviewed by two pathologists. Tissue cores were collected from tumor and control sections of each case (normal fallopian tubes for serous carcinoma) for tissue microarray and represented in duplicate. Antibodies used for this study included BRCA1 (Dako, Carpinteria, CA, USA; Calbiochem (EMD), Darmstadt, Germany; Abcam, Cambridge, MA, USA), HMGA2 (BioChem, CA, USA), MTSS1 (Neomarkers, Fremont, CA, USA) and FOXO3a (Dako). To obtain better immunohistochemical results from *BRCA1*, we examined *BRCA1* expression by immunohistochemistry from three different sources of anti-*BRCA1* (Dako, EMD and Abcam). We found that all antibodies provided variable results for immunointensity, but no statistical significance among antibodies was observed (data not shown). As anti-*BRCA1* antibody (MS110) from EMD provided clear and constant results, and has been used in several different studies (see Results and Discussion), we used this antibody for the current study.

Tissue microarrays were sectioned 4 μm in thickness. After deparaffinization and antigen retrieval, all immunohistochemical staining was performed on a Ventana Nexus automated system (Tucson, AZ, USA). In brief, endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Primary antibodies were detected using standard biotinylated anti-mouse or anti-rabbit secondary antibodies.

MIR182 microRNA *In Situ* Hybridization

The hybridization system and probes of miRCURY LNA, including *MIR182* and *U6*, were purchased from Exiqon (Vedbaek, Denmark). The detailed

procedure for *in situ* hybridization was followed as per the manufacturer's protocol.[15] In brief, 4-μm tissue microarray slides were prepared. Following deparaffinization and deproteinization, the slides were prehybridized with 1X hybridization buffer without probe. The hybridization was carried out overnight in a 1X hybridization buffer (30–70 μl) with predenatured miRCURY LNA, *MIR182 U6* probes. After washing, the slides were blocked and incubated with AP-conjugated anti-DIG Fab fragments (1:1500, Roche, Indianapolis, IN, USA) and visualized for color detection.

Semiquantitative Scores of Immunointensity and Intensity for microRNA *In Situ* Hybridization

One-score system for immunointensity and microRNA intensity was used for the markers *HMGA2*, *BRCA1*, *FOXO3*, *MTSS1* and microRNA *MIR182*. The semiquantitation for intensity was scored on a scale of 0–3, 0: negative, 1: weak, 2: moderate and 3: strong. As tissue cores for all cases were duplicated, the scales for each case and each marker were given by average scores.

Statistical Analysis

Gene expression levels for the entire patient population as well as separated by cases and controls, and by histology and clinical groups were summarized by medians and ranges. Differences between cases and controls, and among histology and clinical groups were assessed using the Wilcoxon rank-sum tests. To determine associations of clinical parameters (optimal bulking, chemoresistance and survival) with gene expression, expression levels were dichotomized according to levels ≤ 1.5 or >1.5. To evaluate the association of pathological findings with the gene expression, we selected the following histological features for analysis, including tumor type (serous and nonserous), grade (low-grade and high-grade), architecture (glandular, papillary, micropapillary and solid), tumor necrosis ($<10\%$ and $>10\%$) and tumor-infiltrating lymphocyte (absent and present). The associations were evaluated according to Fisher's exact test. The overall survival curves were calculated for the expression groups using the Kaplan–Meier method with hazard ratios estimated according to the Cox proportional hazards models. Associations among the gene expression levels were assessed via Spearman's correlation. *P*-values <0.05 were considered statistically significant.

Results

Patients' Clinical and Pathological Information and Case Selection

Cases included in the study were stage III and IV ovarian carcinomas with well-documented histological and clinical information, including tumor types, grade, stage, treatment and follow-up (up to 10 years). The clinical information of these 117 cases is summarized in Table 1. Patients' age ranged from 34 to 90 years (mean age 59). About 85% (100/117) of the cases were high-grade serous carcinoma, 2.5% (3/117) were low-grade serous carcinoma and 12% (14/117) were nonserous carcinomas (including endometrioid, clear cell and mucinous carcinomas). All patients had FIGO stage III and IV disease (stage III = 85%, stage IV = 15%) and all underwent surgical debulking. Nearly 60% (70/117) patients had periaortic and/or pelvic lymph node metastasis. About 60% of patients died of disease, 20% were alive with disease at the time of follow-up and another 20% were disease free during the study period (Table 1). To better estimate the tested gene expression in these 117 cases, we prepared tissue microarrays of 1-mm cores in duplicates, and all cores were randomly distributed throughout tissue microarray blocks. We randomly selected 30 matched fallopian tubes as normal controls and they were also arrayed randomly into the tissue microarray blocks.

