

# Clinical Cancer Research

## BRCA1 mRNA Expression Levels Predict for Overall Survival in Ovarian Cancer after Chemotherapy

Jennifer E. Quinn, Colin R. James, Gail E. Stewart, et al.

*Clin Cancer Res* 2007;13:7413-7420. Published online December 19, 2007.

**Updated Version**

Access the most recent version of this article at:  
doi:[10.1158/1078-0432.CCR-07-1083](https://doi.org/10.1158/1078-0432.CCR-07-1083)

**Cited Articles**

This article cites 42 articles, 16 of which you can access for free at:  
<http://clincancerres.aacrjournals.org/content/13/24/7413.full.html#ref-list-1>

**Citing Articles**

This article has been cited by 20 HighWire-hosted articles. Access the articles at:  
<http://clincancerres.aacrjournals.org/content/13/24/7413.full.html#related-urls>

**E-mail alerts**

[Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions**

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions**

To request permission to re-use all or part of this article, contact the AACR Publications Department at [permissions@aacr.org](mailto:permissions@aacr.org).

## BRCA1 mRNA Expression Levels Predict for Overall Survival in Ovarian Cancer after Chemotherapy

Jennifer E. Quinn, Colin R. James, Gail E. Stewart, Jude M. Mulligan, Patricia White  
Gary K.F. Chang, Paul B. Mullan, Patrick G. Johnston, Richard H. Wilson, and D. Paul Harkin

**Abstract** **Purpose:** We investigated whether *BRCA1* mRNA expression levels may represent a biomarker of survival in sporadic epithelial ovarian cancer following chemotherapy treatment.  
**Experimental Design:** The effect of loss of *BRCA1* expression on chemotherapy response in ovarian cancer was measured *in vitro* using dose inhibition assays and Annexin V flow cytometry. Univariate and multivariate analyses were done to evaluate the relationship between *BRCA1* mRNA expression levels and survival after chemotherapy treatment in 70 fresh frozen ovarian tumors.  
**Results:** We show that inhibition of endogenous *BRCA1* expression in ovarian cancer cell lines results in increased sensitivity to platinum therapy and decreased sensitivity to antimicrotubule agents. In addition, we show that patients with low/intermediate levels of *BRCA1* mRNA have a significantly improved overall survival following treatment with platinum-based chemotherapy in comparison with patients with high levels of *BRCA1* mRNA (57.2 versus 18.2 months;  $P = 0.0017$ ; hazard ratio, 2.9). Furthermore, overall median survival for higher-*BRCA1*-expressing patients was found to increase following taxane-containing chemotherapy (23.0 versus 18.2 months;  $P = 0.12$ ; hazard ratio, 0.53).  
**Conclusions:** We provide evidence to support a role for *BRCA1* mRNA expression as a predictive marker of survival in sporadic epithelial ovarian cancer.

The *BRCA1* tumor suppressor gene is associated with susceptibility to both hereditary breast and ovarian cancer (1, 2). Approximately 5% to 15% of ovarian cancers are inherited and *BRCA1* germ-line mutations account for 90% of these cases conferring a cumulative lifetime risk of 54% compared with 1.8% within the general population (3, 4). Although somatic mutations of *BRCA1* are uncommon in sporadic ovarian tumors (5), down-regulation of *BRCA1* has been reported in >72% of high-grade sporadic ovarian cancers, suggesting that *BRCA1* may also play a major role in the development of sporadic epithelial ovarian cancer (6, 7). Epigenetic inactivation of *BRCA1* is at least partially due to

hypermethylation of the *BRCA1* promoter, which has been observed in up to 15% of cases (8, 9).

Several preclinical breast cancer studies have indicated that *BRCA1* is an important determinant of response to both DNA-damaging and taxane-based chemotherapy. Evidence that *BRCA1* deficiency, whether through inherited mutation or epigenetic down-regulation, confers marked sensitivity to DNA-damaging agents is derived from numerous *in vitro* studies (10–15). Furthermore, several retrospective breast cancer clinical studies are in agreement with these preclinical findings. It has been shown that *BRCA1* mutation carriers gain a significant survival advantage from DNA damage–based chemotherapy compared with non–mutation carriers (16, 17). In addition, reduced *BRCA1* protein expression in sporadic epithelial ovarian cancer was found to correlate with improved survival (18). Finally, a retrospective analysis of *BRCA1* mRNA levels in a cohort of sporadic lung tumors showed that low-*BRCA1*-expressing patients had reduced risk of death following cisplatin treatment (19). Taken together, these studies provide evidence that, in addition to *BRCA1* mutation, reduced expression levels of *BRCA1* mRNA and protein may also predict response to DNA damage–based chemotherapy.

In contrast to the increased response to DNA damage–based chemotherapy observed in *BRCA1* deficient cells, there is now evidence to suggest that loss of functional *BRCA1* confers increased resistance to antimicrotubule agents. Initially, it was observed that overexpression of *BRCA1* in MBR62-bcl2 breast cancer cells enhanced sensitivity to paclitaxel (20). Subsequently, it was shown that reconstitution of exogenous *BRCA1* in the *BRCA1*-mutant HCC1937 breast cancer cell line resulted in

**Author's Affiliation:** Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, United Kingdom

Received 5/7/07; revised 7/2/07; accepted 8/14/07.

**Grant support:** Breast Cancer Campaign (J.E. Quinn and D.P. Harkin); Action Cancer (J.E. Quinn, P.B. Mullan, and J.M. Mulligan); Cancer Research UK (C.R. James, R.H. Wilson, P.G. Johnston, and D.P. Harkin); R&D Office, Department of Health, Social Services and Public Safety for Northern Ireland (P.B. Mullan); Medical Research Council UK (G.E. Stewart); and Queen's University Belfast (P. White and G.K.F. Chang).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Note:** J.E. Quinn and C.R. James contributed equally to this work.

**Requests for reprints:** D. Paul Harkin, Centre for Cancer Research and Cell Biology, Queen's University of Belfast, 97, Lisburn Road, Belfast BT9 7BL, United Kingdom. Phone: 44-28-9097-2760; Fax: 44-28-9097-2776; E-mail: d.harkin@qub.ac.uk.

