Introduction

Ovarian cancers arising in the setting of germ line mutation of the BRCA1 tumor suppressor gene exhibit particular clinical and molecular features, including predominantly serous histology, a high grade suggestive of a more aggressive malignancy, and distinct gene expression profiles (1, 2). Furthermore, these tumors exhibit mutational inactivation of p53 in up to 80% of cases (3). Inactivation of p53 is thought to promote tumor cell survival in the face of BRCA1-dependent defects in DNA repair (3). The unfavorable histologic features and loss of functional p53 in BRCA1-associated ovarian cancers initially suggested that these tumors might be associated with chemoresistance and potentially a poor prognosis (1). Multiple studies, however, have confirmed that patients with BRCA1-associated ovarian carcinomas in fact experience a higher response rate and a more prolonged disease-free interval after platinum-based chemotherapy, as well as improved overall survival (3–5). In vitro studies of human ovarian cancer–derived cell lines have supported the view that loss of BRCA1 function is associated with selective chemosensitivity to platinum agents (6, 7). We and others have previously shown that platinum sensitivity is associated with unrepaired DNA damage in BRCA1-deficient ovarian carcinoma cells (7, 8). Nevertheless, the specific effector pathway that mediates the response to DNA damage in the absence of functional p53 has not been defined.

We have developed a murine model in which defined genetic events induce BRCA1-associated ovarian carcinomas that recapitulate the serous histology, the genetic instability, and the DNA damage sensitivity of human BRCA1-deficient ovarian carcinomas (8–10). We show here that BRCA1-deficient human and murine ovarian carcinoma cells exhibit
selective upregulation of transactivating isoforms of the p53-related gene p73 (TAp73), which is known to function as a mediator of the DNA damage response and chemosensitivity in many cellular contexts (11–14). We provide evidence that this TAp73 upregulation occurs through a novel epigenetic mechanism and that TAp73 serves as an important contributor to the platinum-induced DNA damage response and to chemosensitivity in ovarian carcinoma, both in vitro and in vivo. Our findings, therefore, define an epigenetic regulatory mechanism for p73 that plays a role in the clinical behavior of BRCA1-associated ovarian tumors.

Materials and Methods

Tissue culture and reagents
We (D. Xing and S. Orsulic) generated the murine ovarian carcinoma cell lines (T1, T2, T3, TBR2, TBR5, and TBR6). Western blot analysis was carried out to confirm the absence of whole-length BRCA1 proteins. These lines were maintained in DMEM/F12 containing 10% fetal bovine serum, 100 IU/mL of penicillin, and 100 μg/mL of streptomycin (In-vitrogen). The UWB1.289 human cell line was established (E.M. Swisher) as described (15); reconstituted BRCA1 expression was verified by quantitative reverse transcription–PCR (QRT-PCR). Cells were maintained in 1:1 RPMI 1640/MEGM (Lonza) supplemented with 3% fetal bovine serum. Chemosensitivity assays and antibody reagents are described in Supplementary Materials and Methods.

RNA analysis, lentiviral/retroviral production, and chromatin immunoprecipitation
QRT-PCR analysis was performed as described (13). Specific forward and reverse primer sequences are provided in Supplementary Table S1. High-titer amphotrophic retroviral and lentiviral stocks were generated by cotransfection with packaging vectors into 293T cells as described previously (13, 16). The targeted sequences are provided in Supplementary Table S2. Chromatin immunoprecipitation (ChIP) was carried out as described (16) using primers spanning different regions of p73 intron 1 (Supplementary Table S3).

Bisulfite treatment and methylation analysis
For each cell line and tumor, 200 to 500 ng of genomic DNA were used for bisulfite conversion using the EZ DNA Methylation-Gold kit (Zymo Research Corp.). The 1.6-kb intronic region upstream of exon 2 of p73 was PCR-amplified with primer sets designed using the program EpiderDesignerBETA by Sequenom (Supplementary Table S4). After confirmation of the desired product and quantification, methylation was quantitated in sequenced products by measuring the height of the C and T peaks as described (15) using Chromas Lite version 2.01 (Technelysis Pty. Ltd.) and was expressed as C/(C+T) for each CpG site by averaging the forward and reverse sequence results. Hypermethylation in clinical samples was defined as a value equal to or greater than the methylated fraction in BRCA1-deficient UWB1.289 cells. Methylation status was validated in cell lines and in a representative subset of cases by HpaII/MspI digestion of unconverted DNA after PCR using primers shown in Supplementary Table S4.

