DNA Repair Protein Biomarkers Associated with Time to Recurrence in Triple-Negative Breast Cancer

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Abstract

Purpose: To evaluate the prognostic utility of immunohistochemical assessment of key proteins in multiple DNA repair pathways in triple-negative breast cancer (TNBC; estrogen receptor negative, progesterone receptor negative, and HER2/neu negative by immunohistochemistry).

Experimental Design: Archived clinically annotated tumor specimens from 112 women with TNBC were immunostained with antibodies against DNA repair proteins and scored using digital image analysis. The cohort was divided into training and test sets for development of a multiantibody model. Scores were combined with clinical data to assess association with outcome.

Results: Low XPF (P = 0.005), pMK2 (P = 0.01), MLH; P = 0.002), and FANCD2 (P = 0.001) were each associated with shorter time to recurrence (TTR) in univariate analysis. A 4-antibody model could segregate high-risk and low-risk groups on the basis of TTR in both the training (relative risk [RR] = 3.52; P = 9.05E-07) and test (RR 2.67; P = 0.019) cohorts.

Conclusions: DNA repair proteins may be useful as prognostic markers in TNBC. Further study in larger, uniformly treated cohorts with additional clinical parameters is warranted. Clin Cancer Res; 16(23); 5796–804. ©2010 AACR.

Triple-negative breast cancers (TNBCs), those that are estrogen receptor (ER) negative, progesterone receptor (PR) negative, and HER-2 negative by immunohistochemistry or fluorescent in situ hybridization, comprise approximately 15% of all breast cancers and have an aggressive clinical course with high rates of local and systemic relapse (1). The clinical course appears to reflect the intrinsic biology of this group of tumors as well as the absence of specific targeted treatments to supplement conventional cytotoxic chemotherapy, such as hormonal therapies used for ER- or PR-positive patients and trastuzumab for HER-2 overexpressing tumors. In addition, these cancers may have different sensitivity to common chemotherapeutic agents (2). Thus, there is a great deal of interest in determining novel therapeutic regimens for this aggressive breast cancer subtype. Although TNBCs comprise an established subtype of breast cancer, they are not uniform and relatively little biomarker information is available with which to stratify patients and to direct treatment decisions.

Deficits in DNA repair are a characteristic of at least a subset of TNBCs (3, 4). These tumors exhibit more DNA copy alterations (5) and loss of heterozygosity [LOH; (6)] than other breast cancers—features suggestive of genomic instability. Furthermore, sporadic triple-negative tumors share phenotypic and cytogenetic features with familial BRCA1-associated breast cancers (1), the majority of which are also basal-like using microarray RNA expression data (7). BRCA1 mutant tumors have been shown to be deficient in DNA repair (3, 4), particularly homologous recombination (HR). Taken together, these similarities suggest that a DNA repair deficiency may underlie the development of at least a subset of triple-negative tumors. Deficits in DNA repair may have implications for prognosis and could influence response to both current DNA damage-inducing therapies and novel targeted agents such as poly-ADP ribose polymerase (PARP) inhibitors (8–12).

Given the possible association of TNBCs with DNA repair deficits and the relationship of DNA repair with phenotypic aggressiveness and therapy, we sought to evaluate the prognostic value of key proteins from multiple DNA repair pathways in TNBC. The goal of the study was to evaluate whether a pattern of immunohistochemistry-assessed DNA repair proteins from multiple repair pathways in pretreatment breast cancer specimens was correlated with clinical outcome.
Translational Relevance

This study investigates the use of antibodies against critical proteins in DNA repair pathways as molecular biomarkers. We show that in TNBCs, low expression of several DNA repair proteins (as assessed in FFPE tumor specimens by semiquantitative immunohistochemistry using these antibodies), is associated with worse progression-free survival. We also show the development of a multiantibody model that can further stratify patients into recurrence risk categories.

The results of this study may be applied to the future practice of cancer medicine in a number of ways. First, our data suggest that these biomarkers have prognostic relevance. Second, many therapeutic regimens in oncology involve DNA-damaging agents, so these DNA repair antibody biomarkers could have predictive capacity, as has been suggested by other studies. Finally, these antibodies may prove to be useful as biomarkers in clinical studies of novel therapeutics that target DNA repair such as PARP inhibitors.