© 2007 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-07-1083

enhanced sensitivity to both paclitaxel and vinorelbine (10, 11). In accordance, inhibition of endogenous BRCA1 expression by small interfering RNA (siRNA)-mediated knockdown in both T47D and MCF7 breast cancer cells resulted in resistance to paclitaxel (11, 21). Furthermore, BRCA1-deficient murine cells showed a reduced apoptotic response following treatment with taxanes (14). Finally, a recent clinical study suggested that absence of BRCA1 expression is an independent predictor of time to progression in advanced breast cancer patients treated with taxanes (22).

To date, the role of BRCA1 as a predictor of differential response to chemotherapy in ovarian cancer has been less well established. However, in agreement with *in vitro* breast cancer cell line models, it has been shown that antisense inhibition of BRCA1 in cisplatin-resistant SKOV3 ovarian cancer cells dramatically sensitized these cells by disruption of BRCA1-dependent DNA repair (23). Similarly, overexpression of BRCA1 in ID8 murine ovarian cancer cells reduced sensitivity to cisplatin and other DNA-damaging agents (24). Furthermore, transfection of BRCA1 into the BRCA1-mutated SNU251 ovarian cancer cell line increased resistance to ionizing radiation (25). Finally, clinical studies have reported that BRCA1 mutation status is a significant predictor of survival in patients with advanced ovarian carcinoma, which may be due to increased benefit from platinum-based chemotherapy (26, 27). The role of BRCA1 mRNA expression as a predictive marker of response to both platinum agents and taxanes in sporadic ovarian cancer has not yet been assessed. We therefore evaluated the role of BRCA1 mRNA expression as a predictive marker of survival following chemotherapy in sporadic epithelial ovarian cancer.

## Materials and Methods

**In vitro ovarian cancer cell lines.** The BG1-Neo and BG1-AS4 cell lines were gifts from Dr. Lois Annab (National Institute of Environmental Health Sciences, Research Triangle Park, NC; ref. 28) and OVCAR5 cells were purchased from Cancer Research UK Cell Services. Cells were maintained in DMEM and RPMI, respectively, and supplemented with 10% fetal calf serum, 1 mmol/L sodium pyruvate, and 50 µg/mL penicillin-streptomycin (Life Technologies, Inc.).

**Antibodies and Western blot analysis.** BRCA1 immunoprecipitation-Western blots were done with the mouse monoclonal antibodies AB-1 and AB-4 for immunoprecipitation and immunoblotting, respectively (Calbiochem). Protein lysates (1 mg) were prepared using ELB buffer before immunoprecipitation and separated by 6% SDS-PAGE as previously described (11).

**SiRNA abrogation of BRCA1 expression.** OVCAR5 cells were transfected twice with a BRCA1-specific oligonucleotide and a scrambled oligonucleotide control using Oligofectamine (Invitrogen) as previously described (11). Dose inhibition assays were done 24 h posttransfection as described below.

**Dose inhibition assays.** Assays were done using BG1-Neo and BG1-AS4 cells, and OVCAR5 cells transfected with either scrambled siRNA or BRCA1 siRNA oligonucleotides. Cells were then treated for 48 h with either paclitaxel (Taxol, Bristol Myers Squibb), docetaxel (Taxotere, Sanofi Aventis), carboplatin (Carboplatin, Mayne Pharma, Plc), or cisplatin (Cisplatin, Mayne Pharma) within a concentration range of  $10^{-4}$  and  $10^{-12}$  mol/L as previously described (11). Inhibitory concentrations ( $IC_{50}$ ) were then calculated using Prism software.

**Annexin V-FITC apoptosis analysis.** BG1-Neo and BG1-AS4 were either left untreated or treated for 48 h with 10 nmol/L paclitaxel and 1 µmol/L cisplatin before staining with both propidium iodide and FITC-conjugated Annexin V (Trevigen, Inc.) according to the manu-

facturer's instructions. Flow cytometric analysis was done with a Coulter Epics XL flow cytometer.

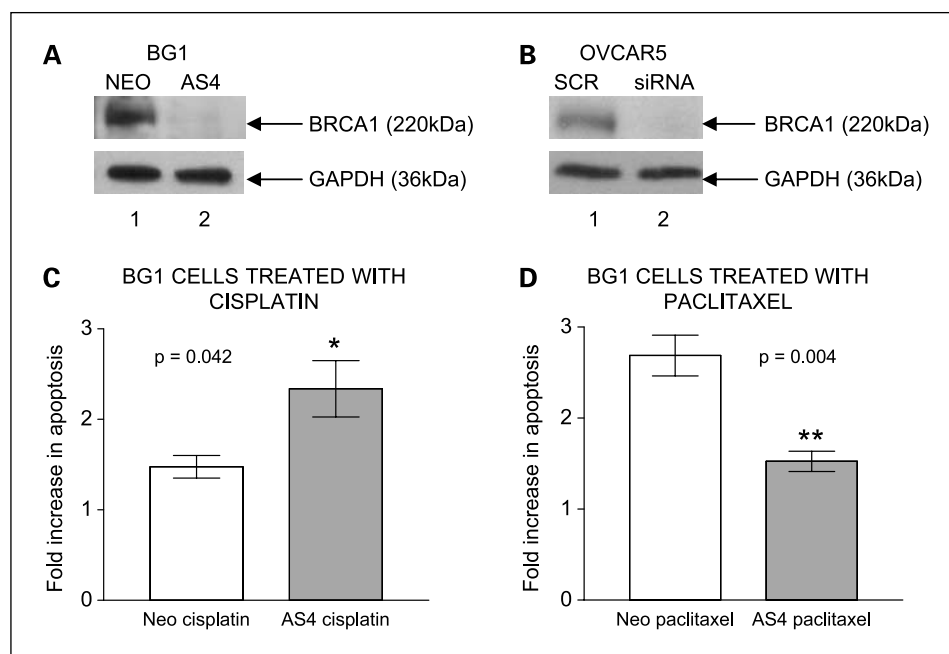
**Patients.** Seventy ovarian cancer cases were identified of patients who had previously been treated with surgery and chemotherapy at the Northern Ireland Cancer Centre, Belfast, United Kingdom. Median age at diagnosis was  $58.9 \pm 2.8$  years and 78.6% of the patients presented with stage III or stage IV. In addition, it was noted that none of the patients were BRCA1 mutation carriers. Further clinicopathologic characteristics of the patient cohort are summarized in Table 1. Ovarian tumor sections were taken at the time of surgery and immediately snap frozen. Samples for analysis were histopathologically verified as having high tumor content and minimal stromal contamination. The median follow-up of patients was 88.5 months (range, 57-174 months). Clinical outcomes were obtained from hospital records; ethical approval was obtained from the Office of Research Ethics Committees in Northern Ireland and National Health Service research governance approval from the Belfast City Hospital Trust.