Patient sample acquisition and processing
Tumor specimens were obtained from the MGH Gynecologic Tissue Repository, Cedars-Sinai Medical Center, and University of Washington. Use of tissues for this study was approved in each case by the respective institutional review boards. Designation of tumors as BRCA1 wild-type or mutant resulted from clinical genetic testing. RNA/DNA was isolated from dry frozen tumor samples that had evidence of >80% tumor volume with the GenElute Mammalian Total RNA and Genomic DNA Miniprep kits following the instructions of the manufacturer (Sigma). For fixed specimens, microdissection or macrodissection of tissue sections was carried out after pathology review, and DNA was extracted using the RecoverAll Total Nucleic Acid Isolation kit (Applied Biosystems), according to the instructions of the manufacturer. Additional methods are detailed in Supplementary Data.

Results

Cisplatin sensitivity of BRCA1-deficient ovarian carcinoma cells
To begin to explore the pathways contributing to chemosensitivity in BRCA1-associated tumors, we first took advantage of our established murine model of BRCA1-deficient ovarian carcinoma (8). This model uses the RCAS avian retroviral system to infect primary ovarian surface epithelium (OSE) cells, which express the TVA retroviral receptor transgene driven by the keratin 5 (K5) promoter (Fig. 1A; ref. 18). To develop a model for BRCA1 loss of function, we established K5-TVA mice harboring homozygous conditional p53lox/lox plus BRCA1flox/flox alleles. By infecting OSE cells derived from these mice with RCAS-Cre, we showed that combined loss of p53 and BRCA1 along with one additional viral expression the c-Myc oncogene was sufficient to induce tumors that exhibit many key features of human metastatic BRCA1-associated ovarian carcinoma (8). Three independent BRCA1-deficient tumor lines were derived from explants of these tumors (TBR2, TBR5, and TBR6). As a control, we compared their phenotypic properties to three tumor lines (T1, T2, and T3) resulting from p53 loss along with different combinations of any two of the oncogenes c-Myc, K-Ras, and AKT, which are required for ovarian tumorigenesis in the absence of BRCA1 deletion (Fig. 1A; ref. 9).

In quantitative chemosensitivity assays, BRCA1-associated tumor lines consistently exhibited significantly increased sensitivity to cisplatin, but not to paclitaxel chemotherapy, compared with BRCA1 wild-type tumor lines (Fig. 1B and Supplementary Fig. S1; ref. 8). The difference in platinum sensitivity was not related to the oncogenes used to transform these cells because all three BRCA1 wild-type tumor cells exhibited significantly reduced sensitivity relative to BRCA1-deficient cells (Fig. 1B), and the expression of either Akt or K-Ras in the BRCA1-deficient tumor cells did not significantly affect cisplatin sensitivity in these cells (data not shown). As expected, chemosensitivity in BRCA1-deficient
tumor cells was associated with substantial unrepaired DNA damage (8). Thus, this model recapitulates the BRCA1-associated chemosensitivity of human ovarian carcinomas.

