Available online at www.sciencedirect.com
SCIENCE DIRECT
Gynecologic Oncology 101 (2006) 403 – 410
Gynecologic Oncology
www.elsevier.com/locate/ygyno

BRCA1 promoter methylation predicts adverse ovarian cancer prognosis ☆

Jing Wang Chiang, Beth Y. Karlan *, Ilana Cass, Rae Lynn Baldwin

Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Cedars–Sinai Medical Center, 8700 Beverly Boulevard, 160W, Los Angeles, CA 90048, USA
Department of Obstetrics and Gynecology, David Geffen School of Medicine, UCLA, Los Angeles, CA 90095, USA

Received 1 July 2005
Available online 19 December 2005

Abstract

Objective. To compare the clinical outcome of ovarian cancer patients whose tumors contain *BRCA1* genes silenced by promoter hypermethylation to patients with germline *BRCA1* mutations and to patients with wild-type *BRCA* genes.

Methods. Ovarian cancers from a hospital-based tumor bank were characterized as having a BRCA1 mutation; or a methylated *BRCA1*, *BRCA1* pseudogene or *MLH1* promotor; or a wild-type *BRCA* gene. Survival of patients with methylated *BRCA1* promoters ($N = 11$) was compared to that of patients with wild-type *BRCA* genes ($N = 30$) and *BRCA1* mutations ($N = 22$). A methylator phenotype was defined to include tumors with hypermethylation of *BRCA1*, *hMLH1* and/or *dBRCA1* pseudogene promoters ($N = 23$).

Results. All cohorts had comparable clinical factors except for age at diagnosis. Median age of methylated *BRCA1* and wild-type *BRCA* patients was older than *BRCA1* mutation carriers (60 and 63 versus 48 years; $P = 0.04$). The median disease-free interval was significantly shorter for patients with a methylated *BRCA1* promoter (9.8 months) than for *BRCA1* mutation carriers (39.5 months; $P = 0.04$). Median overall survival was also significantly shorter for patients with a methylated *BRCA1* promoter (35.6 months) than *BRCA1* mutation carriers (78.6 months; $P = 0.02$). The combined methylator phenotype cohort had significantly shorter survival (36.1 months) compared to wild-type *BRCA* patients (63.3 months; $P = 0.02$).

Conclusion. These data suggest that methylation of the *BRCA1* promoter is associated with poor patient outcome. *BRCA1* may be part of a global panel of methylated genes associated with aggressive disease.

Keywords: DNA methylation; Ovarian cancer; Survival

Introduction

DNA methylation at promoter-associated CpG islands can cause transcription silencing and the loss of tumor suppressor gene function. Approximately 60% of gene promoters contain regions with high CG dinucleotide content called CpG islands [1]. Typically, promoter-associated CpG islands are protected from methylation. The epigenetic inactivation of more than 60 genes including the *BRCA1* tumor suppressor gene has been reported to occur by the aberrant methylation of promoter-region CpG islands, suggesting that DNA methylation may play a role in tumorigenesis [2].

Aberrant methylation of gene promoters appears to be an early and frequent event in carcinogenesis, and it has also been shown to have an impact on clinical outcome. Prognosis of several cancer types including bladder, lung, and ovarian cancers have been shown to be effected by differential CpG island methylation [3–6]. RASSF1A methylation was more frequent in poorly differentiated stage I lung cancers and correlated with adverse survival [4]. Tumors with a higher methylation index have been associated with poor prognosis in bladder cancer, and a high degree of CpG island methylation was also associated with a short disease-free interval in ovarian cancer [3,6]. These data suggest that aberrant DNA methylation profiles may potentially be useful biomarkers for disease onset and progression.

Approximately 10% of epithelial ovarian cancers are hereditary, and the vast majority of these are associated with autosomal dominant inheritance of a mutation in either the

☆ This work was supported in part by NIH R21 CA89137 (RLB), NIH Grant #5 P50 CA83636 and Cedars–Sinai Women's Cancer Research Institute (BYK).
* Corresponding author. Fax: +1 310 423 9753.
E-mail address: beth.karlan@cshs.org (B.Y. Karlan).