Differential Expression of *MIR182* and its Target Genes Between Normal (Fallopian Tube) Controls and Ovarian Cancer Cases

To test whether *MIR182* and its target gene expression are associated with ovarian cancer, we examined expression of *MIR182* and four well-characterized target genes (*BRCA1*, *FOXO3a*, *HMGA2* and *MTSS1*) in 117 advanced ovarian carcinomas and normal fallopian tube controls. We found that *MIR182* expression was significantly higher in ovarian cancer than in fallopian tube epithelia ($P<0.0003$; Table 2, Figure 1). The mean and median of *MIR182* expression were slightly higher in high-grade serous ovarian carcinoma than in nonserous carcinoma, but this difference was not statistically significant ($P>0.05$).

The selected *MIR182* target gene expressions were evaluated by semiquantitative scoring of immunoreactivity. As shown in Table 2, *BRCA1* and *FOXO3a* had significantly lower immunointensity in ovarian cancer than in fallopian tubes ($P<0.001$). In contrast, immunoreactivity for *HMGA2* was significantly higher in tumors than in controls ($P=0.04$). *MTSS1* had low expression in both carcinoma and control tissue and differences in expression between tumors and controls were insignificant ($P>0.05$). The findings suggested that *MIR182* and its associated target genes were differentially expressed between ovarian carcinomas and control tissues (Table 2, Figure 1).

MIR182 and its Target Gene Expression in Association with Tumor Types and Pathological Features

To investigate whether expression of *MIR182* and its target genes is associated with tumor histological

Table 2 Summary statistics for *MIR182* and its target gene expression in normal fallopian tube and ovarian cancer and *P*-values obtained from the Wilcoxon rank-sum test used to compare fallopian tube and ovarian cancer

Markers	*Fallopian tube* (N = *30*)		*Ovarian cancer* (N = *117*)		P-*values*
	Median	*Minimum–maximum*	*Median*	*Minimum–maximum*	
MIR182	1.00	1.00–3.00	2.00	0.50–3.00	**0.0003**
BRCA1	2.00	1.00–3.00	1.00	0.00–3.00	**<0.0001**
FOXO3a	3.00	0.00–3.00	1.50	0.00–3.00	**<0.001**
MTSS1	1.00	0.00–2.00	1.00	0.00–2.50	0.21
HMGA2	1.00	0.00–3.00	1.50	0.00–3.00	**0.04**

The bold *P*-values represent the $P<0.05$, meaning statistical significance.

features, we selected the following pathomorphological parameters for the analyses: serous and non-serous, solid and non-solid (glandular; papillary and micropapillary) growth patterns; tumor-infiltrating lymphocytes; necrosis, lymph node metastasis, ascites and tumor stages. We found solid growth pattern had significantly lower *BRCA1* and *FOXO3a* expression ($P<0.001$ and 0.02, respectively). *HMGA2* overexpression was significantly associated with ascites and serous carcinoma ($P=0.009$ and 0.01, respectively). The latter finding was consistent with our previous study.[16] *MIR182* overexpression and FOXO3a downregulation were significantly associated with advanced stage IV carcinoma ($P<0.02$ and 0.07, respectively). No significant asso-

