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BRCA1-Mutated Estrogen Receptor–Positive Breast Cancer Shows BRCAness, Suggesting Sensitivity to Drugs Targeting Homologous Recombination Deficiency

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Abstract

Purpose: As estrogen receptor–positive (ER^+) breast cancer in *BRCA1* mutation carriers arises at an older age with less aggressive tumor characteristics than ER-negative (ER^-) *BRCA1*-mutated breast cancer, it has been suggested that these tumors are "sporadic" and not *BRCA1* driven. With the introduction of targeted treatments specific for tumors with a nonfunctioning *BRCA1* or *BRCA2* gene, the question whether the *BRCA* genes are impaired in the tumor is highly relevant. Therefore, we performed genomic profiling of *BRCA1*-mutated ER^+ tumors.

Experimental Design: Genomic profiling, *BRCA1* promoter methylation assessment, and loss of heterozygosity analysis were done on 16 *BRCA1*-mutated ER^+ tumors. Results were compared with 57 *BRCA1*-mutated ER^- tumors, 36 *BRCA2*-mutated ER^+ -associated tumors, and 182 sporadic ER^+ tumors.

Results: The genomic profile of *BRCA1*-mutated ER^+ tumors was different from *BRCA1*-mutated ER^- breast tumors, but highly similar to *BRCA2*-mutated ER^+ tumors. In 83% of the *BRCA1*-mutated ER^+ tumors, loss of the wild-type *BRCA1* allele was observed. In addition, clinicopathologic variables in *BRCA1*-mutated ER^+ cancer were also more similar to *BRCA2*-mutated ER^+ and sporadic ER^+ breast cancer than to *BRCA1*-mutated ER^- cancers.

Conclusions: As *BRCA1*-mutated ER^+ tumors show a BRCAness copy number profile and LOH, it is likely that the loss of a functional BRCA1 protein plays a role in tumorigenesis in *BRCA1*-mutated ER^+ tumors. Therefore, we hypothesize that these tumors are sensitive to drugs targeting the *BRCA1* gene defect, providing new targeted treatment modalities for advanced BRCA-deficient, ER^+ breast cancer. *Clin Cancer Res; 23(5); 1236–41. ©2016 AACR.*

Introduction

Most breast cancers arising in *BRCA1* mutation carriers are triple negative, but 10% to 20% are estrogen receptor positive (ER^+; refs. 1–4). These ER^+ *BRCA1*-mutated breast cancers tend to arise in older patients, have less aggressive tumor characteristics, and are sometime believed to be "sporadic" rather than related to BRCA1 deficiency (3, 5, 1). Others, however, showed that loss of the *BRCA1* wild-type allele is equally frequent in *BRCA1*-mutated ER-negative (ER^-) breast cancer as *BRCA1*-mutated ER^+ breast cancer, indicating that BRCA1 is involved in tumorigenesis in these tumors as well (6). Still, others proposed that *BRCA1*-mutated ER^+ tumors are intermediate in pathologic variables between *BRCA1*-mutated ER^- tumors and sporadic ER^+ breast cancers (7). With the revived interest in platinum salts and the introduction of targeted treatments specific for tumors with a nonfunctioning *BRCA1* or *BRCA2* gene (i.e., PARP inhibitors; refs. 8–10), the question whether the *BRCA* genes are impaired in the tumor is highly relevant.

Loss of the remaining wild-type allele in *BRCA1*- or *BRCA2*-mutated tumors results in deficient homologous recombination DNA repair. The normal tissue of the patient does not share this DNA repair defect. This tumor-specific defect can be exploited by PARP inhibitors and double-strand break inducing chemotherapy to induce selective tumor cytotoxicity while sparing normal cells. Indeed, several clinical trials have shown that most evidence for an effect of PARP inhibitors and platinum salts is seen in *BRCA1* and *BRCA2* mutation carriers (11). Should *BRCA1*-mutated ER^+ tumors be more alike sporadic breast cancer and BRCA1 is not impaired in the tumor, these tumors would not be sensitive to PARP inhibitors.

