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Clinical characteristics and outcomes of patients with *BRCA1* or *RAD51C* methylated versus mutated ovarian carcinoma

Sarah S. Bernards [a], Kathryn P. Pennington [a], Maria I. Harrell [a], Kathy J. Agnew [a], Rochelle L. Garcia [b], Barbara M. Norquist [a], Elizabeth M. Swisher [a,*]

[a] Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Washington, 1959 Northeast Pacific Street, Seattle, WA 98195, USA
[b] Department of Pathology, University of Washington, 1959 Northeast Pacific Street, Seattle, WA 98195, USA

HIGHLIGHTS

- Methylation and mutation of *RAD51C* or *BRCA1* were mutually exclusive.
- Methylation of *BRCA1* was associated with younger age at diagnosis.
- Germline *BRCA1* mutations and *BRCA1* methylation were associated with HGS histology.

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ABSTRACT

Objective. In ovarian carcinoma, mutations in homologous recombination DNA repair (HRR) genes, including *BRCA1* and *RAD51C*, are associated with increased survival and specific clinical features. Promoter hypermethylation is another mechanism of reducing gene expression. We assessed whether *BRCA1* and *RAD51C* promoter hypermethylation is associated with similar survival and clinical characteristics.

Methods. Promoter methylation of *BRCA1* and *RAD51C* was evaluated using methylation-sensitive PCR in 332 primary ovarian carcinomas. Damaging germline and somatic mutations in 16 HRR genes were identified using BROCA sequencing.

Results. BRCA1 methylation was detected in 22 carcinomas (6.6%) and *RAD51C* methylation in 9 carcinomas (2.7%). These small numbers limited the power to detect differences in survival and platinum sensitivity. Mutations in one or more HRR genes were found in 95 carcinomas (29%). Methylation of *BRCA1* or *RAD51C* was mutually exclusive with mutations in these genes ($P = 0.001$). Patients whose carcinomas had *BRCA1* methylation (57.7 years ± 2.5) or *BRCA1* mutations (54.1 years ± 1.4) were younger than those without (63.3 years ± 0.8; $P = 0.029$, $P < 0.0001$). *BRCA1* methylation and germline *BRCA1* mutation were associated with high grade serous (HGS) histology ($P = 0.045$, $P = 0.001$). *BRCA1* mutations were associated with increased sensitivity to platinum chemotherapy while *BRCA1* methylation was not ($P = 0.034$, $P = 0.803$). Unlike HRR mutations, methylation was not associated with improved overall survival compared to cases without methylation or mutation.

Conclusions. Patients with *BRCA1*-methylated carcinomas share clinical characteristics with patients with *BRCA1*-mutated carcinomas including younger age and predominantly HGS histology. However, unlike mutation, *RAD51C* and *BRCA1* methylation were not associated with improved survival or greater sensitivity to platinum chemotherapy.

1. Introduction

Ovarian, fallopian tube and primary peritoneal (collectively called ovarian) carcinomas commonly have deleterious mutations in the homologous recombination DNA repair pathway (HRR) genes *BRCA1* and *BRCA2* (*BRCA1/2*), with germline mutations in approximately 15% of patients and somatic mutations in another 6% of carcinomas [1–4]. Germline mutations in these genes are the most common cause of inherited ovarian carcinoma and are associated with increased

* Corresponding author at: University of Washington Medical Center, Department of Obstetrics and Gynecology, Box 356460, Seattle, WA 98195-6460, USA.
E-mail addresses: sarahb18@uw.edu (S.S. Bernards), katypenn@uw.edu (K.P. Pennington), maribel@uw.edu (M.I. Harrell), kagnew@uw.edu (K.J. Agnew), rochelle@uw.edu (R.L. Garcia), bnorquis@uw.edu (B.M. Norquist), swishere@uw.edu (E.M. Swisher).