**Measurement of BRCA1 gene expression in ovarian tissue samples.** Total cellular RNA was extracted from tumor samples using RNA Stat60 (Tel-Test, Inc.) before treatment with DNase I (Invitrogen). Total RNA (2 µg) was then reverse transcribed for cDNA generation using random primers and Moloney murine leukemia virus reverse transcriptase (both from Invitrogen) as described by the manufacturer. A quantitative real-time PCR method was used to measure BRCA1 mRNA expression in fresh-frozen ovarian tumors as previously described (29). Primer sequences for the endogenous reference genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and  $\beta$ -tubulin, were GAPDH, 5'-TCAAGAAGGTGGTGAAGCAG-3' (forward) and 5'-AAAGGTGGAG-GAGTGGGTGT-3' (reverse), and  $\beta$ -tubulin, 5'-CGCAGAAGAGGAG-GAGGATT-3' (forward) and 5'-GAGGAAAGGGGCGAGTTGAGT-3' (reverse). Two BRCA1-specific primer sets, termed BRCA1ss1 and BRCA1ss2, were used and sequences were, for BRCA1ss1, 5'-GGCT-ATCCTCTCAGAGTGACATTT-3' (forward) and 5'-GCCTTATCAGGT-TATGTTGCATGG T-3' (reverse), as previously described (19), and for BRCA1ss2, 5'-GCGTCCCCTCACAAATAAAT-3' (forward) and 5'-CTTGACCATTCTGCTCCGTT-3' (reverse). Quantitative real-time PCR analysis was done on the Opticon 2 System (Bio-Rad) using SYBR Green (Finnzymes) according to the manufacturer's instructions. Relative BRCA1 mRNA expression was quantified using the standard curve method. For accurate normalization purposes, the linearity of the

**Table 1.** Clinicopathologic patient characteristics in retrospective study (N = 70)

Characteristic	No. patients (%)
Stage	
I/II	15 (21.4)
III/IV	55 (78.6)
Grade	
1/2	16 (22.8)
3/4	48 (68.6)
Not known	6 (8.6)
Histology	
Serous	35 (50.0)
Mucinous	7 (10.0)
Endometrioid	15 (21.4)
Clear cell	5 (7.1)
Undifferentiated	7 (10.0)
Not known	1 (1.5)
Surgery	
Optimal	35 (50.0)
Suboptimal	21 (30.0)
Interval	2 (2.9)
Not known	12 (17.1)
Chemotherapy	
Platinum	46 (65.4)
Paclitaxel/platinum	24 (34.6)

**Fig. 1.** Immunoprecipitation-Western blots confirming abrogation of endogenous BRCA1 protein expression in BG1-AS4 (A, lane 2) versus BG1-Neo (A, lane 1) and OVCAR5 BRCA1-siRNA (B, lane 2) versus OVCAR5 scrambled (B, lane 1) epithelial ovarian cancer cell lines. GAPDH controls are included to confirm equal loading. Bar graphs show a 2.5-fold increase in apoptosis in BG1-AS4 cells treated with cisplatin compared with 1.5-fold in BG1-Neo cells (C) and a 2.7-fold increase in apoptosis in BG1-Neo cells treated with paclitaxel compared with 1.5-fold in BG1-AS4 cells (D) as measured in triplicate by Annexin V flow cytometry.



PCR amplification reactions for endogenous reference genes was confirmed as comparable to both BRCA1 primer sets (data not shown). *BRCA1*,  *$\beta$ -tubulin*, and *GAPDH* mRNA levels were then calculated from their respective standard curves. *BRCA1* mRNA was defined as the mean of its quantification by each BRCA1 primer set following normalization to both  *$\beta$ -tubulin* and *GAPDH*. Results obtained were the mean of two independent experiments.

**Statistical analysis.** Patient characteristics were compared using simple descriptive statistics with *t* tests and  $\chi^2$  contingency tables. Overall survival was assessed using Kaplan-Meier curves with the log-rank test and Cox proportional hazards. All statistical analyses were done using Prism and SSPS version 15 software.