Therefore, the question whether ER^+ tumors in patients with a germline *BRCA1* mutation are deficient in BRCA function is clinically important. To answer this question, we performed a detailed genomic analysis and compared histopathologic features

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Translational Relevance

With the revised interest in platinum salts and the introduction of PARP inhibitors that specifically target tumors with a nonfunctioning *BRCA1* or *BRCA2* gene, the question whether the *BRCA* genes are impaired in the tumor is highly relevant. As estrogen receptor–positive (ER^+) tumors in *BRCA1* mutation carriers generally arise at a later age and have less aggressive tumor characteristics than ER-negative (ER^-) *BRCA1*-mutated tumors, it is questioned whether BRCA1 is completely lost. In the current study, we performed genomic analysis on ER^+ *BRCA1*-mutated tumors. ER^+ *BRCA1*-mutated tumors showed a BRCAness genomic profile and loss of heterozygosity at the *BRCA1* locus, indicating complete loss of BRCA1. Therefore, we suggest that these tumors are indeed sensitive to therapies targeting the *BRCA* gene deficiency.

between *BRCA1*-mutated ER^+ tumors, *BRCA1*-mutated ER^- tumors, *BRCA2*-mutated ER^+, and sporadic ER^+ breast cancers.

Materials and Methods

Patients

Four breast cancer groups were selected from the Netherlands Cancer Institute's pathology archive: (i) 16 ER^+ tumors from *BRCA1* mutation carriers; (ii) 57 ER^- tumors from *BRCA1* mutation carriers; (iii) 36 ER^+ tumors from *BRCA2* mutation carriers; and (iv) 182 tumors from women with unknown *BRCA1* or *BRCA2* status. Patients were treated in Dutch hospitals between 1994 and 2014. The first three groups were patients who tested positive for a *BRCA1* or *BRCA2* mutation by one of the clinical genetics centers in the Netherlands. The last group had not been tested for *BRCA1* or *BRCA2* mutations and contains cases treated with neoadjuvant chemotherapy, as well as cases selected from the pathology archive without a breast cancer family history (12–14).

Pathology

ER positivity (antibody from Roche Diagnostics cat. no. 5278406001) and progesterone receptor (PR) positivity (Roche Diagnostics cat. no. 5278392001) were defined by staining at least 1% positive neoplastic cells by IHC. A sample was scored as being HER2 positive when either strong membrane staining (3+) could be observed by IHC (Roche Diagnostics cat. no. 5278368001) or if CISH revealed amplification of HER2 in samples with moderate (2+) membrane staining at IHC. IHC, histology, and grading were revised by an experienced breast cancer pathologist (J. Wesseling).

BRCA1/2 germline testing

BRCA1 mutation status was obtained from patient records, obtained through our family cancer clinic. Briefly, germline DNA was isolated from peripheral blood lymphocytes of affected patients. We used mutation-scanning methods to detect pathogenic mutations. The protein truncation test was used for exon 11 of *BRCA1* and exon 10 and 11 of *BRCA2*. The remaining exons were tested using denaturing gradient gel electrophoresis. Confirmation of aberrant samples was done by Sanger sequencing on an ABI 3730 capillary sequencer (Life Technologies/Applied Biosystems; ref. 15). In addition, multiplex ligation-dependent probe amplification (MLPA) was performed using MLPA Kit P087 (*BRCA1*; MRC-Holland) to detect large genomic deletions or duplications in the genes.

aCGH BRCA-like genomic profile

Array comparative genomic hybridization (aCGH) *BRCA*-like scores were obtained by two different assays. The older samples (2004–2010) were obtained by a 3.5 k BAC aCGH platform. The DNA segments covered the whole genome with an average spacing of 1 MB. The protocols have been described before (16). The more recent samples were analyzed using NimbleGen 128K oligo arrays. For the NimbleGen arrays labeling, hybridization, imaging, and data analysis have been performed according to the manufacturer's protocol (NimbleGen, Roche). Classification of subtypes was performed using the aCGH *BRCA1*-like classifier and the *BRCA2*-like classifier, both developed by Joosse and colleagues. (13, 12). The cutoff to call a tumor *BRCA1*-like or *BRCA2* like is set at 0.5, according to the original publication (13, 12). The *BRCA1*-like and *BRCA2*-like classifier tool is available via http://ccb.nki.nl/software/nkiBRCA/. The translation and concordance between the BAC aCGH *BRCA1*-like and *BRCA2*-like classifiers and the NimbleGen classifier have been described previously (17). Array data are accessible through GEO (GSE77605 for the 16 *BRCA1*-mutated ER^+ tumors, GSE9021, GSE9114, GSE9114, and GSE16511 for the control samples).