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sensitivity to platinum chemotherapy, better five-year survival, high grade serous (HGS) histology, and somatic *TP53* mutations [5–8]. Our group and others have recently shown that somatic mutations in *BRCA1/2* and some non-*BRCA1/2* HRR genes including *RAD51C* are also associated with platinum sensitivity, improved survival, and sensitivity to poly (ADP-ribose) polymerase (PARP) inhibitors [2,9–11].

Promoter hypermethylation (methylation) is another biologic mechanism of reducing gene expression and occurs frequently in cancer [12]. *BRCA1* promoter methylation is common in ovarian carcinoma with rates from 8 to 15% and is associated with both reduced RNA and protein expression [12–19]. In HGS ovarian carcinomas, The Cancer Genome Atlas (TCGA) identified *BRCA1* and *RAD51C* as the only HRR genes in which promoter methylation correlated with reduced RNA expression [1]. *RAD51C* methylation has been described in 1–3% of ovarian carcinomas [1,15].

Given the improved outcomes seen with *BRCA1* mutation and the functional impact of *BRCA1* promoter methylation (decreased RNA and protein expression), we hypothesized that *BRCA1* and *RAD51C* methylation would also be associated with improved outcomes and similar clinicopathological characteristics. However, there are conflicting reports of the prognostic value of *BRCA1* methylation in regards to sensitivity to platinum chemotherapy and survival [1,14,15,20,21]. No previous study except TCGA, which was limited only to ovarian carcinomas of HGS histology, has considered both methylation and mutation in many HRR genes in comparing outcomes [1,14,15,20,21]. We sought to evaluate *BRCA1* methylation and *RAD51C* methylation in ovarian carcinomas of varying histologies and compare outcomes and characteristics of methylated versus mutated cases.

2. Methods

Patients with ovarian, fallopian tube, or peritoneal carcinoma provided IRB-approved informed consent to enroll in the University of Washington gynecologic oncology tissue bank at the time of their primary debulking surgery. Patients diagnosed with carcinoma at the time of planned prophylactic bilateral salpingo-oophorectomy were excluded. Germline and neoplastic DNA was sequenced using BROCA, a targeted capture and massively parallel sequencing platform previously described [2,22,23].

Carcinomas with a damaging germline or somatic mutation in *ATM*, *ATR*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *CDK12*, *CHEK2*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, *RAD51D*, *RBBP8*, *SLX4*, or *XRCC2* were classed as having HRR deficiency.

Methylation of *RAD51C* and *BRCA1* in neoplastic DNA was assessed by bisulfite conversion using the Zymo Research EZ DNA Methylation-Direct kit followed by methylation-specific PCR as previously described [9,17]. Methylation was evaluated in primary carcinomas and, if available, in corresponding recurrent carcinomas.

Patients were considered to have a “strong family history” of cancer if they had a relative with OC, a relative with breast cancer before age 50, or two relatives with breast cancer at any age.

All statistical analyses were pre-planned based on our hypotheses. The Fisher's exact test was used to test the significance of contingency tables (Tables 1 and 2). Progression-free survival (PFS) was defined as the time between study enrollment and disease progression or death. Patients who did not receive chemotherapy and those for whom chemotherapy information was not available were excluded from the PFS analysis. Overall survival (OS) was defined as the time between study enrollment and last follow up visit or death. Patients with stage I disease were excluded from survival analyses. Kaplan Meier curves were generated for OS and PFS and evaluated by the Log Rank test. The study sample size was not large enough to correct for confounders with multivariate analysis.

3. Results

Primary carcinomas from a total of 332 patients were evaluated. Table 1 provides demographic information for all subjects. For 12 patients, paired recurrent neoplasms were also analyzed for methylation.