## Results

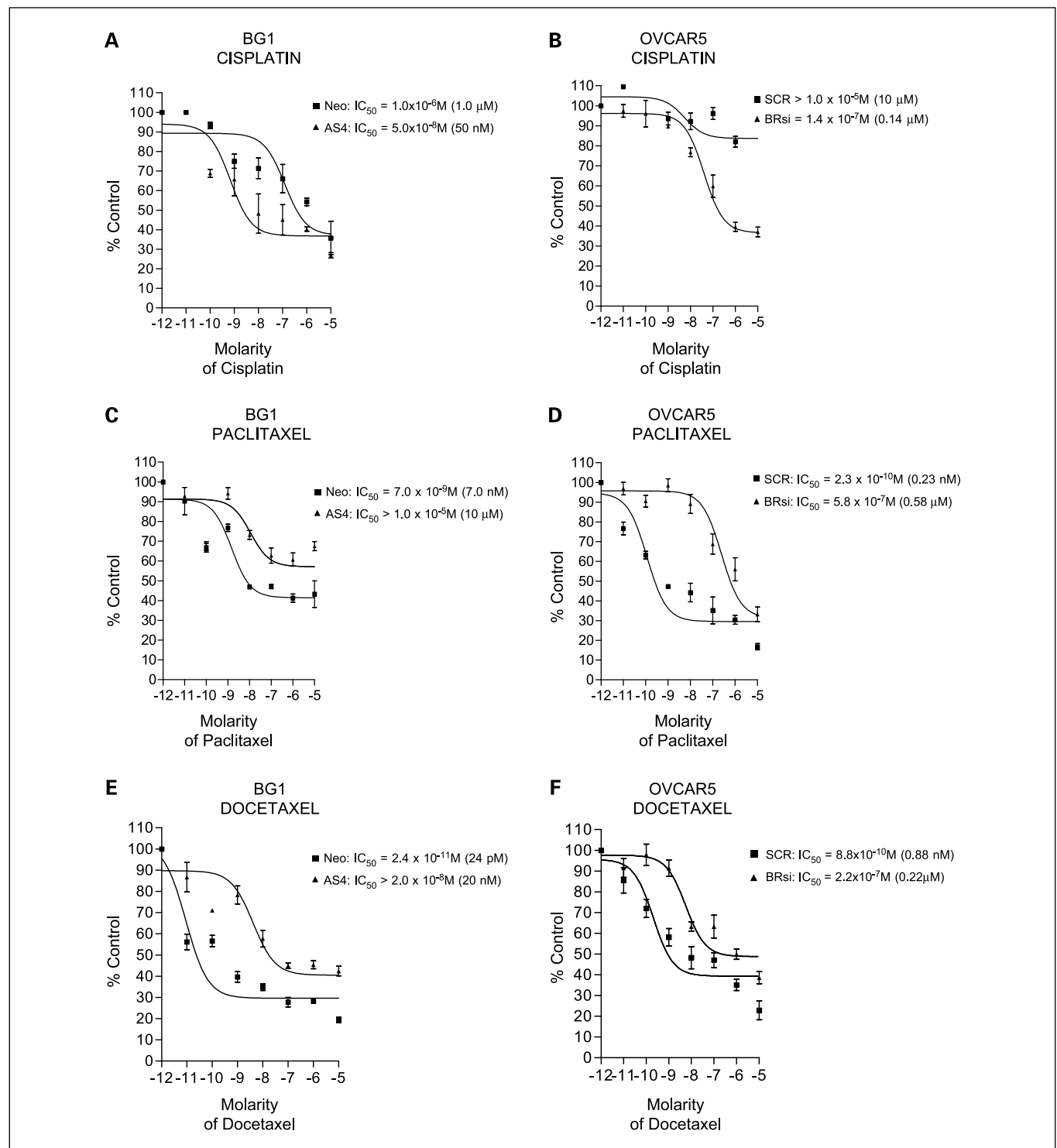
**BRCA1 differentially modulates chemotherapy-induced apoptosis in two in vitro models of sporadic epithelial ovarian cancer.** BRCA1 has previously been implicated as a differential modulator of chemotherapy response in several breast cancer cell line models (30). To investigate the effect of down-regulated BRCA1 expression on chemosensitivity in sporadic ovarian cancer, we used two complementary *in vitro* ovarian cancer cell line models. The BG1 cell line was derived from a patient with stage III, poorly differentiated ovarian adenocarcinoma and is wild-type for both BRCA1 and p53 (31). BG1 cells were stably transfected with a BRCA1 antisense construct or an empty vector control to generate the BG1-AS4 and BG1-Neo cell lines, respectively (28). The OVCAR5 cell line was derived from a patient with advanced ovarian epithelial cancer with no prior cytotoxic therapy and is also wild-type for both BRCA1 and p53 (32). Abrogation of endogenous BRCA1 expression in this cell line was achieved using a siRNA-based approach. Antisense- and siRNA-mediated inhibition of endogenous BRCA1 protein expression in BG1 and OVCAR5 cells, respectively, was confirmed by immunoprecipitation-Western blot analysis (Fig. 1A and B).

We proceeded to investigate the ability of BRCA1 to regulate apoptotic response following platinum- and taxane-based

chemotherapy drugs. To do this, we carried out Annexin V flow cytometry in which cellular uptake of both propidium iodide and FITC-conjugated Annexin V is representative of late apoptosis. BG1-Neo and BG1-AS4 cells were either untreated or treated with 10 nmol/L paclitaxel or 1  $\mu$ mol/L cisplatin for 48 h, and the resulting apoptotic fractions were normalized to their appropriate controls. Following 1  $\mu$ mol/L cisplatin treatment, a significantly higher apoptotic fraction was observed in BG1-AS4 cells compared with BG1-Neo cells [2.3-fold (SE, 0.310) versus 1.5-fold (SE, 0.125); *P* = 0.042; Fig. 1C]. In contrast, treatment of BG1-Neo cells with 10 nmol/L paclitaxel resulted in a 2.7-fold increase in the proportion of these cells undergoing apoptosis, compared with a 1.5-fold increase in BG1-AS4 cells (*P* = 0.004; Fig. 1D). Taken together, these data reveal that reduced BRCA1 expression promotes apoptotic response to cisplatin and inhibits apoptotic response to paclitaxel.

To further assess these phenotypic responses, we carried out dose inhibition assays using both BG1 and OVCAR5 cells. In agreement with the Annexin V flow cytometry data, we showed that antisense inhibition of BRCA1 expression in BG1-AS4 cells results in a >60-fold increase in sensitivity on treatment with cisplatin with *IC*<sub>50</sub> values decreasing from 1.0  $\mu$ mol/L in BG1-Neo cells to 50 nmol/L in BG1-AS4 cells (Fig. 2A). In agreement with this, when compared with scrambled oligonucleotide control, siRNA-mediated inhibition of endogenous BRCA1 expression in OVCAR5 cells results in a >80-fold increase in sensitivity on treatment with cisplatin with *IC*<sub>50</sub> values decreasing from >10 to 0.14  $\mu$ mol/L (Fig. 2B). Similar effects were also observed following treatment with carboplatin in both cell line models (data not shown).

In contrast to the observed increase in sensitivity following treatment with platinum-based compounds, inhibition of endogenous BRCA1 expression resulted in a marked increase in resistance to the antimicrotubule agents paclitaxel and docetaxel. Specifically, antisense inhibition of BRCA1 in BG1-AS4 cells decreased paclitaxel sensitivity by >1,000-fold with *IC*<sub>50</sub> values increasing from 7.0 nmol/L in BG1-Neo cells to



**Fig. 2.** Dose inhibition assays in BG1 (A, C, and E) and OVCAR5 (B, D, and F) epithelial ovarian cancer cell lines using antisense and siRNA knockdown of endogenous BRCA1 expression, respectively, followed by treatment with cisplatin (A and B), paclitaxel (C and D), and docetaxel (E and F). ■, BG1-Neo and OVCAR5-SCR (scrambled oligonucleotide control); ▲, BG1-AS4 and OVCAR5-BRsi (BRCA1 siRNA oligonucleotide).

>10  $\mu M/L$  in BG1-AS4 cells (Fig. 2C). In addition, compared with scrambled control, siRNA-mediated knockdown of BRCA1 in OVCAR5 cells resulted in a >2,000-fold decrease in sensitivity to paclitaxel with  $IC_{50}$  values increasing from 0.23 nmol/L to 0.58  $\mu M/L$  (Fig. 2D).