BRCA1 promoter methylation

Hypermethylation of the *BRCA1* promoter was determined using methylation-specific MLPA analysis, according to the manufacturers' protocol (ME-001, MRC-Holland). For normalization and analysis, we used an in-house–developed Microsoft Excel macro. We used a cutoff of 20% to call a gene promoter methylated.

Loss of heterozygosity analysis

Loss of heterozygosity (LOH) at the *BRCA1* locus for the *BRCA1*-mutated ER^+ tumors was determined by Sanger sequencing of tumor DNA using flanking primers. The incorporation of wild-type versus mutant nucleotide was determined on an ABI sequencer 3730 and used to determine whether there was retention of heterozygosity or LOH and whether the wild-type or mutant allele was underrepresented (lost). For every tumor sample, matched normal DNA, obtained from peripheral blood, was available as a control.

Statistical analysis

The Fisher exact test was used to assess the association between the dichotomized histologic and clinical variables and BRCAness characteristics. $P < 0.05$ was considered statistically significant. IBM SPSS Statistics version 22 was used. Comparison of copy number aberrations between tumor groups was performed in Nexus Copy Number 7.5 (BioDiscovery). To call regions significantly different from each other, the minimum difference in percentage was set at 25% and the *P* value (calculated by Fisher exact test) was below 0.05. As there were multiple tests performed, Q-bound values were calculated, which correct for multiple testing by performing FDR correction.

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Results

Description of the cohort

There were 16 *BRCA1*-mutated ER$^+$ tumors in our database. Average age of patients involved was 45 years. Most tumors were invasive ductal carcinomas, and HER2 negative (Tables 1 and 2). When we applied our previously developed *BRCA1*-like classifier, developed to identify *BRCA1*-associated breast cancer (13), only two *BRCA1*-mutated ER$^+$ tumors showed the characteristic *BRCA1*-like genomic profile. From previous series, we know that 80% to 90% of all *BRCA1*-mutated ER$^-$ tumors show this *BRCA1*-like specific genomic profile (18, 19). Interestingly, 10 of 16 *BRCA1*-mutated ER$^+$ tumors (62.5%) showed a *BRCA2*-like genomic profile, developed to specifically identify *BRCA2*-mutated breast tumors (12). *BRCA1* promoter methylation was absent in all *BRCA1*-mutated ER$^+$ tumors. From 12 tumors, the amount of DNA was sufficient for LOH analysis; 10 of 12 ER$^+$*BRCA1*-mutated tumors showed loss of the wild-type *BRCA1* allele, which is significant (binomial test $P = 0.04$). One sample showed loss of the mutant allele, whereas another sample did not show loss at the *BRCA1* locus (Table 1).

Comparison of copy number profiles

As we observed that *BRCA1*-mutated ER$^+$ tumors more often showed a *BRCA2*-like genomic profile than a *BRCA1*-like profile, we looked at the mean genomic profile of the 16 tumors, with special focus on the *BRCA1*-like and *BRCA2*-like specific genomic regions. For comparison purposes, we also show the aggregate copy number profiles of 57 *BRCA1*-mutated ER$^-$ tumors, 36 *BRCA2*-mutated ER$^+$ tumors, and 182 sporadic ER$^+$ tumors (Fig. 1). Figure 1A highlights the characteristic *BRCA1*-like genomic features, such as 3q gain, 5q loss, and others. Those regions are used in our *BRCA1*-like classifier. They are clearly present in the mean *BRCA1*-mutated ER$^-$ profile, but absent in the *BRCA1*-mutated ER$^+$ profile as well as in the *BRCA2*-mutated tumors or in the sporadic ER$^+$ tumors.