To study cisplatin sensitivity in human ovarian carcinoma cells, we tested UWB1.289 cells, which are derived from an ovarian cancer arising in a germ line BRCA1 mutation carrier and which lack expression of BRCA1 (15). These tumor cells are also deficient in p53 function due to an acquired somatic inactivating mutation (15). We compared cisplatin sensitivity in parental UWB1.289 cells stably reconstituted with either wild-type BRCA1 or the control vector. This reconstitution is physiologically relevant, as it substantially restores defective checkpoint responses after ionizing radiation (15). BRCA1 reconstitution substantially abrogated platinum sensitivity in UWB1.289 cells as assessed in a quantitative dose-response curve (Fig. 1C and Supplementary Fig. S1). Importantly, a clonogenic assay showed the same result, confirming the increased platinum sensitivity of UWB1.289 cells relative to their isogenic BRCA1-expressing counterparts (Supplementary Fig. S1). We then sought to uncover the specific mechanisms involved in the response to cisplatin. We found that cisplatin induces apoptotic cell death in both murine and human BRCA1-deficient cells, as evidenced by cleavage of poly(ADP-ribose) polymerase-1 on Western blot analysis and by Annexin V staining on fluorescence-activated cell sorting analysis (Fig. 1D; data not shown). Given that both the murine and human cells lack functional p53, our findings imply that cisplatin induces cell death in BRCA1-deficient cells, at least in part through an apoptotic mechanism that is independent of p53.

Expression of p73 and its target genes correlate with cisplatin sensitivity in BRCA1-deficient cells

After cisplatin treatment, we observed robust induction of the p53-regulated proapoptotic target genes Noxa, Puma, and p53AIP1 selectively in BRCA1-deficient murine and human cells but little or no induction in their isogenic counterparts expressing wild-type BRCA1, even when using cisplatin doses that cause substantial cell death in both populations (Fig. 2A and B). These findings suggest a distinct cell death pathway in the absence of BRCA1. In particular, the p53-independent induction of these genes led
us to consider that the related p53 family member p73 might contribute to the effects of cisplatin chemotherapy, given that p73 is a direct activator of these particular target genes (19–21). Consistent with a role for p73 in the p53-independent DNA damage response in the specific context of BRCA1 deficiency, we first showed that lentiviral knockdown of BRCA1 led to the induction of p73 after chemotherapy treatment of p53-deficient cells (Supplementary Fig. S2). We next examined p73 expression in wild-type and BRCA1-deficient murine ovarian carcinoma cells using an isoform-specific QRT-PCR approach to distinguish between proapoptotic transactivating p73 isoforms (TAp73) and NH2 terminally truncated isoforms (ΔNp73). Remarkably, both murine and human BRCA1-deficient tumor lines showed substantially higher expression of TAp73 than any of the BRCA1-expressing lines (Supplementary Fig. S2). In contrast, levels of ΔNp73 isoforms were comparable irrespective of BRCA1 status. Taken together, these data show that loss of BRCA1 function and platinum sensitivity correlate with both increased TAp73 and induction of TAp73 target genes in human and murine ovarian carcinoma.