Similar effects were observed following docetaxel treatment with antisense to BRCA1 in BG1-AS4 cells, decreasing docetaxel sensitivity by >800-fold with  $IC_{50}$  values increasing from 24 pmol/L in BG1-Neo cells to 20 nmol/L in BG1-AS4 cells (Fig. 2E). In accordance, when compared with scrambled



control, abrogation of BRCA1 using siRNA in OVCAR5 cells resulted in a >250-fold decrease in sensitivity to paclitaxel with IC<sub>50</sub> values increasing from 0.88 nmol/L to 0.22  $\mu$ mol/L (Fig. 2F). Taken together, these data show that reduced BRCA1 expression in both BG1 and OVCAR5 cells confers increased sensitivity to the DNA-damaging agents cisplatin and carboplatin and increased resistance to the taxanes paclitaxel and docetaxel.

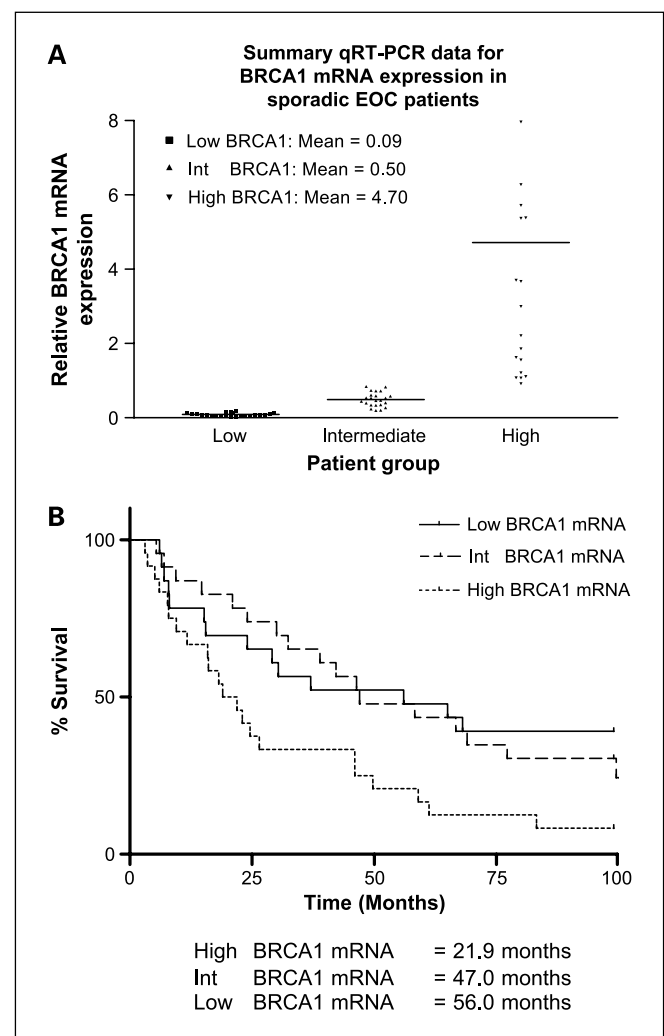
**BRCA1 is a predictive marker of survival following chemotherapy in epithelial ovarian cancer patients.** Based on our preclinical data, we investigated if BRCA1 mRNA expression levels might predict survival outcome *in vivo*. To do this, we carried out a retrospective clinical analysis of seventy sporadic ovarian cancer patients and correlated BRCA1 mRNA expression levels with overall survival following treatment with platinum or a combination of platinum and paclitaxel chemotherapy.

Clinicopathologic characteristics of the patient cohort are described in Table 1. It was noted that 46 patients received platinum-based chemotherapy as first-line treatment (Table 1). This included single-agent carboplatin or cisplatin or a combination of both cisplatin and cyclophosphamide. Twenty-four patients received paclitaxel in combination with either cisplatin or carboplatin. The median survival for all patients was 37.0 months [95% confidence interval (95% CI), 19.7-54.3 months].

Quantitative real-time PCR was done on all 70 samples using BRCA1-specific primers relative to  $\beta$ -tubulin and GAPDH controls. BRCA1 mRNA expression was measurable in all samples and considerable variability in expression levels was observed on mean normalization of BRCA1 to both endogenous reference genes. Patients were subdivided into three groups based on low (0.015-0.17; mean, 0.09), intermediate (0.20-0.84; mean, 0.5), and high (0.90-14.1; mean, 4.7) levels of BRCA1 mRNA expression (Fig. 3A) and the clinical characteristics of these three subdivisions were then compared. In a univariate analysis of overall survival, patients who had low levels of BRCA1 mRNA had a median survival of 56.0 months (95% CI, 1.8-110.2 months) whereas those with intermediate levels of BRCA1 mRNA had 47.0 months (95% CI, 21.9-72.1 months) and those with high levels of BRCA1 mRNA had a median survival of 21.9 months (95% CI, 10.7-27.3 months; Fig. 3B). Because patients in the low and intermediate groups had similar levels of BRCA1 mRNA (normalized to <1; SD, 0.26) and because there was no significant difference in their median survival ( $P = 0.85$ ), these two groups were combined for further statistical analysis. Clinicopathologic characteristics of these two patient groups (low/intermediate BRCA1 expressors versus high BRCA1 expressors) are summarized in Table 2.

Univariate analysis of patients who received a platinum-based chemotherapy regimen (Fig. 4A) revealed that patients who had low/intermediate levels of BRCA1 mRNA in comparison with patients with high levels of BRCA1 mRNA had a statistically significant reduction in risk of death, which related to a survival advantage of 39 months [57.2 months (95% CI, 25.7-86.3) versus 18.2 months (95% CI, 8.0-28.4);  $P = 0.0017$ ; hazard ratio, 2.9]. This suggests that patients with ovarian tumors displaying lower levels of BRCA1 mRNA expression may gain greatest benefit in terms of increased survival when treated with platinum-containing chemotherapy regimens.