Figure 1B highlights specific *BRCA2*-associated genomic regions, like 3p loss, 6q loss, and other regions. They are clearly present in the *BRCA2*-mutated ER$^+$ tumors. Interestingly, most genomic regions specific for *BRCA2*-mutated tumors are also observed in the *BRCA1*-mutated ER$^+$ tumors and, to a somewhat lesser extent, in *BRCA1*-mutated ER$^-$ tumors. We have to make clear that in the original classifier, the combination of multiple loci could discriminate *BRCA2* mutated from sporadic tumors; however (12), in later research, it appears that the profile was also observed in other genomically instable tumors, such as *BRCA1*-mutated tumors. However the percentage of these changes is lower in non-*BRCA2*–mutated cancers. In the sporadic tumors, most specific *BRCA2*-like copy number changes are absent or seen at a low frequency, for example, 3p loss, 11q loss, 13q loss, etc. From these observations, we can conclude that the copy number profiles of *BRCA1*-mutated ER$^+$ tumors are different from the copy number profiles of *BRCA1*-mutated ER$^-$ tumors, but that they are more alike *BRCA2*-mutated tumors. From the comparison with sporadic ER$^+$ tumors, we can conclude that the genomic profile of *BRCA1*-mutated ER$^+$ tumors is like the specific *BRCA2*-related profile, and not a standard sporadic luminal copy number profile (Supplementary Fig. S1; Supplementary Tables S1 and S2).

Comparison of clinicopathologic and molecular markers

We compared genomic and histopathologic markers of *BRCA1*-mutated ER$^+$ tumors with *BRCA1*-mutated ER$^-$ tumors,

Table 1. Patient and tumor characteristics

Patient characteristics				Histopathology and IHC						Molecular analyses					
		Germline BRCA1 mutation													
ID	Age	Exon	Mutation (c.)	Tumor type	Grade	ER%	PR%	HER2	p53%	KI67%	BRCA1-like	BRCA1 score	BRCA2-like	BRCA2 score	Methylation	LOH analysis
1	53	11	c.2210delC	ILC	1	100	0	Neg	0	ND	SP-like	0	SP-like	0.28	No meth	Loss wt allele
2	55	11	c.2210delC	IDC	3	100	0	Neg	0	ND	SP-like	0	B2-like	0.94	No meth	Loss wt allele
3	40	13	c.4186-1632_4357+2031del3835	IDC	2	100	100	Neg	100	ND	SP-like	0.019	SP-like	0.002	No meth	Loss wt allele
4	39	20	c.5266dupC	Mixed	3	90	70	Neg	<10	ND	SP-like	0.002	B2-like	0.69	No meth	Loss wt allele
5	38	22	c.5333-36_5406+400del510	IDC	ND	100	30	Neg	20	ND	Sp-like	0.16	B2-like	1	No meth	ND
6	72	14	c.4416_4417delTTinsG	IDC	1	100	ND	ND	ND	ND	SP-like	0	SP-like	0	No meth	ROH
7	41	11	c.3820dupG	ILC	ND	75	10	Neg	95	5	Sp-like	0.45	Sp-like	0.16	ND	Loss wt allele
8	66	11	c.2989_2990dupAA	IDC	ND	100	60	Neg	1	15	B1-like	0.76	B2-like	0.59	No meth	Loss wt allele
9	47	22	c.5333-36_5406+400del510	IDC	ND	90	<5	Neg	90	80	Sp-like	0.35	Sp-like	0.001	No meth	Loss wt allele
10	42	22	c.5333-36_5406+400del510	IDC	ND	5	0	Neg	80	90	Sp-like	0.41	Sp-like	0	No meth	Loss wt allele
11	62	20	c.5266dupC	IDC	3	20	0	Neg	1	25	B1-like	1	B2-like	1	No meth	Loss mut allele
12	58	17	c.4987-?_5074+?del	IDC	3	100	5	Pos	ND	30	Sp-like	0.01	B2-like	0.997	ND	ND
13	68	5	c.181T>G	IDC	3	100	1	Neg	ND	40	Sp-like	0.01	B2-like	0.998	ND	Loss wt allele
14	39	22	c.5333-36_5406+400del510	IDC	ND	90	80	Neg	0	10	SP-like	0.003	B2-like	0.67	No meth	ND
15	43	5	c.181T>G	IDC	2	100	0	Neg	ND	ND	SP-like	0	B2-like	1	ND	ND
16	43	22	c.5333-36_5406+400del510	IDC	2	100	100	Neg	100	ND	SP-like	0.04	B2-like	0.68	ND	Loss wt allele

