VOLUME 28 · NUMBER 29 · OCTOBER 10 2010

JOURNAL OF CLINICAL ONCOLOGY

CORRESPONDENCE

BRCA1 CpG Island Hypermethylation Predicts Sensitivity to Poly(Adenosine Diphosphate)-Ribose Polymerase Inhibitors

TO THE EDITOR: Recently, Fong et al[1] reported the antitumor activity of the poly(adenosine diphosphate)-ribose polymerase (PARP) inhibitor olaparib (AZD2281; KU-0059436) in patients with *BRCA1/BRCA2* germline mutated ovarian cancer. Female *BRCA1* and *BRCA2* mutation carriers have a significantly elevated lifetime risk of breast and ovarian cancer. BRCA1 and BRCA2 proteins play major roles in DNA double-strand break repair through homologous recombination, and inhibition of DNA single-strand break repair leads to the accumulation of double-strand breaks. These potentially lethal events in homologous recombination-deficient cells could be exploited for therapeutic purposes. The PARP-1 protein is essential for

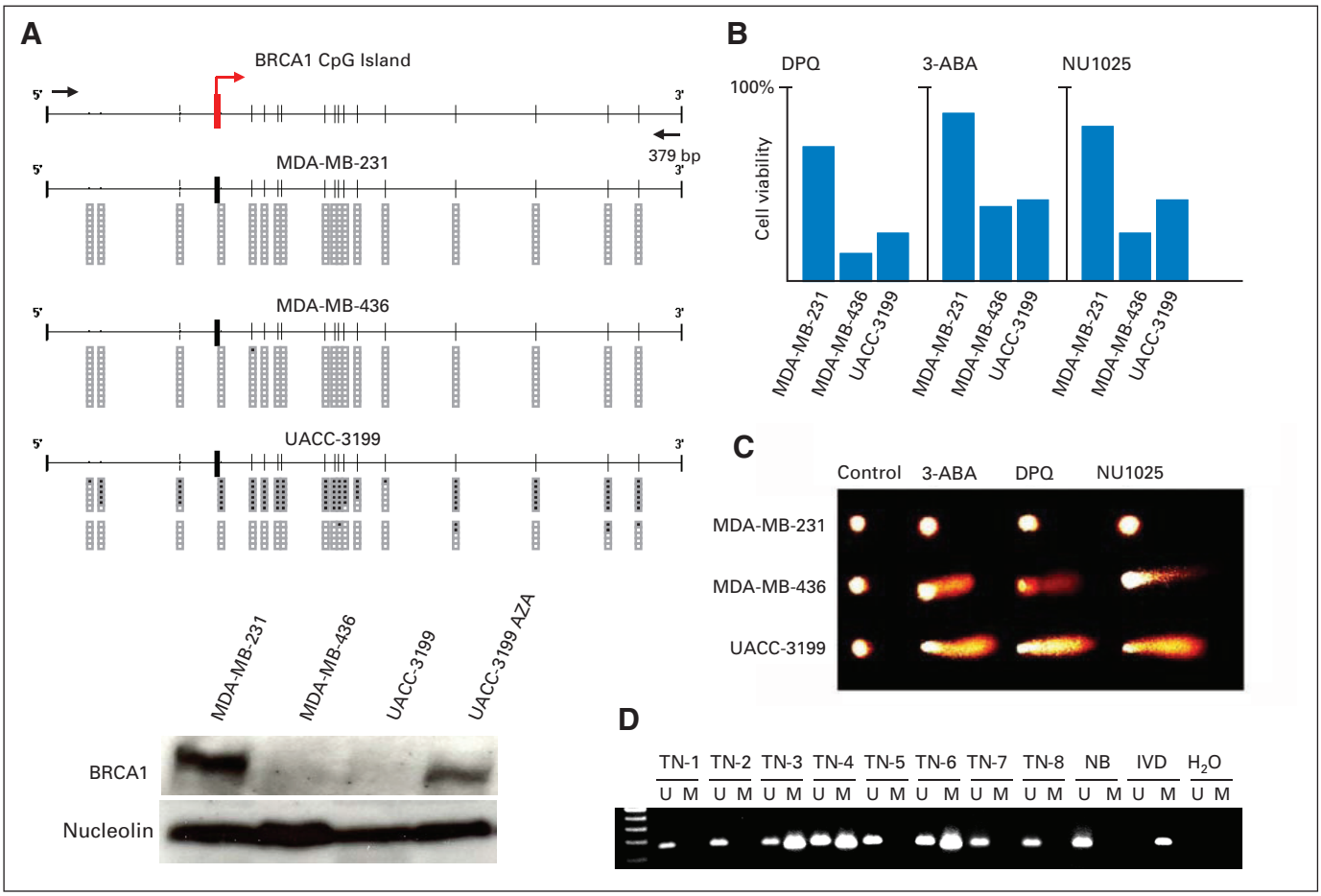

Fig 1. BRCA1 CpG island hypermethylation predicts sensitivity to poly(adenosine diphosphate)-ribose polymerase (PARP) inhibitors. (A) BRCA1 CpG island DNA methylation status (top) and protein expression (bottom) in breast cancer cells. Results of bisulfite genomic sequencing of 10 individual clones in the BRCA1 promoter CpG island are shown. Presence of a methylated or unmethylated cytosine is indicated by a black- or white-filled square, respectively. Black arrows indicate the position of the bisulfite genomic sequencing primers, and the red arrow shows the transcription start site. BRCA1 expression was determined by Western blot, and the nucleolin protein was used as a loading control. The UACC3199 breast cancer cells show a hypermethylated CpG island in association with the loss of the BRCA1 protein, whereas treatment with a DNA demethylating agent (5-azacytidine lane) restores BRCA1 expression. (B) BRCA1-hypermethylated cells are sensitive to the following three PARP inhibitors: 4-dihydro-5-(4-[1-piperidinyl]butoxy)-1(2H)-isoquinolinone (DPQ; 30 μmol/L), 3-aminobenzamide (3-ABA; 1 μmol/L) and 8-hydroxy-2-methylquinazolin-4-(3H)one (NU1025; 500 μmol/L). Cell viability at 48 hours assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays demonstrates that both methylated (UACC3199) and mutant (MDA-MB-436) BRCA1 cells display an enhanced sensitivity to the compounds compared with wild-type and unmethylated MDA-MB-231 breast cancer cells. (C) BRCA1-hypermethylated cells are not able to repair DNA damage. Representative comet assays demonstrate that both methylated and mutant BRCA1 cells experience permanent DNA damage when PARP inhibitors are used, whereas damage is not observed in BRCA1 wild-type and unmethylated cells (MDA-MB-231). The intensity of the tail reflects the amount of DNA damage sustained. (D) BRCA1 hypermethylation is a common finding in patients with triple-negative breast cancer. The image shows the results of analysis of BRCA1 methylation by methylation-specific polymerase chain reaction. The presence of a polymerase chain reaction band under lane M indicates methylated genes, whereas the presence under lane U indicates unmethylated genes. Normal breast (NB) and in vitro–methylated DNA (IVD) are used as negative and positive controls for unmethylated and methylated genes, respectively. BRCA1 hypermethylation is observed in the triple-negative primary breast tumors TN-3, TN-4, and TN-6.