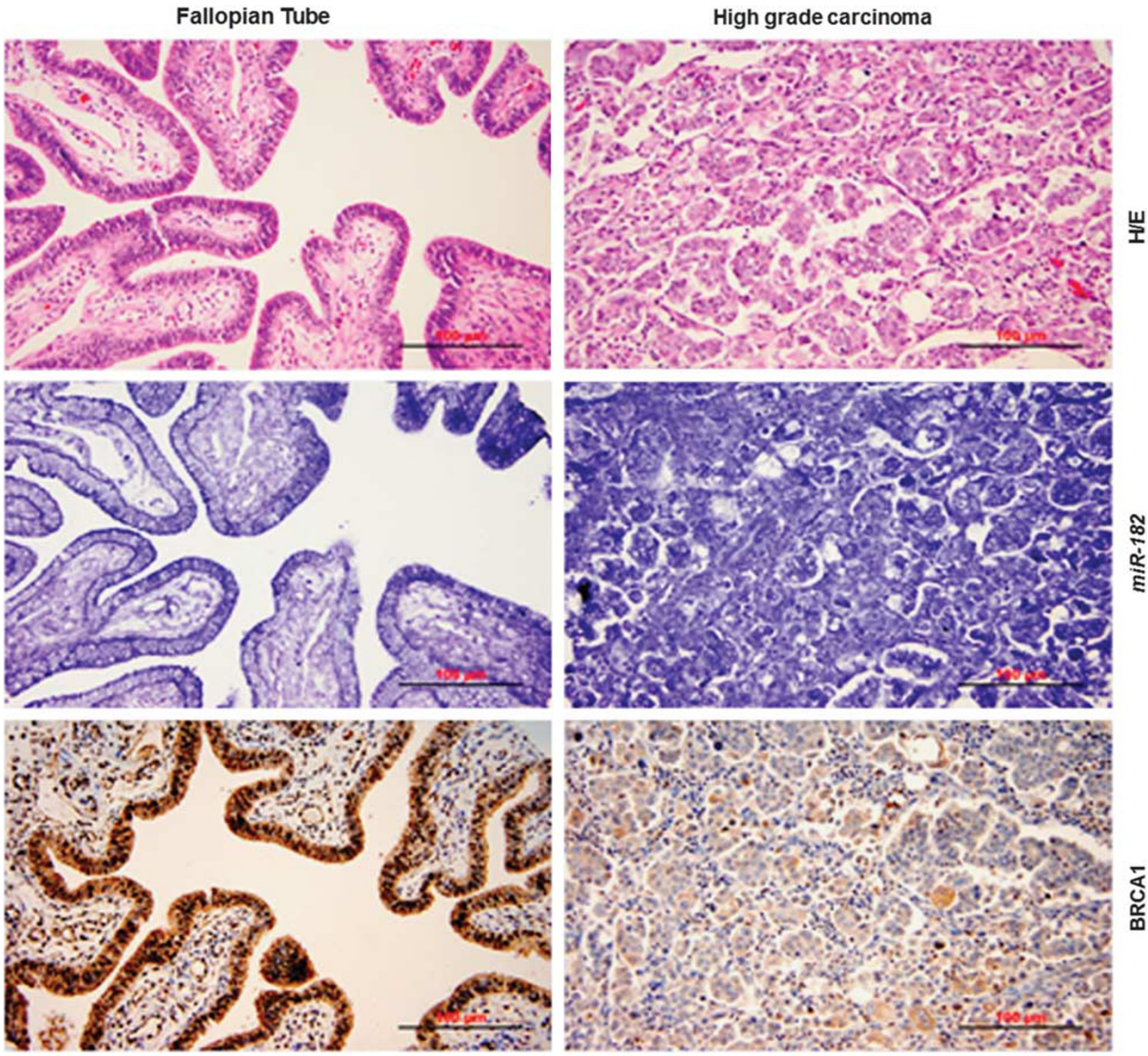

Figure 1 Photomicrographs illustrate examples of immunoreactivity for HMGA2, BRCA1, FOXO3, MTSS1 and *in situ* hybridization of *MIR182* in high-grade serous ovarian carcinoma and normal control of fallopian tube. Hematoxylin and eosin (H/E) and immunohistochemical stains for the selected markers were performed in serial sections of tissue core with high magnification (× 20).