0090-8258/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2005.10.034

BRCA1 or *BRCA2* genes [7–9]. The *BRCA1* gene acts as a tumor suppressor and regulates cellular proliferation and DNA repair [10,11]. In vitro and animal model data have demonstrated that diminished *BRCA1* gene product is associated with chromosomal instability and an increased proliferative rate [10,12,13]. This observation led investigators to hypothesize that *BRCA* mutations may impact on tumor biology and clinical behavior. Studies have shown that ovarian cancer patients with hereditary mutations in *BRCA1* and *BRCA2* have a more favorable prognosis than their counterparts without germline mutations. *BRCA1* and *BRCA2* mutation carriers with ovarian cancer were shown to have increased progression-free and overall survival that was likely due to an improved response to chemotherapy [14,15].

Prior studies have shown that *BRCA1* is silenced by promoter hypermethylation in 10–19% of ovarian adenocarcinoma [16,17]. Here, we tested the hypothesis that silencing of *BRCA1* expression by promoter CpG island hypermethylation has an impact on the clinical outcome of ovarian cancer patients. We compared the outcomes of ovarian cancer patients whose tumors contained *BRCA1* genes silenced by aberrant CpG island methylation to ovarian cancer patients with inherited *BRCA1* mutations and to the ovarian cancer patients whose tumors had wild-type *BRCA* genes.

Materials and methods

Specimens

Snap-frozen ovarian cancer tumor specimens were initially harvested in the operating suites obtained from the Gynecological Oncology Tissue Bank at Cedars–Sinai Medical Center. All ovarian tumor tissues and blood samples were collected under an IRB approved protocol from consenting patients. Low malignant potential tumor specimens were excluded. Sixty-five Jewish ovarian cancer patients were enrolled between 1986 and 2001 and screened for the three germline founder mutations in *BRCA1* and *BRCA2* seen in the Ashkenazi Jewish population [15]. The *BRCA* mutation status of these 65 Jewish ovarian cancer patients was determined as previously described [15]. Briefly, all specimens were screened for the three Jewish founder mutations commonly occurring in the Ashkenazi Jewish population: *BRCA1* 185delAG (exon 2), *BRCA1* 5382insC (exon 20), and *BRCA2* 6174delT (exon 11) by heteroduplex analysis of *BRCA1* exon 2, single-strand conformational polymorphism (SSCP) analysis for *BRCA1* exon 20, and protein truncation testing of *BRCA2* exon 11 [18]. Mutations were confirmed by complete sequence analysis. Ashkenazi Jewish patients with a *BRCA1* founder mutation or without *BRCA1* or *BRCA2* founder mutations comprised the *BRCA1* mutant (MUT) and *BRCA* wild-type (WT) cohorts, respectively.

Patients with a methylated *BRCA1* promoter were also identified from our hospital-based ovarian tumor bank of snap-frozen tumor tissue. The only selection criterion used in choosing these cases was that there was enough tissue available to complete all analysis [16]. The analysis of *BRCA1* promoter methylation status was performed for 95 sporadic ovarian tumor samples as previously described [16]. Briefly, *BRCA1* promoter hypermethylation was determined by a combination of methylation-sensitive restriction enzyme digestion followed by Southern blot analysis and methylation-specific PCR (MSP). The probe used for Southern blot analysis also detected methylation of the *BRCA1* pseudogene [19]. *BRCA1* promoter methylation is determined by the cleavage pattern. Two or more completely or partially uncleaved AvaII fragments are generated by further cleavage with methylation-sensitive enzymes, HpaII and CfoI. (Fig. 1). *MLH1* promoter methylation status was determined for sporadic ovarian cancer patients from the same hospital-based cohort of patients by MSP analysis using previously published PCR primers [20].