single-strand break repair, and inhibition of PARP leads to persistence of DNA lesions normally repaired by homologous recombination. These observations lead to the seminal discovery that BRCA1- and BRCA2-deficient cells are highly sensitive to PARP inhibition in comparison with BRCA1 and BRCA2 wild-type cells,[2] resulting in clinical trials in patients with breast and ovarian cancer to test the efficacy of PARP inhibitors. At least one of these compounds, olaparib, has shown antitumor activity in breast tumors from *BRCA1* and *BRCA2* mutation carriers, with few of the adverse effects associated with conventional chemotherapy.[3] The current article by Fong et al[1] extends the activity of this compound to *BRCA1/2* familial ovarian cancers. However, because *BRCA1* and *BRCA2* mutation carriers represent only a minority of patients with breast and ovarian cancer, there is the question of how to select patients most likely to benefit from PARP inhibitors in larger sporadic breast and ovarian cancer populations.[4] Herein, we propose that inactivation of the *BRCA1* gene by CpG island hypermethylation[5,6] could be such a marker.

Epigenetic inactivation of tumor suppressor genes by the aberrant addition of methyl groups in their CpG-rich regulatory regions (promoter CpG islands) is a common hallmark of human tumors.[6] The ability of these alterations to be useful as predictive biomarkers has been demonstrated in the case of the CpG island hypermethylation of another DNA repair gene, O^6-methylguanine–DNA methyltransferase (*MGMT*), which predicts sensitivity to temozolomide in gliomas.[7] For *BRCA1*, numerous reports have described DNA methylation–associated inactivation of this gene in sporadic breast and ovarian tumors.[5,6] That this event produced a tumor with a BRCA1 phenotype was reinforced by the demonstration that it leads to the same pattern of gene expression as inherited *BRCA1* mutations.[8] This suggested that it might be possible to extend the observation of enhanced PARP inhibitor sensitivity of BRCA1/BRCA2 familial tumors to sporadic BRCA1-hypermethylated tumors. To this end, we determined the CpG island methylation status of the *BRCA1* and *BRCA2* genes in seven common breast cancer cell lines (MCF7, SK-BR-3, T47D, Hs578T, UACC3199, MDA-MB-231, and MDA-MB-436) by bisulfite genomic sequencing. BRCA2 methylation was not found in any case, but the breast cancer cell line UACC3199 exhibited dense BRCA1 CpG island hypermethylation with associated loss of BRCA1 protein expression, which was restored with a DNA demethylating agent (Fig 1). We next analyzed the sensitivity of BRCA1-hypermethylated tumor cells to three PARP inhibitors, 3,4-dihydro-5-(4-[1-piperidinyl]butoxy)-1(2H)-isoquinolinone, 3-aminobenzamide, and 8-hydroxy-2-methylquinazolin-4-(3H)one, comparing them to *BRCA1* mutant (MDA-MB-436) and *BRCA1* wild-type (MDA-MB-231) breast cancer cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. BRCA1 hypermethylation conferred the same degree of sensitivity to the three PARP inhibitors as did the *BRCA1* mutation (Fig 1). Furthermore, BRCA1-deficient cells (hypermethylated or mutated) experienced massive DNA damage, as assessed by the comet assay, when treated with PARP inhibitor, which was not observed in BRCA1-proficient cells (Fig 1).

What effect might these findings have in the clinical setting? We used methylation-specific polymerase chain reaction to determine the frequency of BRCA1 hypermethylation in triple-negative noninherited breast cancer tumors where PARP inhibitors have begun to be studied. We found that 36.7% (25 of 68 tumors) of these breast tumors had BRCA1 methylation (Fig 1), which would be expected to be more sensitive to therapy with PARP inhibitors. Therefore, we suggest that the analysis of BRCA1 hypermethylation be included in current and prospective clinical trials of patients with breast and ovarian cancer treated with PARP inhibitors as a potential biomarker of sensitivity to these compounds. This approach could be used to further improve treatment response and provide a more personalized therapy.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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DOI: 10.1200/JCO.2010.30.1010; published online ahead of print at www.jco.org on August 2, 2010