**TAp73 is required for cisplatin sensitivity in BRCA1-deficient ovarian carcinoma cells**

We then sought to address directly whether TAp73 itself is an important mediator of the response to cisplatin in BRCA1-deficient cells *in vitro* and *in vivo*. We first ablated TAp73 expression using lentiviral RNA interference in both human and murine ovarian carcinoma cells and then quantitatively assayed cisplatin sensitivity. Consistently, knockdown of TAp73 induced significant cisplatin resistance in human BRCA1-deficient UWB1.289 cells, although it did not affect proliferation or cell viability in the absence of cisplatin (Fig. 3A and Supplementary Fig. S3). TAp73 does not play a role in BRCA1-expressing cells, which exhibit low levels of TAp73, as knockdown of TAp73 had little or no effect on chemosensitivity over a 5-log dose range of cisplatin (Fig. 3A). Similarly, knockdown of TAp73 in all three murine BRCA1-deficient ovarian carcinoma lines using a distinct short hairpin RNA (shRNA) construct also induced substantial cisplatin resistance.
Figure 3. A TAp73-dependent transcriptional program is required for cisplatin sensitivity in ovarian carcinoma cells and tumors. A, ablation of TAp73 consistently induces cisplatin resistance in BRCA1-deficient but not BRCA1-reconstituted UWB1.289 cells. Lentiviral transduction expressing a control (Vec) or TAp73-directed shRNA (TAp73si) was followed by an MTT assay performed at 72 h after cisplatin. Error bars represent SD for representative experiments performed in triplicate. B, TAp73 is important for cisplatin sensitivity in murine BRCA1-deficient (TBR) but not BRCA1 wild-type (T) carcinoma cells. Lentiviral RNA interference, cisplatin treatment, and MTT assay as in A. C, TAp73-dependent Noxa induction by cisplatin is observed selectively in BRCA1-deficient lines. Cells described in B were treated with cisplatin (1 μmol/L, 72 h) followed by RNA analysis using QRT-PCR. Error bars represent SD for two experiments performed in triplicate. D, TAp73 is highly expressed in responsive (R; >6 mo recurrence-free survival) vs. unresponsive (NR) tumors. QRT-PCR analysis from unselected primary tumors, expressed as log2 TAp73 value. Note the mean TAp73 level is 10-fold higher (23.3) in responsive cases.
whereas it showed no effect in BRCA1 wild-type cells at any dose (Fig. 3B and Supplementary Fig. S3). Further supporting the specificity of these effects, TAp73 knockdown essentially abolished cisplatin-induced Noxa expression in the three murine BRCA1-deficient ovarian carcinoma lines (Fig. 3C), whereas little or no effect was observed in BRCA1 wild-type cells (Fig. 3C). The ability of TAp73 ablation to induce resistance to cisplatin was then confirmed in UWB1.289 cells using a clonogenic assay (Supplementary Fig. S3). Finally, retroviral overexpression of TAp73(δ) was sufficient to induce significant cisplatin sensitivity in BRCA1-expressing cells (Supplementary Fig. S3). These experiments together argue that a TAp73-dependent transcriptional program is an important contributor to the chemosensitivity pathway in BRCA1-deficient ovarian carcinoma cells.

Given that a subset of sporadic ovarian cancers are thought to phenotypically resemble tumors arising in BRCA1/2 mutation carriers (22, 23), we hypothesized that some of these ovarian cancers would show increased expression of TAp73 in association with platinum sensitivity. To test this prediction, we examined a series of unselected tumors from ovarian carcinoma patients treated with platinum-based chemotherapy after surgical resection. We first quantitated TAp73 expression by isoform-specific qRT-PCR in chemotherapy-responsive tumors (>6 months recurrence-free survival, a standard clinical measure) versus unresponsive cases (24). The two groups of patients were otherwise well-matched for clinical characteristics, including tumor histology, tumor grade, and stage (Supplementary Table S5). Remarkably, we observed a mean 10-fold higher level of TAp73 expression in responsive versus unresponsive tumors, a finding that was statistically significant (Fig. 3D). Thus, TAp73 expression is associated with clinical platinum sensitivity, consistent with the data in our model systems showing a direct role for p73 as a mediator of the cellular effects of cisplatin-induced DNA damage.

The ZEB1 transcriptional repressor binds the TAp73 locus and represses TAp73 levels selectively in BRCA1-expressing cells

We then sought to uncover the regulatory mechanism by which TAp73 was differentially expressed in BRCA1-deficient and BRCA1-proficient human and murine ovarian carcinoma cells. Several mechanisms that may contribute to the regulation of p73 expression in tumor cells, including 5′ promoter methylation (25), EGR-1 expression (26), and E2F1 dysregulation (27), have been identified. We detected no significant methylation of the p73 promoter in any of our ovarian carcinoma cells, consistent with prior reports (Supplementary Fig. S4; ref. 28). Furthermore, we did not find large differences in mRNA or protein levels of EGR-1 or E2F family members by microarray analysis or immunoblot, respectively (data not shown).