Abbreviations: IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; meth, methylation; mut, mutant; ND, not determined; ROH, retention of heterozygosity; wt, wild type.

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Table 2. Comparison of clinicopathologic characteristics between B1mutER$^+$, B1mutER$^-$, B2mutER$^+$, and sporadic ER$^+$ tumors

		B1mutER$^+$ ($n = 16$) *n* (%)	**B1mutER$^-$ ($n = 57$)** *n* (%)	*P*[a]	**B2mutER$^+$ ($n = 36$)** *n* (%)	*P*[a]	**SporER$^+$ ($n = 182$)** *n* (%)	*P*[a]
BRCA1-like	BRCA1-like	2 (12.5%)	49 (86%) 2		2 (5.6%)		4 (2.6%)	
	Sporadic-like	14 (87.5%)	8 (14%)	<0.001	34 (94.4%)	0.39	147 (97.4%)	0.04
	Not tested	0	0		0		31	
BRCA2-like	BRCA2-like	10 (62.5%)	31 (60.8%)		30 (83.3%)		49 (32.5%)	
	Sporadic-like	6 (37.5%)	20 (39.2%)	0.90	6 (16.7%)	0.10	102 (67.5%)	0.02
	Not tested	0	6		0		31	
BRCA1 Meth	Methylated						1 (2%)	
	Nonmethylated	10 (100%)	44 (100%)	ND	0 90%)	ND	49 (98%)	0.65
	Not done	6	13		36		132	
PR IHC	Negative	8 (57.1%)	48 (100%)		21 (60%)		51 (30.9%)	
	Positive	6 (42.9%)	0 (0%)	<0.001	14 (40%)	0.85	114 (69.1%)	0.05
	Unknown	2	9		1		17	
HER2 IHC	Negative	14 (93.3%)	46 (100%)		31 (93.9%)		131 (80.4%)	
	Positive	1 (6.7%)	0 (0%)	0.08	2 (6.1%)	0.94	32 (19.6%)	0.22
	Unknown	1	11		3		19	
Histology	IDC	10 (83.3%)	24 (100%)		35 (100%)		139 (87.4%)	
	ILC	2 (16.7%)	0 (0%)	0.04	0 (0%)	0.01	20 (12.6%)	0.68
	Unknown	4	33		1		23	
Grade	Grade 1/2	4 (50%)	3 (13.6%)		22 (62.9%)		71 (65.7%)	
	Grade 3	4 (50%)	19 (86.4%)	0.04	13 (37.1%)	0.50	37 (34.3%)	0.37
	Unknown	8	35		1		74	
Age (median ± SD)		45 ± 11.7	43 ± 9.4	0.04	41 ± 11.7	0.04	48 ± 8.6	0.65

NOTE: Patients with unknown characteristics were not included in *P* value calculations; therefore, percentages for these groups are not depicted in the table.
Abbreviations: B1mutER$^+$, *BRCA1*-mutated ER$^+$; B1mutER$^-$, *BRCA1*-mutated ER$^-$; B2mutER$^+$, *BRCA2*-mutated ER$^+$; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ND, not determined; SporER$^+$, sporadic ER$^+$.
[a]*P* value for comparison of *BRCA1*-mutated ER$^+$ tumors with *BRCA1*-mutated ER$^-$ tumors, *BRCA2*-mutated ER$^+$ tumors, and sporadic ER$^+$ tumors, respectively.

