A Marker of Homologous Recombination Predicts Pathologic Complete Response to Neoadjuvant Chemotherapy in Primary Breast Cancer

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Abstract

Purpose: To assess the prevalence of defective homologous recombination (HR)-based DNA repair in sporadic primary breast cancers, examine the clinicopathologic features that correlate with defective HR and the relationship with neoadjuvant chemotherapy response.

Experimental Design: We examined a cohort of 68 patients with sporadic primary breast cancer who received neoadjuvant anthracycline-based chemotherapy, with core biopsies taken 24 hours after the first cycle of chemotherapy. We assessed RAD51 focus formation, a marker of HR competence, by immunofluorescence in postchemotherapy biopsies along with geminin as a marker of proliferative cells. We assessed the RAD51 score as the proportion of proliferative cells with RAD51 foci.

Results: A low RAD51 score was present in 26% of cases (15/57, 95% CI: 15%–40%). Low RAD51 score correlated with high histologic grade (P = 0.031) and high baseline Ki67 (P = 0.005). Low RAD51 score was more frequent in triple-negative breast cancers than in ER- and/or HER2-positive breast cancer (67% vs. 19% respectively; P = 0.0036). Low RAD51 score was strongly predictive of pathologic complete response (pathCR) to chemotherapy, with 33% low RAD51 score cancers achieving pathCR compared with 3% of other cancers (P = 0.011).

Conclusions: Our results suggest that defective HR, as indicated by low RAD51 score, may be one of the factors that underlie sensitivity to anthracycline-based chemotherapy. Defective HR is frequent in triple-negative breast cancer, but it is also present in a subset of other subtypes, identifying breast cancers that may benefit from therapies that target defective HR such as PARP inhibitors.

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Chemotherapy for primary breast cancer is frequently given neoadjuvantly (prior to surgery) to reduce tumor size with the aim of avoiding mastectomy (14, 15). Although the majority of patients receiving neoadjuvant therapy achieve a clinical response to chemotherapy, only a minority of the patients achieve a pathologic complete response (pathCR)—absence of invasive tumor cells in the breast and axillary lymph nodes. Achieving a pathCR is a strong predictor of good outcome, with substantially improved outcomes in comparison with patients who do not enter a pathCR (16, 17).

In this study, we examined a cohort of patients with sporadic primary breast cancer who had tumor biopsy 24 hours after the first cycle of neoadjuvant anthracycline-based chemotherapy (18, 19). RAD51 focus formation was assessed in the postchemotherapy biopsy to determine the prevalence of defective HR in sporadic primary breast cancer, investigate whether defective HR predicts for pathCR to chemotherapy, and identify clinicopathologic features that correlate with defective HR.

**Materials and Methods**

**Clinical material**

Patients and tumor material have been described previously (18, 19). Briefly, we identified all patients who, between 1995 and 2002, received neoadjuvant chemotherapy for primary breast cancer at Royal Marsden Hospital and had a tumor biopsy 24 hours after the first cycle of chemotherapy. All patients with formalin-fixed and paraffin-embedded (FFPE) material available were included in this study. Pathologic complete response to chemotherapy was defined as the absence of invasive tumor cells in the breast and axillary lymph nodes in the surgical resection specimen. Clinicopathologic characteristics of patients included in the study are shown in Table 1. No patients were known to carry germline mutations in *BRCA1* or *BRCA2*. Estrogen receptor (ER) and Ki67 (assessed with MIB1) were assessed as reported previously (18, 19). To assign tumor subtype, progesterone receptor (PR) expression was assessed on ER-negative/HER2-negative cancers (Dako clone PgR 636). A single ER-negative/HER2-negative cancer expressed PR and was assigned as ER-positive/HER-negative subtype, with the remainder being triple negative (12 cases). Patients gave informed consent for collection of biopsies, and research was approved by the Royal Marsden Hospital Research Ethics Committee.

**Translational Relevance**

Here, we show that a substantial proportion of sporadic breast cancers have evidence of defective homologous recombination (HR). Our findings have potential significance for the development of therapies that target defective HR, such as PARP inhibitors, identifying new tumor subtypes that might benefit from therapy.

**Cell lines, materials, and antibodies**

Capan1 cells and derived PIR2 PARP inhibitor–resistant cell line were described previously (20). Cald1 cells were obtained from ATCC and maintained in phenol red–free DMEM or RPMI with 10% FBS (fetal bovine serum; PAA gold) and 2 mmol/L of l-glutamine (Sigma-Aldrich). Antibodies RAD51 (Clone 14B4; GeneTex), γH2AX (phospho-Histone H2AX Ser139 clone 20E3; Cell Signaling), and geminin (10802-1-AP, ProteinTech Group).