An additional mechanism that is thought to contribute to endogenous p73 regulation involves the transcriptional repressor ZEB1 (also known as δEF1/zfhx1a). ZEB1 is a zinc finger and homeodomain-containing factor that binds to a regulatory region within the first intron of the TAp73 transcription unit and potently represses TAp73 mRNA expression (29, 30). However, we observed no consistent difference in ZEB1 expression levels between BRCA1-deficient and BRCA1-expressing cells (Supplementary Fig. S5). To test directly whether ZEB1 contributed to p73 regulation in these cells, we inhibited endogenous ZEB1 using lentivirally expressed shRNA constructs (Fig. 4A). Remarkably, knockdown of ZEB1 induced a dramatic (5-fold to 15-fold) upregulation of TAp73 in all three ovarian lines expressing wild-type BRCA1 but had no effect on TAp73 expression in any of the three BRCA1-deficient lines (Fig. 4A). Similarly, in human cells, we observed a high-level induction of TAp73 after ZEB1 knockdown in reconstituted UWB1.289 cells expressing wild-type BRCA1 but no induction in parental cells lacking BRCA1 function (Fig. 4B). Thus, endogenous ZEB1 potently represses TAp73 only in cells expressing wild-type BRCA1. These results suggest that high levels of TAp73 observed in BRCA1-deficient cells might be due to the loss of the repressive effect of ZEB1.

To pursue this hypothesis, we next tested whether differential binding of ZEB1 to TAp73 regulatory sequences might explain a loss of ZEB1-mediated repression in BRCA1-deficient cells. ZEB repressors contain dual zinc fingers that bind to bipartite E boxes (CACCT and CACCTG), the orientation and spacing of which vary in different target genes (31, 32). To identify the endogenous ZEB1 binding site in murine cells, we performed ChIP in ovarian carcinoma cells expressing wild-type BRCA1. We mapped robust and specific binding of ZEB1 to a 1-kb region within the first intron of the TAp73 transcription unit that contains such a bipartite E-box motif (Fig. 4C). Although significant binding of ZEB1 was consistently observed within this region in murine BRCA1 wild-type cells, in the isogenic BRCA1-deficient ovarian carcinoma cells, ZEB1 binding was reduced to background levels (Fig. 4C). Similarly, in human cells expressing wild-type BRCA1, we observed robust and specific binding of endogenous ZEB1 to a 550-bp region containing bipartite E-box motifs (29, 30), yet virtually no specific binding was detected in isogenic BRCA1-deficient cells (Fig. 4D). Taken together, these findings show that endogenous ZEB1 represses TAp73 expression in murine and human ovarian carcinoma cells expressing wild-type BRCA1 and that loss of BRCA1 function is associated with a loss of ZEB1 binding and its repressive effect on TAp73.

Methylation of the ZEB1 binding locus controls ZEB1 binding and TAp73 expression in human and murine cells

Because ZEB repressors are thought to bind directly to DNA (32), we asked whether chemical modification of DNA itself (e.g., methylation) might mediate differential binding of ZEB1 in cells expressing or lacking wild-type BRCA1. We first examined methylation of the ZEB1 binding region in these cells by digesting genomic DNA with either methylation-sensitive or -insensitive isoschizomeric restriction enzymes (HpaII or MspI, respectively), followed by PCR amplification. All three murine BRCA1 wild-type lines showed hypomethylation of this locus, as assessed by the absence of a significant PCR product after HpaII digestion (Fig. 5A). In contrast, in
all BRCA1-deficient lines, this locus was highly methylated, and the DNA was therefore resistant to HpaII digestion. A remarkably similar pattern was observed upon analysis of the ZEB1 binding locus in human ovarian carcinoma cells: hypomethylation in BRCA1-reconstituted UWB1.289 cells and hypermethylation in the BRCA1-deficient parental cells (Fig. 5B).

To characterize the methylation of this locus in detail, we performed bisulfite sequencing across the entire ZEB1 binding region identified by ChIP in UWB1.289 cells (Fig. 5C). This region contains ~20 CpG dinucleotides, and we observed substantial hypomethylation of each CpG within this binding region in BRCA1-reconstituted cells compared with parental BRCA1-deficient cells (Fig. 5C), in agreement with our results from MspI/HpaII digestion analysis in both murine and human cells. Based on these findings, it is reasonable to hypothesize that hypermethylation might inhibit binding of the ZEB1 repressor, leading to a loss of ZEB1-mediated repression and a consequent increase in TAp73 levels.