Tables 1 and 2 summarize the methylation and germline mutation status of the primary carcinomas. Mutation information for all but three patients was previously published [2]. Sixty-nine (20.8%) carcinomas had a germline mutation in one or more of the HRR genes assayed (1 *ATM*, 2 *BARD1*, 38 *BRCA1*, 13 *BRCA2*, 4 *BRIP1*, 3 *CHEK2*, 1 *NBN*, 1 *PALB2*, 3 *RAD51C*, and 4 *RAD51D*). Twenty-eight (8.4%) carcinomas had a somatic mutation in one or more HRR genes (2 *ATM*, 16 *BRCA1*, 5 *BRCA2*, 1 *BRIP1*, 4 *CHEK2*, 1 *MRE11A*, 1 *RAD51C*, 1 *SLX4*). Among these mutated cases, six (1.8%) had mutations in more than one gene. These cases were a germline *BRCA1* mutation with a somatic *CHEK2* mutation, a germline *BRCA1* mutation with a somatic *ATM* mutation, a germline *BRCA1* mutation with a germline *BARD1* mutation, a germline *CHEK2* mutation with a somatic *BRCA2* mutation, a somatic *CHEK2* mutation with a somatic *SLX4* mutation, and a case with somatic mutations in *BRCA1*, *BRIP1*, and *MRE11A*.

A total of 31 primary carcinomas were found to have methylation of either *BRCA1* (22, 6.6%) or *RAD51C* (9, 2.7%). Two hundred seven (62.3%) carcinomas had neither methylation nor mutation. No carcinoma had both a germline mutation in a HRR gene and methylation of either *BRCA1* or *RAD51C* ($P = 0.0008$). One carcinoma with a somatic mutation in *BRIP1* had methylation of *BRCA1*.

When available, paired recurrent carcinomas were also evaluated for somatic mutations and methylation. All paired carcinomas had concordant methylation status: eleven of the recurrent carcinomas were unmethylated as were their corresponding primary neoplasms, and one recurrent carcinoma was *BRCA1* methylated, as was the primary neoplasm associated with it.

Table 1 describes the clinical features of the study population by methylation and mutation status. At time of diagnosis, patients with *BRCA1* methylation of their carcinoma were on average 5.6 years younger (mean 57.7 years ± 2.5) than patients without germline or somatic mutations and without methylation (mean 63.3 years ± 0.79) ($P = 0.029$). Patients with germline or somatic *BRCA1* mutations were younger at diagnosis than patients without mutations and without methylation by 9.3 years ± 1.7 (mean 54.1 years ± 1.4) ($P < 0.0001$). The difference in age at diagnosis was more pronounced when the analysis was restricted to patients with germline *BRCA1* mutations, who were on average 10.9 years younger (mean 52.4 years ± 1.4) than patients without mutations and without methylation ($P < 0.0001$). Patients with germline or somatic mutations in HRR genes other than *BRCA1* or *RAD51C* were also younger at diagnosis than patients without mutations and without methylation by 5.83 ± 2.04 years (mean 57.5 ± 1.9 years) ($P = 0.005$).

Germline *BRCA1* mutations and *BRCA1* methylation were both associated with HGS histology when compared to carcinomas without mutation or methylation (95% vs 71%, $P = 0.001$; 91% vs 71%, $P = 0.045$), but this was not true for *BRCA1* somatic mutations. Of the 16 somatically mutated *BRCA1* carcinomas, eight were HGS, four were undifferentiated, two were endometrioid, one was clear cell, and one was a carcinosarcoma. A strong family history of breast or ovarian carcinoma was more common in patients with germline HRR mutations including mutations in *BRCA1* and *RAD51C* (55% vs 17% of patients whose carcinomas were neither mutated nor methylated, $P < 0.0001$). Patients with germline HRR gene mutations had a significantly higher incidence of a personal history of breast cancer (21.7% vs 3.4% of those with neither mutation nor methylation, $P < 0.0001$). Advanced stage disease (stage III or IV), utilization of neoadjuvant chemotherapy, and achievement of optimal primary surgical debulking was similar in carcinomas with or without mutation or methylation. As previously published, germline or somatic *BRCA1* mutations were associated with increased platinum sensitivity compared to carcinomas without mutation or methylation (80% vs

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