**Screening of RAD51 focus antibodies**

Capan1 and PIR2 cells (6 × 10⁶ cells) were injected, admixed 1:1 with Matrigel, into the lateral flanks of 18 Ncr-nude female mice and for the CAL51 cells (2 × 10⁶) were injected into both flanks of 11 Ncr-nudes. When tumors were visible, the mice were divided into 2 groups, 1 group was irradiated with 8 Gy IR, and the other not, the tumors were excised 6 hours later and formalin fixed.

**RAD51 immunofluorescence assay**

Immunofluorescence analysis was carried out on 3-μm sections of FFPE tumor material. Following antigen retrieval by microwaving at pH 9 (DAKO pH 9 buffer) for 18 minutes followed by 20 minutes cooling in buffer, sections were treated with Triton 0.2% for permeabilization for 20 minutes, washed in phosphate-buffered saline (PBS), treated with 100 μL of DNAse I (Roche) for 1 hour at 37°C and blocked with immunofluorescence buffer (IFF; 1% bovine serum albumin, 2% FBS in PBS) for 30 minutes at room temperature (RT). Sections were stained with geminin antibody 1:400 in IFF for 1 hour at RT, washed with PBS, followed by anti-rabbit Alexa488 conjugate 1:1,000 in IFF for 1 hour at RT, washed with PBS, fixed with 4% paraformaldehyde (PFA) solution for 15 minutes, stained with RAD51 antibody 1:100 in IFF for 1 hour at RT, washed with PBS, followed by anti-mouse Alexa555 conjugate 1:1,000 in IFF for 1 hour at RT, washed with PBS, fixed with 4% paraformaldehyde (PFA) solution for 15 minutes, stained with RAD51 antibody 1:100 in IFF for 1 hour at RT, washed with PBS, followed by anti-mouse Alexa555 conjugate 1:1,000 in IFF for 1 hour at RT, washed with PBS, fixed with 4’, 6 diamidino 2 phenylindole (DAPI; 1:10,000) for 15 minutes, and sections were fixed again with 4% PFA. The protocol for γH2AX staining was similar, with primary antibody 1:200 in IFF for 1 hour at RT.

**Scoring RAD51 focus assay**

Images were captured on a Leica TCS confocal microscope. We scored the nuclear foci staining as follows. Between 100 and 500 invasive tumor cells were counted at representative areas across the section. Cores with only DCIS (ductal carcinoma in situ) were deemed not assessable. A cell was counted as being geminin positive with any
A cell was counted as being RAD51 positive if there was at least 1 distinct focus per nucleus. This threshold was selected after examining baseline, pre-chemotherapy, cores in which greater than 99.9% of cells lacked any RAD51 foci. Proliferative fraction in the section was taken as the number of geminin-positive cells (S- and G2-phase marker) divided by total number of cells. The raw RAD51 count was taken as the number of RAD51-positive cells divided by the total number of cells expressed as a percentage. The RAD51 score was assessed as the percentage of geminin-positive cells that were also positive for RAD51. The γH2AX staining was performed on consecutive slide to that assessed for RAD51, with a cell considered positive with at least 1 foci.

Assessment of all immunofluorescence was performed blinded to clinicopathologic and chemotherapy response data. A tumor was defined as being deficient in HR with a RAD51 score less than 10% (i.e., <10% of geminin-positive cells had RAD51 foci).

### Statistical analysis

All statistical analyses were 2 sided and performed with GraphPad Prism version 5.0.

### Results

#### Establishment and validation of RAD51 focus formation assay

We set out to identify a RAD51 antibody for use in FFPE tissues. We screened 8 RAD51 antibodies and identified GeneTex Clone 14B4 to be the optimal antibody for use in FFPE material (Supplementary Table 1 and Fig. 1A). To validate the RAD51 antibody, we generated xenografts with the CAPAN1 BRCA2 mutant cell line (HR deficient), the
CAPAN1 derived "PIR" (HR competent) cell line that carries a revertant mutation in BRCA2 (20), and the CAL51 breast cancer cell line (HR competent). Established xenografts were irradiated, and 6 hours postirradiation tumors fixed in formalin. FFPE sections were subjected to RAD51 focus assay. Both PIR and CAL51 xenografts demonstrated a substantial induction of RAD51 foci that was not seen in CAPAN1 (Fig. 1B).