Furthermore, there was no evidence that patients with low/intermediate levels of BRCA1 gained any benefit from the addition of a taxane (data not shown). However, univariate analysis did suggest a trend toward additional benefit from taxanes in patients with higher levels of BRCA1 mRNA, with a 4.8-month increase in median survival when compared with those who received platinum-only chemotherapy [23.0 months (95% CI, 10.2-33.6) versus 18.2 months (95% CI, 8.0-28.4);  $P = 0.12$ ; hazard ratio, 0.53]. Although nonsignificant, these data suggest that there may be a reduction in the risk of death for patients with high levels of BRCA1 when paclitaxel is used in combination with a platinum agent (Fig. 4B). Therefore, patients with high levels of BRCA1 mRNA in tumors may gain a survival advantage on treatment with taxane-based chemotherapy. Further univariate analysis revealed that stage was also significantly associated with survival and patients with stage III/IV had a worse survival ( $P = 0.001$ ; hazard ratio, 3.84). Multivariate analysis using Cox regression revealed that both



**Fig. 3.** A, diagram showing the stratification of patients into three groups based on low BRCA1 mRNA (0.015-0.17; mean, 0.09), intermediate (Int) BRCA1 mRNA (0.20-0.84; mean, 0.5), and high BRCA1 mRNA (0.90-14.1; mean, 4.7). B, Kaplan-Meier overall survival curves of sporadic epithelial ovarian cancer patients classified as having low, intermediate, or high levels of BRCA1 mRNA expression (low versus intermediate BRCA1 mRNA,  $P = 0.85$ ; intermediate versus high BRCA1 mRNA,  $P = 0.016$ ; low versus high BRCA1 mRNA,  $P = 0.016$ ).

**Table 2.** Clinicopathologic patient characteristics according to BRCA1 mRNA

Characteristic	Low/Intermediate BRCA1 mRNA (n = 47)	High BRCA1 mRNA (n = 23)	P
Age (y)	60.5	60.1	0.730
Stage III/IV	36	19	0.112
Grade 3/4	39	15	0.456
Surgery			
Optimal	24	11	
Suboptimal	14	7	
Interval	1	1	0.790
Treatment			
Platinum	35	11	
Paclitaxel/platinum	12	12	0.027

BRCA1 mRNA expression ( $P = 0.006$ ; hazard ratio, 2.63) and stage ( $P = 0.023$ ; hazard ratio, 3.013) continued to be of statistical significance.

## Discussion

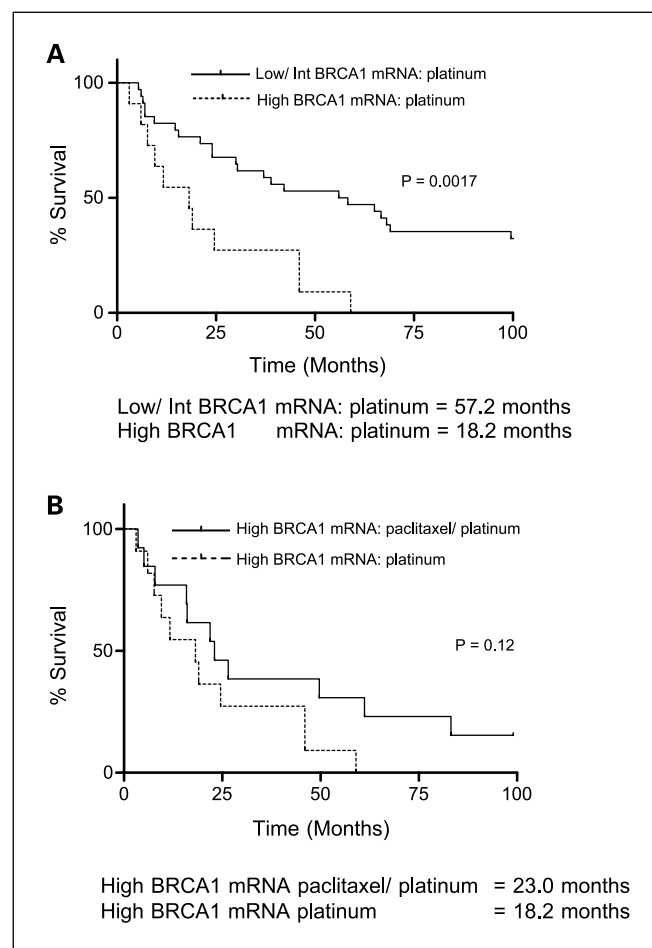
We provide both preclinical and clinical evidence to suggest that BRCA1 is a differential modulator of survival in sporadic ovarian cancer. Specifically, we show in both BG1 and OVCAR5 cells that inhibition of endogenous BRCA1 expression results in a significant increase in resistance to the antimicrotubule agents paclitaxel and docetaxel and, conversely, an increase in sensitivity to the DNA cross-linking agents cisplatin and carboplatin. In addition, we show that the observed differential sensitivity is due to differential regulation of apoptosis by BRCA1. Finally, we provide retrospective clinical data to suggest that low levels of BRCA1 mRNA correlate significantly with improved outcome on treatment with single-agent platinum and that patients with high levels of BRCA1 mRNA may gain greater benefit from combination chemotherapy involving platinates and taxanes.

In accordance with our observation that reduced BRCA1 expression confers enhanced sensitivity to cisplatin, antisense inhibition of BRCA1 in SKOV3 ovarian cancer cells results in increased cisplatin cytotoxicity (23). Several *in vitro* murine ovarian cancer models are also in agreement. First, overexpression of a BRCA1 dominant negative mutant in ID8 murine ovarian cancer cells correlates with increased sensitivity to cisplatin (24). Second, BRCA1-deficient murine ovarian surface epithelial cells were more sensitive to cisplatin-induced cell death when compared with corresponding BRCA1 wild-type cells (33). A third report also showed that conditional inactivation of BRCA1 in the mouse ovarian surface epithelium results in enhanced cisplatin sensitivity (34). Finally, a recent study using genome-wide siRNA screening revealed that increased cisplatin cytotoxicity was observed in BRCA1 dysfunctional tumor cells, and this effect was enhanced with p53 deficiency (35).

Fewer studies have investigated the potential role of BRCA1-mediated response to taxanes. We provide the first preclinical evidence that wild-type BRCA1 confers a significant increase in sensitivity to the antimicrotubule agents paclitaxel and docetaxel in ovarian cancer cell lines. This observation is consistent with numerous other breast cancer studies (36). Several clinical studies have shown that BRCA1 mutation carriers have a better

clinical response to anthracycline- and cyclophosphamide-containing chemotherapy than corresponding sporadic breast cancer patients (16). In support of this, BRCA1 carriers are also more sensitive to radiation (17). In agreement with these breast cancer studies, BRCA1 germ-line mutation carriers with epithelial ovarian cancer have a significantly more favorable outcome than patients with sporadic ovarian cancer (26, 37, 38). This increased survival may be potentially explained by the enhanced response of BRCA1 associated patients to platinum-based chemotherapy (27), which is likely due to loss of BRCA1-mediated DNA damage response and DNA repair.