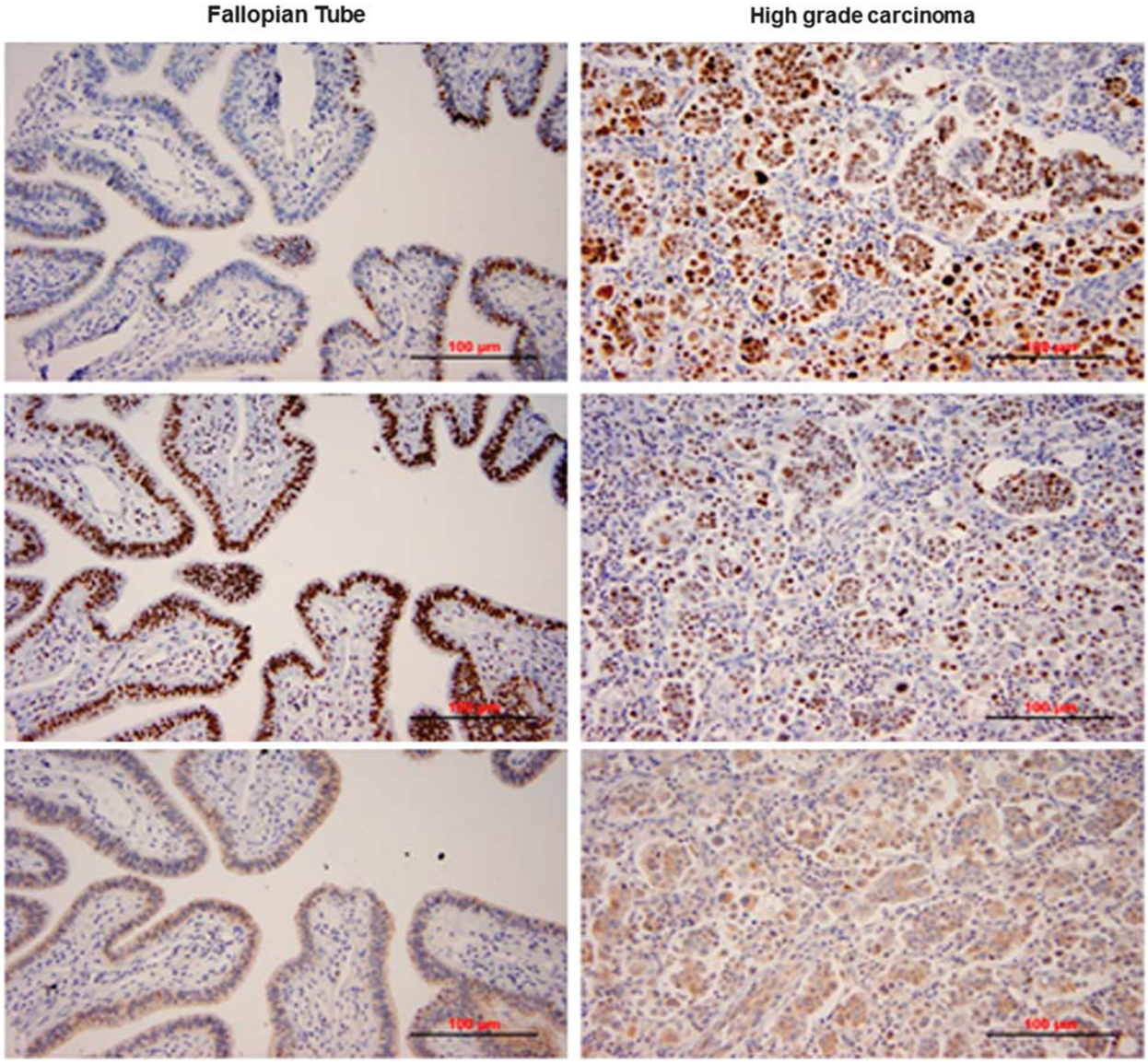

Figure 1 Continued.

ciation of tumor-infiltrating lymphocytes with any marker was found (Table 3).

MIR182 and its Target Gene Expression in Association with Clinical Features

We selected three clinically most relevant parameters for the analysis: optimal debulking status post surgery, chemoresistance and progression-free survival. To better define the cutoff for the scores of each markers, we compared the differences in the scales of: (1) positive (1–3)/negative (0); (2) negative + weakly positive (0 + 1)/positive (2–3); or (3) low positive (<1.5)/high positive (1.5). No significant association was found between *MIR182* or the other selected protein expression and optimal debulking status post surgery or chemoresistance.

For the dichotomized expression scores (≤ 1.5 or >1.5), there was no significant association between *BRCA1* protein expression and overall survival or *MIR182* expression and progression-free survival (for *BRCA1*, adjusted hazard ratio = 0.89 (0.52–1.54), $P = 0.68$; for *MIR182*, adjusted hazard ratio = 0.89 (0.53–1.51), $P = 0.68$). In the 117 patients, there was a significant association between *HMGA2* protein expression and progression-free survival (adjusted hazard ratio = 1.79 (1.09–2.95), $P = 0.02$; Figure 2). The results of other markers are summarized in Table 4. In further analyses classifying patients with minimal residual disease into low (*BRCA1* ≤ 2.5) and high (*BRCA1* >2.5) expression groups patients with low *BRCA1* expression had a more favorable outcome (median progression-free survival was 24.7 and 16.6 months in patients with low and high *BRCA1*, respectively; hazard ratio = 0.56 (0.35–0.89), $P = 0.01$).

Table 3 Summary statistics for *MIR182* and its target gene expression in different stages, ascites status, tumor types and architecture, and *P*-values obtained from the Wilcoxon rank-sum test used to compare values between groups