Patient and tumors included in the study

To examine the ability of the RAD51 focus assay to predict response to neoadjuvant chemotherapy and assess the prevalence of HR defects in primary breast cancer, we examined a series of 68 women who had received neoadjuvant chemotherapy for primary breast cancer and had core biopsies taken at 24 hours after the first cycle of anthracycline-based chemotherapy. The clinicopathologic characteristics are described in Table 1.

Assessment of RAD51 focus formation in baseline biopsies

We initially examined RAD51 focus formation in the baseline biopsies of 35 patients. RAD51 foci were observed in baseline biopsies in only 2 of the 35 cancers, and in these cancers, only 2% of cells had RAD51 foci (Fig. 2). Therefore, although low levels of RAD51 foci may form during the repair of endogenous DNA damage (21), these were below the level of sensitivity of this assay. This was advantageous, as it implied that assessment of RAD51 foci was only required in the postchemotherapy biopsy, as RAD51 foci present in postchemotherapy biopsies would reflect induction of RAD51 foci by damage. We restricted all further assessment to biopsies taken 24 hours after the first cycle of neoadjuvant chemotherapy.

Assessment of RAD51 focus induction at 24 hours postchemotherapy

Both the expression of RAD51 and the ability to perform HR, are cell cycle–regulated; HR is suppressed in G0/G1 and M phases of the cell cycle, and RAD51 foci form only in S and G2 phases of the cell cycle (12). To control for differences in proliferation between tumors, we costained each section for geminin; geminin is expressed only in S and G2 phases of the cell cycle (22) and is involved in the inhibition of replication licensing (23). This correction was important as proliferation is decreased in many tumors at 24 hours postchemotherapy (18, 19) and without correction, a tumor may be falsely attributed as HR deficient if proliferation is low or absent. Tumor sections from 11 women (16%, 11/68) taken 24 hours after chemotherapy did not score for geminin, and these tumors were excluded from further analysis (Supplementary Fig. 1). There was a
significant correlation between geminin and Ki67 assessed on the same biopsy 24 hours after chemotherapy ($r = 0.68$, 95% CI: 0.49–0.81; $P < 0.0001$; Spearman’s correlation coefficient; data not shown).

We initially examined the raw RAD51 count, the proportion of all tumor cells positive for RAD51 foci. The relationship between raw RAD51 count and proliferative fraction, assessed in the same section as the proportion of tumor cells geminin positive, was biphasic (Fig. 3A). In general, raw RAD51 count and proliferative fraction were highly significantly positively correlated in tumors with greater than 2% raw RAD51 count ($r = 0.67$, 95% CI: 0.45–0.82; $P < 0.0001$; Spearman’s correlation coefficient; Fig. 3A). However, for tumors with low raw RAD51 count (<2%) the relationship between raw RAD51 count and proliferation was lost ($r = -0.2$; $P =$ not significant, NS), with these cancers having substantially raised proliferative fraction (Fig. 3A). Although a post hoc analysis, these data suggested that the majority of tumors were HR competent, with appropriate cell-cycle regulation of HR. In contrast, a minority of tumors displayed potential evidence of deficient HR (Fig. 3A).

We investigated whether there was a pharmacokinetic explanation for lack of RAD51 foci, that is, that biopsied tumor had not been exposed to sufficient concentrations of chemotherapy to induce DNA damage. To do this, we measured DNA damage by examining for induction of phosphorylated H2AX ($\gamma$H2AX), a marker of DNA double-strand breaks (24), in 3 tumors with RAD51 foci and in 4 tumors without RAD51 foci postchemotherapy (Supplementary Figs. 2 and 3). No difference in the induction of $\gamma$H2AX was found between tumors with and without RAD51 foci (median increase in $\gamma$H2AX 18% vs. 38% respectively; $P = 0.09$; Student’s t test), with $\gamma$H2AX induction actually being numerically greater in tumors without RAD51 foci (Supplementary Fig. 2). This strongly suggested...
that the observed differences reflected tumor biology, as opposed to poor drug exposure.

**Cell cycle–adjusted RAD51 score**

To control for the effect of proliferation on RAD51, we assessed RAD51 foci only in geminin-positive cells, therefore assessing RAD51 foci only in tumor cells in the correct cell-cycle phase. We termed this the RAD51 score (the percentage of geminin-positive cells also positive for RAD51). In all tumors analyzed, RAD51 foci were almost exclusively observed in geminin-positive cells (median of 40% geminin-positive cells were RAD51 positive compared with 0% geminin-negative cells; \( P < 0.0001 \); Mann–Whitney U test; Fig. 3B). The strength of this anticipated relationship provided further validation of the immunofluorescent staining.