The consequence of epigenetic inactivation of BRCA1 on taxane-based chemotherapy response in sporadic epithelial ovarian cancer has not yet been reported. This is a somewhat surprising fact because BRCA1 is epigenetically inactivated in 72% to 90% of sporadic ovarian tumors (6, 7). To date, only one sporadic breast cancer study has investigated levels of BRCA1 mRNA expression and response to anthracycline-based chemotherapy, and it suggested that high levels of BRCA1 mRNA correlate with response to anthracycline-based chemotherapy (39). It is unclear why such a different effect was



**Fig. 4.** A, Kaplan-Meier overall survival curves of sporadic epithelial ovarian cancer patients treated with platinum only—based chemotherapy comparing low/intermediate levels of BRCA1 mRNA expression with high levels of BRCA1 mRNA expression. B, Kaplan-Meier overall survival curves of sporadic epithelial ovarian cancer patients with high levels of BRCA1 mRNA expression following treatment with either platinum only—based or paclitaxel/platinum only—based chemotherapy.

observed in this study, which is in contrast to that observed in the inherited form of breast cancer or in the *in vitro* models of breast cancer already reported in the literature (40). It may possibly relate to the small sample size or because the study measured rates of response rather than overall survival rates as in the current study. However, in accordance with our current findings, *BRCA1* mRNA expression levels were reported to be important in predicting outcome following cisplatin-containing chemotherapy in sporadic non-small-cell lung cancer (19). Specifically, it was shown that patients with low levels of *BRCA1* mRNA gained greatest benefit from cisplatin-based chemotherapy treatment whereas higher-*BRCA1*-expressing patients had the poorest outcome. The authors concluded that levels of *BRCA1* mRNA expression were indicative of differential cisplatin sensitivity in non-small-cell lung cancer, which is consistent with our current study.

We have also observed that functional *BRCA1* can enhance the response of ovarian cancer cells to paclitaxel *in vitro*. Previously published phase III ovarian cancer clinical trials show that the addition of paclitaxel to platinum provides significant improvements in response rates and overall survival when compared with cyclophosphamide and cisplatin (41, 42). Our retrospective clinical data suggest that high levels of *BRCA1* mRNA may predict which patients gain survival benefits following paclitaxel-based chemotherapy. In addition, a recent breast cancer study supports this observation because *BRCA1*-

expressing tumors had improved clinical outcome following taxane chemotherapy as assessed by time to progression (22).

In conclusion, we provide both preclinical and retrospective clinical data to suggest that *BRCA1* may function as a predictive biomarker of response to chemotherapy agents used in the treatment of ovarian cancer. Our data suggest that sporadic epithelial ovarian cancer patients with low levels of *BRCA1* mRNA expression may gain the majority of therapeutic benefit from platinum chemotherapy agents, whereas patients with higher levels of *BRCA1* potentially have a better response to regimens containing taxanes. These observations suggest that *BRCA1* mRNA levels may be an important therapeutic biomarker in treatment decisions involving sporadic epithelial ovarian cancer patients. These preliminary findings will need to be further validated in a larger retrospective cohort of epithelial ovarian cancer patients and may, in future, potentially be used to stratify patients for choice of therapy most likely to result in benefit.

## Acknowledgments

We thank our funders for their generous support; the Belfast City Hospital gynecological oncologists for harvesting tumor samples and our patients for consenting to provide these; Dr. Susan Farragher (Almac Diagnostics, Craigavon, United Kingdom) for technical advice; and Dr. Richard Kennedy (Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, MA) for critical review of the manuscript.