Markers	*Stage III* (N = *94*)		*Stage IV* (N = *17*)		P-*values*
	Median	*Minimum–maximum*	*Median*	*Minimum–maximum*	
MIR182	2.00	0.50–3.00	1.50	1.00–2.50	**0.02**
BRCA1	1.00	0.00–3.00	1.50	0.50–3.00	0.53
FOXO3a	1.50	0.00–3.00	1.00	0.00–2.50	**0.07**
MTSS1	1.00	0.00–2.50	0.50	0.00–2.00	0.27
HMGA2	1.50	0.00–3.00	1.00	0.00–3.00	0.85
	Ascites (N = *81*)		*No ascites* (N = *20*)		
	Median	*Minimum–maximum*	*Median*	*Minimum–maximum*	
MIR182	2.00	0.50–3.00	2.00	1.00–3.00	0.92
BRCA1	1.50	0.00–3.00	1.00	0.00–3.00	0.43
FOXO3a	1.50	0.00–3.00	1.50	0.00–2.00	0.30
MTSS1	1.00	0.00–2.50	0.75	0.00–2.00	0.30
HMGA2	2.00	0.00–3.00	0.50	0.00–3.00	**0.01**
	HGSOC (N = *97*)		*NON* (N = *14*)		
	Median	*Minimum–maximum*	*Median*	*Minimum–maximum*	
MIR182	2.00	0.50–3.00	2.00	1.00–3.00	0.19
BRCA1	1.00	0.00–3.00	1.25	0.00–2.50	0.82
FOXO3a	1.50	0.00–3.00	2.00	1.00–3.00	0.24
MTSS1	1.00	0.00–2.50	0.75	0.00–2.00	0.37
HMGA2	1.50	0.00–3.00	0.00	0.00–0.00	**0.01**
	Papillary/glandular (N = *52*)		*Solid* (N = *60*)		
	Median	*Minimum–maximum*	*Median*	*Minimum–maximum*	
MIR182	2.00	1.00–3.00	2.00	0.50–3.00	0.51
BRCA1	1.50	0.00–3.00	1.00	0.00–3.00	**0.001**
FOXO3a	2.00	0.00–3.00	1.50	0.00–3.00	**0.06**
MTSS1	1.00	0.00–2.50	1.00	0.00–2.00	0.55
HMGA2	1.50	0.00–3.00	1.50	0.00–3.00	0.25

The bold *P*-values represent the $P<0.05$, meaning statistical significance.

BRCA1 Immunohistochemical Scores in Ovarian Cancer

Immunohistochemical stain for *BRCA1* is not well established. As described in Materials and methods, we have examined several anti-*BRCA1* antibodies from different vendors. We could not get reliable results from Abcam's antibody, but we did obtain a comparable result from EMD and Dako antibodies. Figure 3 provides the examples of side-by-side comparison of immunoreactivity for *BRCA1* from these two antibodies. By reviewing the literatures, we found that most authors used MS110 for the study of *BRCA1* expression in ovarian cancer (Table 5), we therefore used EMD anti-*BRCA1* antibody (MS110) for this study. We found that immunoreactivity for *BRCA1* was completely negative in 13% (14/105), weakly positive in 38% (40/105), moderately positive in 39% (41/105) and strongly positive in only 10% (10/105) of cases

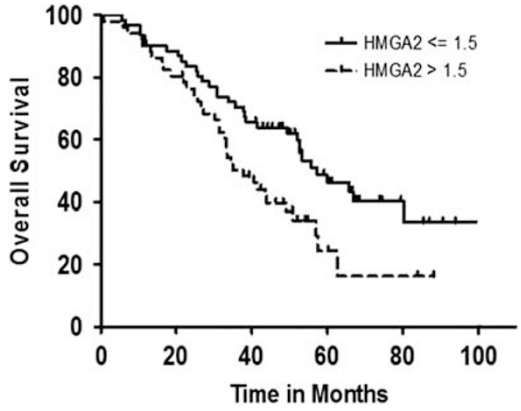

Figure 2 Kaplan–Meier survival curve analysis of HMGA2 in 117 advanced high-grade serous ovarian cancer patients.

(Table 5). Among the 117 ovarian cancer cases, only 6 patients were documented with either *BRCA1* or *BRCA2* mutations. As not all cases were tested for

Table 4 *MIR182* and its target genes in overall survival

Prognostic factors	*No. of cases*	*No. of events*	*Overall survival*[a] *hazard ratio (95% CI)*	P-*value*
MIR182 >1.5	107	64	0.89 (0.53, 1.51)	0.68
BRCA1 >1.5	105	64	0.89 (0.52, 1.54)	0.68
FOXO3 >1.5	108	66	0.84 (0.51, 1.37)	0.47
MTSS1 >1.5	106	64	0.51 (0.12, 2.10)	0.35
HMGA2 >1.5	108	66	1.79 (1.09, 2.95)	**0.02**

[a]The median time and 95% CI for the whole cohort is: 52.07 (38.14, 57.43) months; the median time and 95% CI for patients with HMAGA2 score ≤1.5 is: 57.33 (49.45, 100.17) months; and the median time and 95% CI for patients with HMAGA2 score >1.5 is: 37.75 (30.16, 50.83) months.
The bold *P*-values represent the $P<0.05$, meaning statistical significance.

BRCA mutations, comparison of *BRCA* mutations with immunohistochemistry for *BRCA1* could not be further evaluated in this study.