To test this model directly, we asked whether treatment with a 5-azacytidine (a demethylating agent) would alter the binding of ZEB1 to this locus. We observed a >100-fold increase in ZEB1 binding in BRCA1-deficient cells after 5-azacytidine treatment compared with mock-treated cells, as assessed by quantitative ChIP (Fig. 5D). Furthermore, 5-azacytidine treatment of BRCA1-expressing cells (which are already relatively hypomethylated) only increased ZEB1 binding by <2-fold (Fig. 5D). Taken together, these data show that the binding of ZEB1 to the TAp73 regulatory region is controlled through an epigenetic mechanism and that ZEB1 is bound and suppresses TAp73 expression in BRCA1-expressing but not BRCA1-deficient cells.
Correlation of ZEB1 binding site methylation and TAp73 expression with BRCA1 status and with clinical response in primary ovarian tumors

Our findings predict that ovarian cancers arising in patients with germ line BRCA1 mutations will exhibit hypermethylation of the ZEB1 binding site within the \( p73 \) transcription unit compared with cancers arising in patients without such mutations. To test this prediction, we obtained a series of such tumors that were well matched in terms of both clinical stage and histologic subtype (Supplementary Table S6). Bisulfite sequencing within the ZEB1 binding region showed higher methylation in BRCA1-associated tumors than in those expressing wild-type BRCA1 (Fig. 6A). This finding was confirmed in a subset of cases by an independent method involving \( MspI/HpaII \) digestion of primary tumor DNA followed by PCR as shown in Fig. 5 (data not shown). These findings support the association of BRCA1 deficiency with epigenetic regulation of the \( p73 \) locus in ovarian cancers \textit{in vivo}.

Recent reports have shown that one mechanism by which both BRCA1- and BRCA2-associated ovarian cancers acquire resistance to chemotherapy \textit{in vivo} is through re-expression of a functional protein (33–35). We therefore tested methylation of the ZEB1 binding site in patient-matched primary and recurrent tumors, in which the primary tumor contained only the mutant BRCA1 allele (185delAG) and exhibited no BRCA1 protein expression, whereas the recurrent, chemoresistant tumor exhibited the expression of a wild-type sequence and detectable BRCA1 protein (35). We performed methylation-specific PCR at the ZEB1 binding locus within \( p73 \) (Fig. 6B) after validating that this technique correlates with results obtained by both \( MspI/HpaII \) analysis and direct bisulfite sequencing (data not shown). Remarkably, a substantial decrease in methylation was observed in the platinum-resistant, recurrent tumor relative to the hypermethylated primary tumor (Fig. 6B). Thus, these results support our findings and provide \textit{in vivo} evidence that functional BRCA1 status, which affects platinum chemosensitivity, is associated with epigenetic regulation of the proapoptotic \( TAp73 \) locus.

Finally, we tested the association of TAp73 expression with methylation of the ZEB1 binding locus in the entire cohort of unselected and BRCA1-associated tumors (Figs. 3D and 6A). Thus, we performed bisulfite sequencing across the entire 500-bp ZEB binding region, as well as QRT-PCR for TAp73. As predicted, those tumors exhibiting hypermethylation of this locus were more likely to express high levels of TAp73.

![Figure 5. BRCA1-associated differential methylation of the ZEB1 binding locus controls ZEB1 repressor binding and TAp73 expression.](image-url)
In contrast, hypomethylated tumors almost exclusively expressed low levels of TAp73, comparable with the level expressed in BRCA1-reconstituted UWB1.289 cells. The pattern of methylation in the tumors recapitulated that seen in the UWB1.289 cell line (Fig. 5C), in that differences in methylation were observed across all the CpG residues in region rather than being restricted to a few residues (data not shown). Altogether, these findings support the view that TAp73 is regulated through this epigenetic pathway and is an important mediator of the response to platinum-induced DNA damage in a subset of ovarian carcinomas (Fig. 6D).