## References

- Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science* 1994;266:66–71.
- Cannistra SA. Cancer of the ovary. *N Engl J Med* 2004;351:2519–29.
- King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in *BRCA1* and *BRCA2*. *Science* 2003;302:643–6.
- Pal T, Permuth-Wey J, Betts JA, et al. *BRCA1* and *BRCA2* mutations account for a large proportion of ovarian carcinoma cases. *Cancer* 2005;104:2807–16.
- McCoy ML, Mueller CR, Roskelley CD. The role of the breast cancer susceptibility gene 1 (*BRCA1*) in sporadic epithelial ovarian cancer. *Reprod Biol Endocrinol* 2003;1:72.
- Russell PA, Pharoah PD, De Foy K, et al. Frequent loss of *BRCA1* mRNA and protein expression in sporadic ovarian cancers. *Int J Cancer* 2000;87:317–21.
- Wang C, Horiuchi A, Imai T, et al. Expression of *BRCA1* protein in benign, borderline, and malignant epithelial ovarian neoplasms and its relationship to methylation and allelic loss of the *BRCA1* gene. *J Pathol* 2004;202:215–3.
- Baldwin RL, Nemeth E, Tran H, et al. *BRCA1* promoter region hypermethylation in ovarian carcinoma: a population-based study. *Cancer Res* 2000;60:5329–33.
- Chan KY, Ozcelik H, Cheung AN, Ngan HY, Khoo US. Epigenetic factors controlling the *BRCA1* and *BRCA2* genes in sporadic ovarian cancer. *Cancer Res* 2002;62:4151–6.
- Tassone P, Tagliaferri P, Perricelli A, et al. *BRCA1* expression modulates chemosensitivity of *BRCA1*-defective HCC1937 human breast cancer cells. *Br J Cancer* 2003;88:1285–91.
- Quinn JE, Kennedy RD, Mullan PB, et al. *BRCA1* functions as a differential modulator of chemotherapy-induced apoptosis. *Cancer Res* 2003;63:6221–8.
- Lafarge S, Sylvain V, Ferrara M, Bignon YJ. Inhibition of *BRCA1* leads to increased chemoresistance to microtubule-interfering agents, an effect that involves the JNK pathway. *Oncogene* 2001;20:6597–606.
- Moynahan ME, Chiu JW, Koller BH, Jasin M. *Brcal* controls homology-directed DNA repair. *Mol Cell* 1999;4:511–8.
- Fedier A, Steiner RA, Schwarz VA, Lenherr L, Haller U, Fink D. The effect of loss of *brcal* on the sensitivity to anticancer agents in p53-deficient cells. *Int J Oncol* 2003;22:1169–73.
- Scagias MK, Wagner KU, Hamik B, et al. *Brcal*-deficient murine mammary epithelial cells have increased sensitivity to CDDP and MMS. *Cell Cycle* 2004;3:1451–6.
- Chappuis PO, Goffin J, Wong N, et al. A significant response to neoadjuvant chemotherapy in *BRCA1/2* related breast cancer. *J Med Genet* 2002;39:608–10.
- Kirova YM, Stoppa-Lyonnet D, Savignoni A, Sigal-Zafrani B, Fabre N, Fourquet A. Risk of breast cancer recurrence and contralateral breast cancer in relation to *BRCA1* and *BRCA2* mutation status following breast-conserving surgery and radiotherapy. *Eur J Cancer* 2005;41:2304–11.
- Thrall M, Gallion HH, Kryscio R, Kapali M, Armstrong DK, DeLoia JA. *BRCA1* expression in a large series of sporadic ovarian carcinomas: a Gynecologic Oncology Group study. *Int J Gynecol Cancer* 2006;11:166–71.
- Taron M, Rosell R, Felip E, et al. *BRCA1* mRNA expression levels as an indicator of chemoresistance in lung cancer. *Hum Mol Genet* 2004;13:2443–9.
- Mullan PB, Quinn JE, Gilmore PM, et al. *BRCA1* and GADD45 mediated G<sub>2</sub>/M cell cycle arrest in response to antimicrotubule agents. *Oncogene* 2001;20:6123–31.
- Chabalier C, Lamare C, Racca C, Privat M, Valette A, Larminat F. *BRCA1* down-regulation leads to premature inactivation of spindle checkpoint and confers paclitaxel resistance. *Cell Cycle* 2006;5:1001–7.
- Kurebayashi J, Yamamoto Y, Kurosaki M, et al. Loss of *BRCA1* expression may predict shorter time-to-progression in metastatic breast cancer patients treated with taxanes. *Anticancer Res* 2006;26:695–701.
- Husain A, He G, Venkatraman ES, Spriggs DR. *BRCA1* up-regulation is associated with repair-mediated resistance to *cis*-diamminedichloroplatinum(II). *Cancer Res* 1998;58:1120–3.
- Sylvain V, Lafarge S, Bignon YJ. Dominant-negative activity of a *Brcal* truncation mutant: effects on proliferation, tumorigenicity *in vivo*, and chemosensitivity in a mouse ovarian cancer cell line. *Int J Oncol* 2002;20:845–53.
- Zhou C, Smith JL, Liu J. Role of *BRCA1* in cellular resistance to paclitaxel and ionizing radiation in an ovarian cancer cell line carrying a defective *BRCA1*. *Oncogene* 2003;22:2396–404.
- Rubin SC, Benjamin I, Behbakht K, et al. Clinical and pathological features of ovarian cancer in women with germ-line mutations of *BRCA1*. *N Engl J Med* 1996;335:1413–6.
- Cass I, Baldwin RL, Varkey T, Moslehi R, Narod SA, Karlan BY. Improved survival in women with *BRCA*-associated ovarian carcinoma. *Cancer* 2003;97:2187–95.
- Annab LA, Hawkins RE, Solomon G, Barrett JC, Afshari CA. Increased cell survival by inhibition of *BRCA1* using an antisense approach in an estrogen responsive ovarian carcinoma cell line. *Breast Cancer Res* 2000;2:139–48.
- Kroupis C, Stathopoulou A, Zygaki E, Erekidou L, Talieri M, Lianidou ES. Development and applications of a real-time quantitative RT-PCR method (QRT-PCR) for *BRCA1* mRNA. *Clin Biochem* 2005;38:50–7.
- James CR, Quinn JE, Mullan PB, Johnston PG, Harkin DP. *BRCA1*, a potential predictive biomarker in the treatment of breast cancer. *Oncologist* 2007;12:142–50.
- Geisinger KR, Kute TE, Pettenati MJ, et al. Characterization of a human ovarian carcinoma cell line with estrogen and progesterone receptors. *Cancer* 1989;63:280–8.
- Hamilton TC, Young RC, Louie KG, et al. Characterization of a xenograft model of human ovarian carcinoma which produces ascites and intraabdominal carcinomatosis in mice. *Cancer Res* 1984;44:5286–90.
- Xing D, Orsulic S. A mouse model for the molecular characterization of *brcal*-associated ovarian carcinoma. *Cancer Res* 2006;66:8949–53.



34. Clark-Knowles KV, Garson K, Jonkers J, Vanderhyden BC. Conditional inactivation of *brca1* in the mouse ovarian surface epithelium results in an increase in preneoplastic changes. *Exp Cell Res* 2007;313:133–45.
35. Bartz SR, Zhang Z, Burchard J, et al. Small interfering RNA screens reveal enhanced cisplatin cytotoxicity in tumor cells having both BRCA network and TP53 disruptions. *Mol Cell Biol* 2006;26:9377–86.
36. Mullan PB, Gorski JJ, Harkin DP. BRCA1-A good predictive marker of drug sensitivity in breast cancer treatment? *Biochim Biophys Acta* 2006;2:205–16.
37. Boyd J, Sonoda Y, Federici MG, et al. Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. *JAMA* 2000;283:2260–5.
38. Ben David Y, Chetrit A, Hirsh-Yechezkel G, et al. Effect of BRCA mutations on the length of survival in epithelial ovarian tumors. *J Clin Oncol* 2002;20:463–6.
39. Egawa C, Motomura K, Miyoshi Y, et al. Increased expression of BRCA1 mRNA predicts favorable response to anthracycline-containing chemotherapy in breast cancers. *Breast Cancer Res Treat* 2003;78:45–50.
40. Kennedy RD, Quinn JE, Mullan PB, Johnston PG, Harkin DP. The role of BRCA1 in the cellular response to chemotherapy. *J Natl Cancer Inst* 2004;96:1659–68.
41. McGuire WP III. Current status of taxane and platinum-based chemotherapy in ovarian cancer. *J Clin Oncol* 2003;21:133–5.
42. Piccart MJ, Bertelsen K, James K, et al. Randomized intergroup trial of cisplatin-paclitaxel versus cisplatin-cyclophosphamide in women with advanced epithelial ovarian cancer: three-year results. *J Natl Cancer Inst* 2000;92:699–708.