Discussion

TP53 mutations are common in high-grade serous ovarian carcinoma[17] and are important in the tumorigenesis of this cancer. However, *TP53* mutations are neither sufficient to trigger a sequence of neoplasia nor rate-limiting.[4] In fact, *TP53* mutations are commonly seen in preneoplastic fallopian tube secretory epithelial cells (called a *p53* signature)[2,3] and are equally distributed in fallopian tubes of at-risk and non-at-risk populations.[18] In contrast, *BRCA1* and *BRCA2* mutations are a hallmark of high-grade serous ovarian carcinoma tumorigenesis.[4] Based on a recent genome-wide molecular study of ovarian carcinoma from the Cancer Genome Atlas Research Network, 20.25% ovarian carcinomas had either germ line (14.56%) and somatic (6.01%) mutations of *BRCA1* and *BRCA2*.[19] If we combined those ovarian cancer with epigenetic inactivation of *BRCA1* and *BRCA2* (via methylation),[7] nearly 30% of ovarian serous carcinoma may have either defects or altered expression of *BRCA1/2*. However, based on several studies of *BRCA1* protein expression analyses (by immunohistochemistry), the absence of or low *BRCA1* expression was found in about 41–65% of ovarian cancer[20–22] (Table 5), indicating that there are additional as yet undefined mechanisms of *BRCA1* inactivation involved in ovarian cancer development. Some of these data were further validated and correlated with *BRCA1* mRNA expression.[20–22]

Recent identification of *MIR182* repression of *BRCA1* expression[10] provides a new venue for the study of microRNA-mediated *BRCA1* gene alteration. In the global profiling analyses of high-grade serous ovarian carcinoma, we[11] and others[23] found that *MIR182* was significantly overexpressed in both early and late stages of this cancer. The finding prompted us to hypothesize that *MIR182* may have an important role in the tumorigenesis of high-grade serous ovarian carcinoma; partially through negative regulation of *BRCA1* expression and other target genes. In this study, using microRNA *in situ* hybridization analysis, we found that nearly 70% of advanced ovarian cancers had upregulation of *MIR182* expression in comparison with normal fallopian tubal epithelia (Figure 1, Table 2). This finding suggests that *MIR182* overexpression may be responsible for some of the additional cases of low or absent *BRCA1* expression in ovarian cancer via *BRCA1* downregulation. However, because of the lower sensitivity of semiquantitative immunostaining, no significant inverse correlation between *MIR182* and *BRCA1* expression was identified in this study.

HMGA2 was one of oncogenes significantly overexpressed in high-grade serous ovarian carcinoma.[16,17] The major tumorigenic functions of *HMGA2* in the pathogenesis of high-grade serous ovarian carcinoma likely include regulation of epithelial mesenchymal transition.[24] A study by Ahmed *et al*[25] shows that *BRCA1*, *ZBRK1* and *CtIP* form a repression complex that coordinately inhibits *HMGA2* expression via a *ZBRK1* recognition site in the *HMGA2* promoter. We found that *MIR182* repression of BRCA1 expression will release a negative regulation and result in *HMGA2* overexpression.[11,25] We propose that *MIR182* overexpression in high-grade serous ovarian carcinoma may be in part or completely responsible for *HMGA2* overexpression. Although *MIR182* and *HMGA2* overexpression are found in nearly 70% of high-grade serous ovarian carcinoma (this study and Mahajan *et al*[16] and Wei *et al*[17]), low correlation of expression of these two genes was found ($r=0.23$) in this study. This could be due to the fact that *HMGA2* can be regulated by many microRNAs, such as the well-characterized ones in the let-7 family.[26,27] Nevertheless, we found *HMGA2* overexpression is significantly associated with high-grade serous ovarian carcinoma[16] in this study (Table 4).

FOXO3a is considered a tumor suppressor and is involved in a large series of functions, including cellular proliferation, transformation, differentiation, DNA damage response and longevity.[28] *FOXO3a* expression in ovarian cancer is largely unknown. Fei *et al*[29] studied a total of 63 ovarian cancer cases and found that low *FOXO3a* expression was significantly associated with worse clinical outcome. As a *MIR182*-specific target gene,[14] *FOXO3a* expression is of great interest in ovarian cancer. In this study, we did find significant downregulation of *FOXO3a* in most ovarian cancer cases. The role of *FOXO3a* in ovarian cancer thus deserves further investigation.

MTSS1 is a potential metastasis suppressor gene[30] and is downregulated in many solid tumors. *MTSS1* downregulation enhances the growth, invasion and mobility of breast cancer cells, and is associated with poor prognosis.[31] We confirmed that *MTSS1* is

a specific target of *MIR182* and downregulation of *MTSS1* is strongly associated with aggressive invasion of ovarian cancer cells.[11] This study reveals no significant correlation of *MTSS1* expression with pathological and clinical parameters (Table 2).