Discussion

Using murine and isogenic human ovarian carcinoma models, we show that BRCA1 is associated with a pathway for epigenetic regulation of TAp73, which we find to be an important contributor to chemosensitivity in BRCA1-deficient tumors. Previous studies have documented that TAp73 mediates cellular sensitivity to DNA damage in a variety of contexts (11, 12, 36, 37). Furthermore, we and others have shown that TAp73 is activated specifically in the context of platinum chemotherapy (11, 13). We show here that TAp73 expression is substantially increased in BRCA1-deficient human and murine ovarian carcinoma cells. Treatment with cisplatin induces the proapoptotic transcriptional target genes of TAp73 selectively in these cells, and ablation of TAp73 promotes chemoresistance exclusively in BRCA1-deficient cells and blocks induction of these target genes. Together, these findings strongly argue that a direct, TAp73-dependent effector pathway contributes to the chemotherapy response in these cells. Further support for this concept is
provided by recent clinical studies that find a correlation between poor clinical outcome and increased expression of mRNA for nontransactivating p73 isoforms, which are thought to function as TAp73 antagonists, in ovarian and other cancers (38–40).

We provide substantial evidence that upregulation of TAp73 in BRCA1-deficient ovarian carcinoma cells is mediated through an epigenetic mechanism that controls DNA binding of the ZEB1 transcriptional repressor. The ability of DNA methylation to block binding by a transcriptional regulatory factor has been described in several other contexts (41). Regulation of a transcriptional repressor such as ZEB1 through this mechanism seems less common, however, as most described cases involve differential binding of transcriptional activators. It is also notable that methylation of the TAp73 proximal promoter was not correlated with methylation of the intronic ZEB1 binding region, consistent with prior reports that TAp73 promoter methylation is quite rare in ovarian carcinomas (28). The differential regulation of DNA methylation at these two loci is in accordance with the emerging data from genome-wide studies that have uncovered distinct patterns of regulation in CpG-rich versus CpG-poor regions (42). Thus, the proximal TAp73 promoter is a CpG-rich region that is bound by polycomb factors (43) and that exhibits a tumor-specific pattern of hypermethylation (44). In contrast, the intronic ZEB1 binding locus is a relatively CpG-poor region that may be subject to more dynamic changes in methylation status (42). Although BRCA1 has not been previously associated with the regulation of site-specific methylation, a putative BRCA1-regulated gene, GADD45, has been implicated in a demethylation pathway in zebrafish (45). However, we did not find a difference in GADD45α or GADD45β expression between BRCA1-deficient and reconstituted ovarian carcinoma cells, and knockdown studies did not yield a GADD45-dependent difference in methylation at the ZEB1 binding locus (data not shown). It will be of interest to determine whether the ability of BRCA1 to control the site-specific methylation that we have uncovered is mechanistically related to its reported regulation of the facultative heterochromatin formation on the X chromosome (46).

We show that TAp73 expression is correlated with the clinical response in unselected ovarian cancers, and we provide evidence that TAp73 is regulated in at least some of these tumors through the BRCA1-associated epigenetic mechanism. These observations are consistent with data showing that chemosensitivity of a subset of sporadic ovarian carcinomas is associated with the inactivation of a BRCA1/2-dependent pathway that also involves the Fanconi anemia proteins (22, 23, 47). Intriguingly, a recent report suggests that TAp73 is upregulated in cells from Fanconi anemia patients through a ZEB1-dependent epigenetic mechanism (48). An ongoing challenge for the field is to identify and classify the many mechanisms by which the BRCA1/2 pathway may be abrogated during ovarian epithelial tumorigenesis. Nevertheless, our findings do suggest that low levels of TAp73 may identify patients who will not benefit from standard platinum-based therapy and who might therefore pursue other treatment options. Additionally, this work supports an emerging consensus that the TAp73 pathway might represent an attractive therapeutic target (14). Thus, future targeted therapeutics that trigger p73 expression and/or activation might be developed, thereby inducing apoptosis or sensitizing otherwise insensitive cells to DNA-damaging agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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BRCA1-Associated Epigenetic Regulation of p73 Mediates an Effector Pathway for Chemosensitivity in Ovarian Carcinoma

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