There was a wide range of RAD51 score at 24 hours postchemotherapy, from 0% to 100% (Fig. 4). We determined the optimal cutoff to assign low RAD51 score by examining the point at which the association between raw RAD51 count and proliferation broke down (Fig. 3A), suggesting that a RAD51 score of less than 10% was the most appropriate to reflect deficient HR, potentially identifying 2 distinct populations (Fig. 3A). Overall, 26% of primary breast cancers had a RAD51 score less than 10% (15/57, 95% CI: 15%–40%; Fig. 4).

**Correlations of RAD51 score with clinical features**

We examined the relationship between RAD51 score and clinicopathologic features (Table 2 and Fig. 4). Low RAD51 score was associated with high histologic grade, ER-negative tumors, and high proliferation in baseline core biopsy and 24-hour biopsy, potentially reflecting an association between loss of HR and tumor proliferation (Table 2). We examined the prevalence of defective HR according to tumor subtype. Triple-negative cancers had a lower RAD51 score than other cancers (median RAD51 score; TN cancers 2.3% vs. other subtypes 42.5%; \( P = 0.0077 \); Mann–Whitney U test; Fig. 4), and a low RAD51 score was observed in 67% (8/12) TN breast cancers compared with 19% non-TN (7/37; \( P = 0.0036 \); Fisher’s exact test). We conducted multivariate analysis of the features associated with RAD51 score. In a model incorporating tumor subtype and baseline Ki67, TN subtype was a significant predictor of low RAD51 score (Supplementary Table 3).

**Inability to form RAD51 foci correlates with pathologic CR**

Overall there was no association between clinical response to chemotherapy and low RAD51 score (Table 2), although all tumors with a low RAD51 score achieved a clinical response compared with 76% without a low score (14/14 vs. 31/41; \( P = 0.051 \); Fisher’s exact test). Clinical response to chemotherapy is a poor surrogate for outcome, and we therefore examined pathCR to chemotherapy.

Tumors that achieved a pathCR with neoadjuvant chemotherapy had lower RAD51 scores than those that did not achieve pathCR (median RAD51 score; pathCR 2.6% vs. non-pathCR 44%; \( P = 0.028 \); Mann–Whitney U test; Fig. 4). Of the tumors with low RAD51 score 33% (4/12) achieved pathCR, compared with 3% (1/36) of tumors with RAD51 foci (\( P = 0.011 \); Fisher’s exact test), corresponding to a sensitivity of 80% and specificity of 81% for low RAD51 score as a predictive marker of pathCR.

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**Fig. 3.** Raw RAD51 count shows a biphasic relationship with proliferation in 24-hour postchemotherapy biopsies. A, plot of raw RAD51 counts (x-axis, percentage of tumor cells that have RAD51 foci) against proliferative fraction assessed on same section (y-axis, percentage of tumor cells with nuclear geminin expression) in core biopsies from 57 tumors taken at 24 hours postchemotherapy. In tumors with a raw RAD51 count greater than 2% there is a positive correlation between RAD51 count and proliferation (\( P = 0.67 \), 95% CI: 0.45–0.82; \( P < 0.0001 \); Spearman’s correlation coefficient), but not in tumors with raw RAD51 count less than 2% (\( P = 0.21 \); \( P = 0.11 \); Mann–Whitney U test; Fig. 3B). The strength of this anticipated relationship provided further validation of the immunofluorescent staining.
Discussion

In this study, we have used a functional RAD51 assay to provide a comprehensive assessment of HR in sporadic breast cancer. We provide evidence that a functional defect in HR is common in TN breast cancer and also provide evidence for defective HR in a subset of high-grade ER- and/or HER2-positive breast cancer. Other recent studies have used RAD51 to examine breast and ovarian cancer samples (25–27). One study examined RAD51 foci in breast cancers irradiated ex vivo (25), and a further study examined RAD51 in primary breast cancers without considering the confounding factor of proliferation in the biopsy, identifying only moderate predictive power for clinical response to chemotherapy (26). Clinical response to chemotherapy is a poor surrogate for outcome, and here we have investigated for predictors of pathCR, which is strongly predictive of good outcome (16, 17).

Our study illustrates the importance of considering proliferation when assessing a cell cycle–regulated marker such as RAD51 foci. Without making any assessment of proliferation, RAD51 would have less predictive power for pathCR (P = 0.07; Mann–Whitney U test; data not shown) and no association with TN phenotype (P = 0.13; Mann–Whitney U test, data not shown), primarily as the 11 cases with no geminin expression would all have been, potentially falsely, assigned as HR deficient. Lack of geminin expression following chemotherapy is likely to reflect lack of proliferation resulting from the engagement of cell-cycle checkpoints. However, an alternative explanation would be failure of immunofluorescence due to poor fixation of the tissue core. Assessment of geminin may, therefore, improve RAD51 assessment by both screening out cores in which proliferation is absent, as well as providing an internal control.