In summary, this study intends to disclose the expression patterns of *MIR182* and its major target

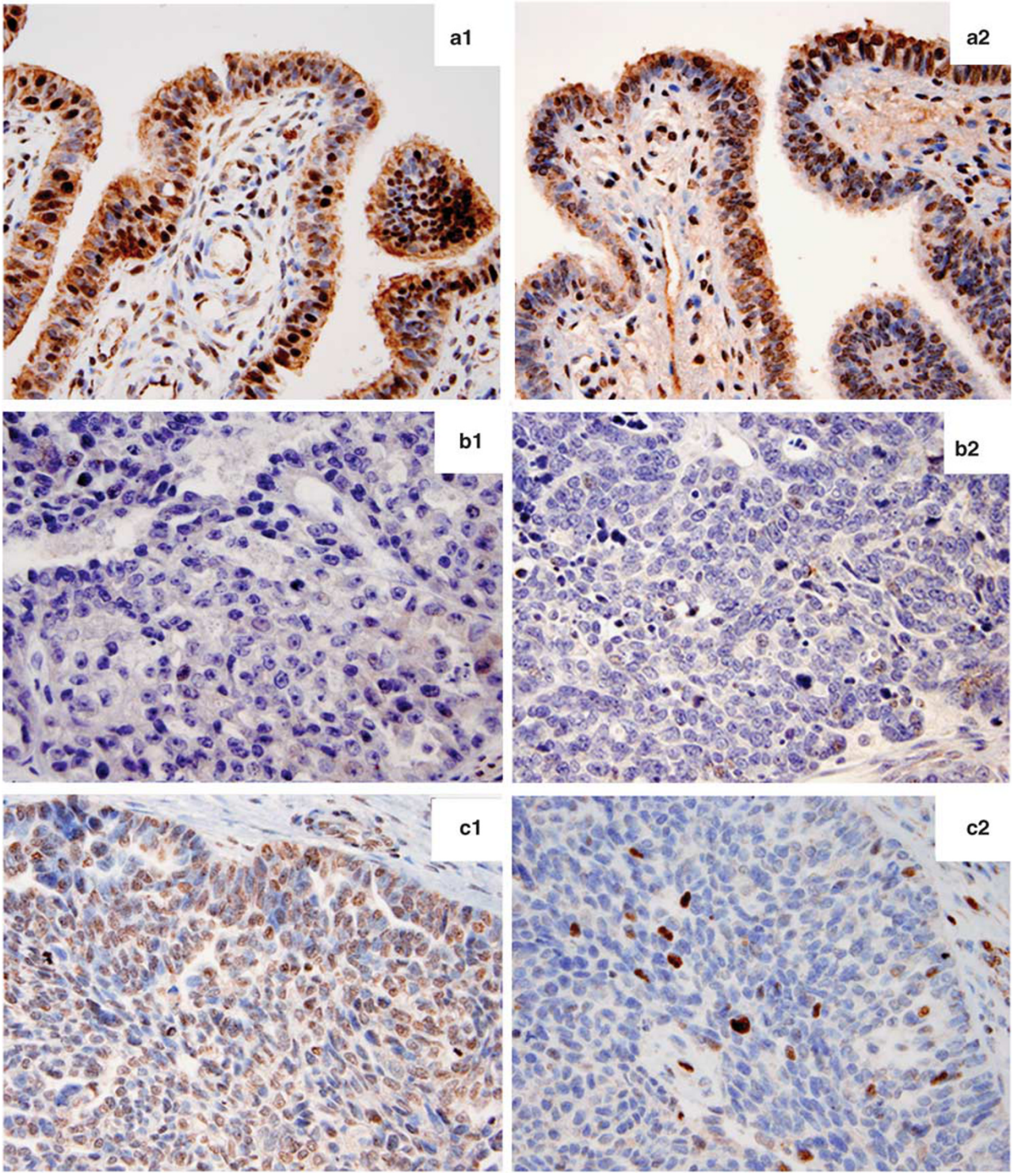

Figure 3 Photomicrographs illustrate examples of immunoreactivity for *BRCA1* detected by two different antibodies in normal fallopian tube (**a**) and high-grade serous ovarian carcinomas (**b–e**). Immunostain by MS110 from EMD (a1–e1) and GLK from Dako (a2–e2) are shown in side-by-side comparison. Immunointensity for *BRCA1* was scored based on MS110 as negative (**b**), weak (**c**), moderate (**d**) and strong (**e**) expressions.