BRCA2-mutated ER$^+$ tumors, and sporadic ER$^+$ breast cancer (Table 2). As expected, a *BRCA1*-like profile was frequently found in *BRCA1*-mutated ER$^-$ tumors, but not in *BRCA1*-mutated ER$^+$ tumors, *BRCA2*-mutated ER$^+$ tumors, or sporadic ER$^+$ tumors (86%, 12.5%, 5.6%, and 2.6%, respectively, had a BRCA1-like profile). A *BRCA2*-like profile was frequently observed in *BRCA2*-mutated tumors (83.3%). It was also frequently observed in *BRCA1*-mutated ER$^+$ tumors (62.5%), as well as in *BRCA1*-mutated ER$^-$ tumors (60.8%), indicating that it is less specific than the *BRCA1*-like pattern and also depicts tumors with a high amount of genomic instability.

BRCA1 methylation was not observed in *BRCA1*-mutated tumors, neither in *BRCA1*-mutated ER$^+$ tumors nor in *BRCA1*-mutated ER$^-$ tumors. Previously, it has been shown that a mutation in *BRCA1* and *BRCA1* promoter methylation are two mutually exclusive events in ER$^-$ breast cancer (18), this is apparently also true for ER$^+$ tumors. *BRCA1* promoter methylation was not observed in *BRCA2*-mutated tumors. Only one of the sporadic ER$^+$ tumors showed *BRCA1* promoter methylation. As *BRCA1* methylation is frequently observed in non-*BRCA*–mutated triple-negative tumors, it appears mainly related to this breast cancer subtype (18).

BRCA1-mutated ER$^+$ tumors more often showed positive PR staining, HER2 amplification, invasive lobular histology, and grade 1–2 tumors and occurred at a higher age than *BRCA1*-mutated ER$^-$ tumors. Pathologic characteristics were similar between *BRCA1*-mutated ER$^+$ tumors and *BRCA2*-mutated ER$^+$ tumors, except for histology. Age was also higher in *BRCA1*-mutated ER$^+$ tumors compared with *BRCA2*-mutated ER$^+$ (45 years vs. 41 years, respectively; $P = 0.04$). Sporadic breast cancer cases more often had positive immune staining for PR than *BRCA1*-mutated ER$^+$ tumors.

Discussion

BRCA1-mutated ER$^+$ tumors have been suggested to be "sporadic" tumors and not related to *BRCA1* deficiency. In the current study, we showed that most *BRCA1*-mutated ER$^+$ tumors have a BRCAness copy number profile and LOH, indicating that the loss of a functional BRCA1 protein plays a role in their tumorigenesis. Interestingly, the genomic profile of these tumors is highly similar to *BRCA2*-mutated ER$^+$ tumors, but not to *BRCA1*-mutated ER$^-$ tumors. Therefore, we hypothesize that these tumors are sensitive to drugs targeting the *BRCA1* gene defect, such as PARP inhibitors.

In a study in 2007, Melchor and colleagues concluded that ER status is an important marker of changes in the tumor genome and independent of the underlying mutation status (20). Interestingly, both the *BRCA1*-mutated ER$^+$ tumors and *BRCA2*-mutated ER$^+$ tumors in our series showed a similar specific copy number profile, quite distinct from the ER$^+$ sporadic breast cancer profile. So, although the specific type of the mutation does not determine the specific profile, tumors with a hereditary component have a clearly different genomic appearance than sporadic tumors, representing BRCAness. This is also demonstrated by the work from Nik-Zainal and colleagues, showing that *BRCA1*- and *BRCA2*-associated cancers have clearly distinct mutational and rearrangement signatures (21, 22).