Predicting the responsiveness of an individual tumor to chemotherapy has been a major challenge of breast cancer research (28). A number of prior studies have examined pathologic markers in biopsies 24 to 48 hours postchemotherapy. Assessment of apoptosis at 48 hours postchemotherapy may predict for benefit to chemotherapy (29, 30), but another study has not confirmed this at 24 hours postchemotherapy (18). Similarly, assessment of Ki67 postchemotherapy has only weak predictive power (18, 19, 31), and these studies have not identified a consistent predictor of response to chemotherapy. Here, we have shown that the inability to form RAD51 foci is strongly associated with pathCR to neoadjuvant chemotherapy.
suggesting that defective HR may be a mechanism underlying pathCR to anthracycline-based chemotherapy. Our data suggest that it may be possible to identify patients who are less likely to benefit from anthracycline-based chemotherapy as soon as 24 hours following the first cycle of chemotherapy. In this study, high RAD51 score (induction of RAD51 by chemotherapy) had a 97% negative predictive value for failure to achieve pathCR. Potentially, after further validation of this test, tumors with a high RAD51 score may be better treated with taxane-based chemotherapy or switched to hormonal therapy. However, it is important to emphasize that pathCR is a surrogate endpoint, and only a very substantially larger validation study could assess the negative predictive value for outcomes such as recurrence-free survival. In future clinical trials, it may also be possible to assess HR competency by treating with a single-agent PARP inhibitor prior to assessing RAD51 foci.

Inhibitors of PARP are showing great promise in the treatment of hereditary BRCA1-/BRCA2-related breast and ovarian cancer (6). Similarly, a PARP inhibitor has demonstrated substantial activity in TN breast cancers in combination with carboplatin/gemcitabine chemotherapy (32). We have previously shown that basal-like cancers have suppressed BRCA1 expression (7). Here, we extend this observation to suggest that a substantial proportion of TN breast cancers have evidence for defective HR, and this potentially explains the benefit seen with PARP inhibitors in this subtype of cancers. In addition, certain chemotherapy drugs, such as interstrand cross-linking drugs including platinum, are highly toxic to HR-deficient cells and defective HR may also explain the high sensitivity of platinum chemotherapy seen in TN breast cancer (9, 33). However, our data suggest that approximately 30% to 40% of TN breast cancers do not have defective HR, providing further evidence for the heterogeneity of TN breast cancers.

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<th>Table 2. Clinicopathologic features according to RAD51 score</th>
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<tr>
<td>Low RAD51 score (HR deficient)</td>
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<tr>
<td>Median RAD51 score</td>
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<td>Pathology</td>
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<td>Invasive ductal carcinoma</td>
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<td>Invasive lobular carcinoma</td>
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<td>Median tumor size, mm</td>
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<td>Axillary node positive</td>
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NOTE: Statistical analysis was with Fisher’s exact test, unless indicated. Pathologic response data were not available on 9 cases as discussed in Supplementary Figure 1.

aMann–Whitney U Test or bChi squared test.
breast cancer and raising the possibility that these 30% to 40% TN cancers would not benefit from PARP inhibitors. However, mechanisms of sensitivity to PARP inhibitors do exist other than loss of HR. For example, cells with loss of Fanconi anemia genes are sensitive to PARP inhibitors in vitro (13), and PARP inhibitors have the potential to improve outcomes via chemopotentiation (34). Nevertheless, our data emphasize the importance of assessing biomarkers in ongoing PARP inhibitor trials to identify the subset of patients who benefit.

Our data suggest that approximately 20% of non-TN breast cancers have defective HR and might be expected to benefit from PARP inhibitors. Although all low RAD51 score cancers responded clinically to chemotherapy, the majority did not achieve a pathCR (Table 2). Anthraccline-/cyclophosphamide-based chemotherapy generates multiple cytotoxic lesions, of which only a small fraction results in DNA damage that would specifically target HR-deficient tumors. It is likely that HR-deficient tumors would benefit from DNA damage that is targeted more specifically at defective HR, to increase the therapeutic window. This observation potentially suggests that a combination of chemotherapy and PARP inhibitor might increase the fraction achieving pathCR and improve the long-term outcome of these patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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