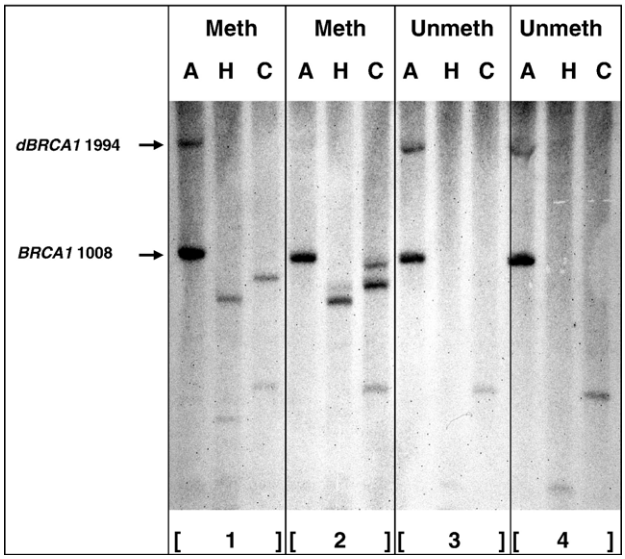

Fig. 1. Representative Southern blot analysis. Representative Southern blot of 4 ovarian cancer patients demonstrating methylated and unmethylated tumor samples. Lanes labeled A contain DNA digested with AvaII alone; H, with AvaII and HpaII; C, with AvaII and CfoI. The 1008 bp AvaII fragment contains the *BRCA1* promoter. The 1994 bp fragment contains the *BRCA1* pseudogene promoter. The last 2 specimens are completely cleaved with AvaII in combination with methylation-sensitive enzymes HpaII and CfoI, demonstrating these are unmethylated, while in the first 2 specimens the majority of HpaII and CfoI cleavage sites are uncleaved and methylated.

P53 mutation

p53 mutation status as indicated by protein over-expression was characterized by immunohistochemical staining of paraffin-embedded tissues. Slides were incubated with anti-human *p53* monoclonal antibody AB-6 (Clone DO-1: Oncogene Research Products, Cambridge, MA) at a 1:100 dilution. Control slides were incubated with normal mouse IgG. A Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA) and diaminobenzidine as color reagent were used to detect antibody binding. Extent and intensity of antibody staining were evaluated by 2 researchers, independently. For the purpose of this study, any staining was considered positive for *p53* mutation.

Clinical–pathologic review

Following the determination of *BRCA1* mutation and promoter methylation status, the ovarian cancer patients were divided into three cohorts: (1) patients with a methylated *BRCA1* promoter and no *BRCA* mutation (MET, $n = 11$); (2) Jewish patients with founder germline *BRCA1* mutations, and no methylation (MUT, $n = 22$); and (3) Jewish patients without a germline *BRCA1* or *BRCA2* mutation and without *BRCA1* methylation were considered as wild type (WT, $n = 30$). These 63 patients comprise the first part of our data analysis. The 23 patients with hypermethylation at *BRCA1*, *BRCA1* pseudogene, and/or *MLH1* promoters comprised the methylator phenotype cohort in the second part of the analysis.

A retrospective chart review was conducted and patient demographic, clinical, and pathologic characteristics were extracted from patient medical records, operative and pathologic reports. Survival data on recurrence, death, and follow-up duration were obtained from a review of hospital records, tumor registry reports, and the use of the social security death index http://ssdi.genealogy.rootweb.comunder an IRB approved protocol.

Independent variables collected for each patient included patient age at diagnosis, year of diagnosis, histological type, pathologic grade and stage, optimal debulking status, *p53* expression, and first chemotherapy regimen. Histological types were grouped as papillary serous histology or others. Pathologic grade was categorized into low (grade 1) and high (grades 2 and 3). Surgical staging was categorized into early (stage 1 and 2) and advanced (stage 3 and 4). Outcome measures included interval to disease recurrence, duration of