Strong proof for the involvement of *BRCA1* loss in the origin of these tumors was obtained by LOH analysis. An assumption is that LOH of the wild-type allele is the second hit in *BRCA1*-mutated breast cancer and responsible for complete silencing of the *BRCA1* gene. In the vast majority of *BRCA1*-mutated ER$^+$ tumors in this series, LOH analysis indeed showed loss of the wild-type allele. In line with published literature, only two of 12

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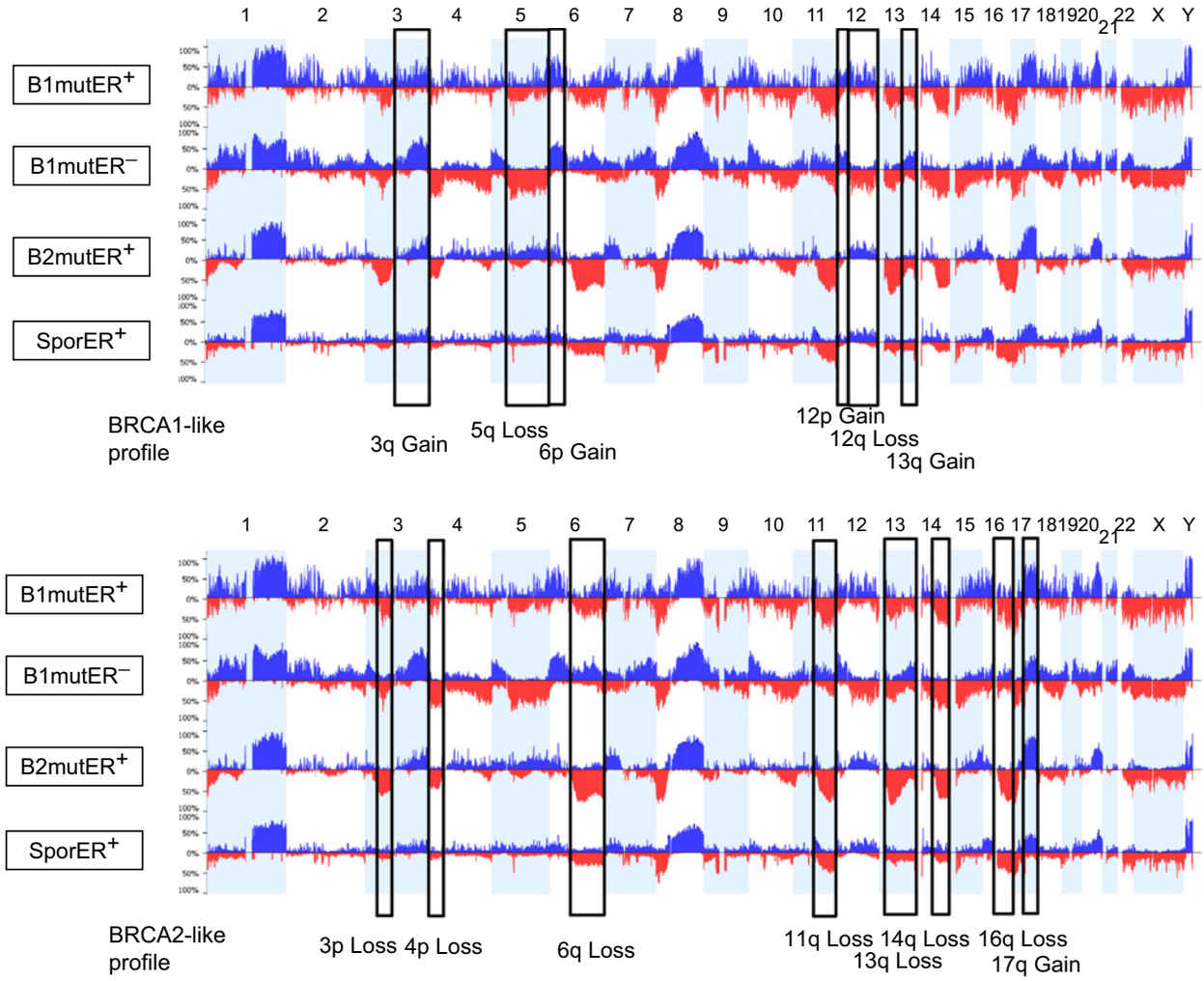

Figure 1.