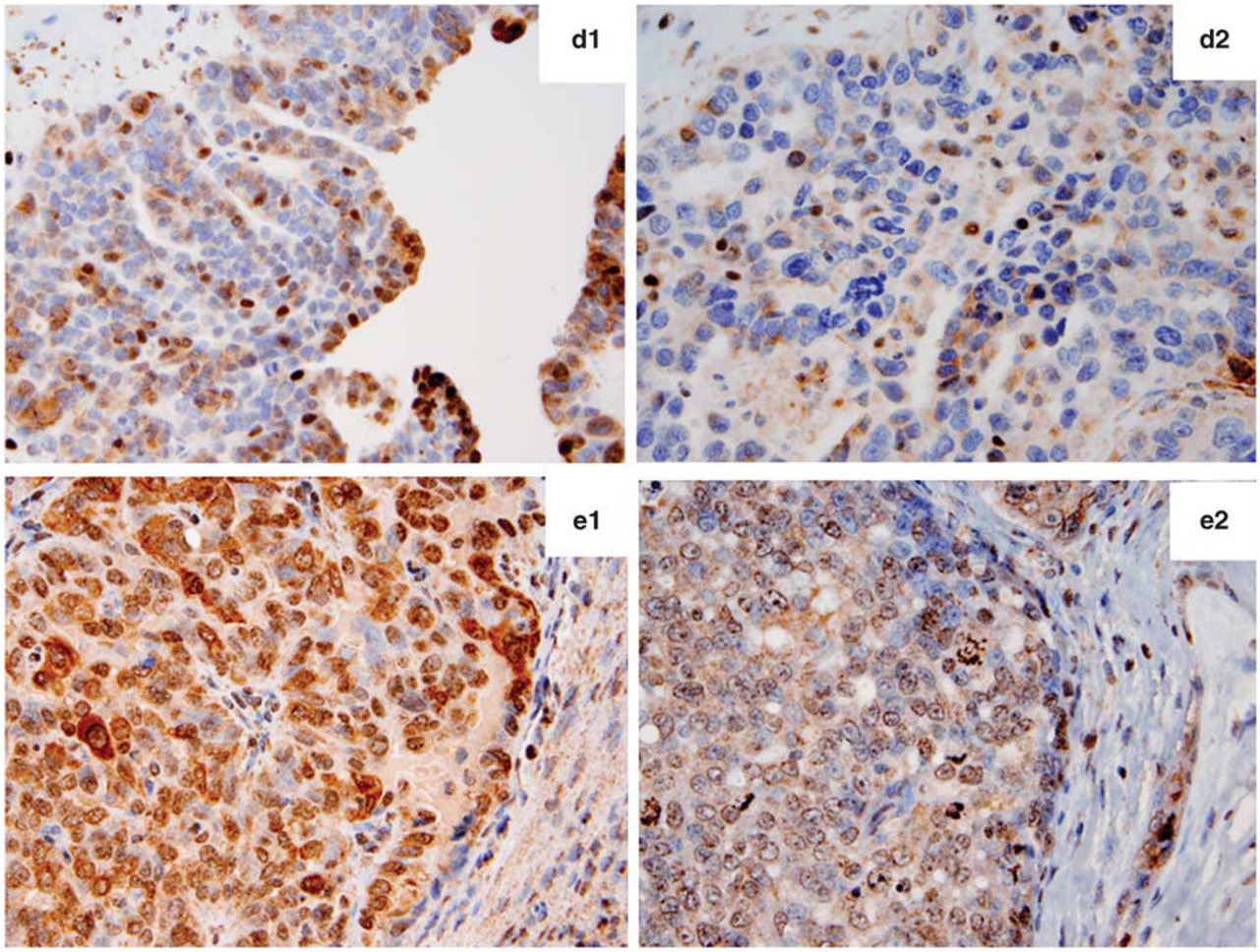

Figure 3 Continued.

Table 5 BRCA1 protein expression analyses scored by immunohistochemistry in this study and studies published recently

No. of cases	*BRCA1 antibody*	*Immunoreactivity*				*References*
		Negative (%)	*Weak (%)*	*Moderate (%)*	*Strong (%)*	
292	MS110	41		59		Carser *et al*[21]
27	MS110	44		56		Radosa *et al*[20]
251	MS110	16	49	24	11	Weberpals *et al*[22]
117	MS110	13	38	39	10	McMillen *et al* (this study)

genes in ovarian cancer. We found that at least 50% of ovarian cancer cases had either absent or low *BRCA1* protein expression. These findings are consistent with several similar studies. Among the *MIR182* target genes studied, *HMGA2* is the only gene that is significantly associated with high-grade and aggressive ovarian cancer in our series. Although we found a significant dysregulation of other selected genes in ovarian cancer, correlation between *MIR182* and its target genes has not been established, indicating a complex regulation of these genes in ovarian cancer and lower sensitivity for semiquantitative immunohistochemical scores.

Acknowledgements

We thank Mrs Bella Shmaltsuyeba for her technical support. This study is supported in part by the Marsha Rivkin Ovarian Cancer Research Award and the Dixon Innovation Translational Research Award. A part of this work was presented in the 101st

United States and Canadian Academy of Pathology in Vancouver 2012.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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