This figure compares the genomic profiles of the *BRCA1*-mutated ER$^+$ (B1mutER$^+$), *BRCA1*-mutated ER$^-$ (B1mutER$^-$), *BRCA2*-mutated ER$^+$ (B2mutER$^+$), and sporadic ER$^+$ (SporER$^+$) tumor samples, respectively. Top, regions from the *BRCA1*-like signature (black squares); bottom, regions from the *BRCA2*-like signature. Top, we can see that *BRCA1*-mutated ER$^+$ does not show the characteristic *BRCA1*-like features, as seen in the *BRCA1*-mutated ER$^-$ tumors. Bottom, we can see that several *BRCA2*-like features are clearly observed in the *BRCA1*-mutated ER$^+$, like 3p loss, 6q loss, 11q loss, 16q loss, and 17q gain.

tumors did not show LOH of the wild-type allele. Tung and colleagues (7) reported a percentage of 81% LOH with loss of the wild-type allele in *BRCA1*-mutated ER$^+$ tumors, comparable with our series and comparable with *BRCA1*-mutated ER$^-$ tumors. *BRCA1* promoter methylation is another mechanism for silencing of the *BRCA1* gene. However, *BRCA1* promoter methylation was not observed in our series of *BRCA1*-mutated ER$^+$ tumors. This observation is similar to the lack of *BRCA1* promoter methylation in *BRCA1*-mutated ER$^-$ tumors (18).

Histopathologic features and age at diagnosis were different between *BRCA1*-mutated ER$^+$ and ER$^-$ tumors. *BRCA1*-mutated ER$^+$ tumors were more similar to *BRCA2*-mutated ER$^+$ tumors and to sporadic ER$^+$ tumors. Tung and colleagues compared histopathologic features between *BRCA1*-mutated ER$^+$ and ER$^-$ tumors and concluded that ER$^+$ cancers appear pathologically intermediate between *BRCA1*-mutated ER$^-$ tumors and sporadic ER$^+$ breast cancers (7). This would suggest that either some *BRCA1*-mutated ER$^+$ tumors are incidental or that there is a unique mechanism by which these cancers develop. In our series, we observed that patients with *BRCA1*-mutated ER$^+$ tumors had a higher age at diagnosis, even compared with patients with *BRCA2*-mutated ER$^+$ tumors. One of the patients without loss of the wild-type allele was 72 years old and did not show a BRCAness copy number profile, suggesting that her tumor was indeed a "sporadic" one.

PARP inhibitors are now studied in multiple clinical trials (11). Most support for their use is for *BRCA1*- and *BRCA2*-mutated tumors. Therefore, it is essential to know whether PARP inhibitors are effective irrespective of the hormonal status of a tumor. The loss of the wild-type *BRCA1* allele as well as a BRCAness copy number profile was shown in a vast majority of the cases, indicating complete homologous recombination deficiency. Therefore, these data suggest that *BRCA1*-mutated ER$^+$ tumors are sensitive to drugs targeting this DNA repair defect, like PARP

inhibitors. In the ongoing clinical trials on PARP inhibitors in *BRCA1* mutation carriers, we suggest to include ER$^+$ BRCA1-mutated tumors and to specifically monitor their treatment response. LOH analysis and genomic profiling could be additional biomarkers to select patients for treatment with drugs targeting homologous recombination deficiency. In addition to PARP inhibitors, *BRCA1*-mutated ER$^+$ tumors would also be good candidates for treatment with other DNA-damaging drugs, such as platinum salts or alkylating agents.

A limitation of the current study is that the number of ER$^+$ *BRCA1*-mutated tumors is small and that treatment data are unavailable. Therefore, we can only speculate how these tumors would respond. Another limitation is that the samples are obtained from our pathology archive and are not a well-described cohort, but rather a mixture of patient groups.

In conclusion, our genetic and genomic analysis confirms a role for *BRCA1* in tumorigenesis *BRCA1*-mutated ER$^+$ tumors and suggests homologous recombination deficiency in these tumors. Our findings are clinically highly relevant, as targeting the DNA damage repair defect, for example, by PARP inhibitors, is an effective and promising strategy to treat homologous recombination–deficient tumors successfully.

Disclosure of Potential Conflicts of Interest

G.S. Sonke reports receiving teaching activity fees from AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

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Conception and design: E.H. Lips, F.B.L. Hogervorst, P.M. Nederlof
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