Materials and Methods

Patients

We identified 143 women with a diagnosis between 1991 and 2006 whose primary breast cancers had been reported to be negative for estrogen and progesterone receptors and HER2/neu by clinical immunohistochemistry at the time of their initial visit to the Breast Oncology Center at the Dana Farber Cancer Institute, Boston, MA. Women were eligible if they had signed the informed consent document, provided a blood specimen to the Dana Farber/Harvard Cancer Center SPORE in Breast Cancer specimen bank, and given permission to use clinical data, a blood specimen, and their unused stored tumor specimens for research. Pathology was reviewed for histology and ER/PR/Her2 status by a breast pathologist at the Brigham and Women’s Hospital or Beth Israel Deaconess Medical Center, and all grades were confirmed. Clinical data including date of diagnosis, age at diagnosis, clinical TNM stage, BRCA1 mutation status, adjuvant chemotherapy regimen, and dates of administration and pathologic data including histologic type, histologic grade, lymphovascular invasion, and pathologic nodal status were collected and transferred with identifiers removed in accordance with procedures specified in a protocol approved by the Dana Farber/Harvard Cancer Center Institutional Review Board. BRCA1 mutation carriers had been identified previously by exon grouping analysis, a method based on conformation specific gel electrophoresis (13, 14). Of the 143 eligible women identified with triple-negative tumors in the Breast Cancer specimen bank, 112 had adequate tissue and clinical follow-up data available for analysis.

Specimen characteristics

A tissue microarray (TMA) was constructed from stored formalin-fixed, paraffin-embedded (FFPE) breast tumor specimens obtained from either biopsy tissue taken prior to neoadjuvant chemotherapy (n = 6) or primary surgical excisions performed before the administration of adjuvant radiation or chemotherapy (n = 105). The 1 patient whose specimen had been obtained after neoadjuvant chemotherapy was ultimately excluded from immunohistochemical (IHC) analysis. The TMA was created to efficiently evaluate the markers and minimize the effects of artifact-based staining variation between slides. For TMA construction, 3 0.6-mm cores were obtained from a paraffin block of each breast cancer and were inserted into the recipient TMA block.

Assay methods

Immunohistochemistry was conducted on 5-μm sections cut from the TMA block, using primary antibodies against proteins in multiple DNA repair pathways, including XPF [nucleotide excision repair (NER); AbCam, Ab17798], FANCD2 [Fanconi anemia/homologous recombination (FA/HR); Santa Cruz, sc-20022], MLH1 [mismatch repair (MMR); BioCare Medical, CM220], PARP1 [base excision repair (BER); AbD Serotec, MCA1522G], PAR [BER; Millipore, MAB3192], pMK2 [MAPKAPKinase2, DNA damage response (DDR); Cell Signaling Technology, 3007], p53, and Ki67. These downstream DNA repair pathway proteins were chosen as surrogates for the integrity of the entire pathway. For example, in the FA pathway, a critical step is the monoubiquitination of FANCD2 and the formation of nuclear foci (15–17). Therefore, antibodies against FANCD2, assessing for nuclear staining, were used to represent all of the upstream events that occur in the FA pathway. XPF is a protein that forms a heterodimer with ERCC1 to create an endonuclease that is critical in the removal of helix-distorting DNA lesions through the NER pathway (18). Cells with absent XPF are hypersensitive to ultraviolet irradiation and chemicals that cause interstrand cross-links (19–22). Likewise, PAR, MLH1, and pMK2 are proteins that are critical actors in the BER and MMR pathways (23, 24) and DDR (25), respectively. Ki-67 was added to investigate the possible dependence of DNA repair protein expression on proliferation rate.

Positive assay controls, consisting of human breast cancer sections previously determined to express each antigen of interest, were run for each antibody, as was a negative control lacking primary antibody. Tissue sections were deparaffinized and rehydrated using standard techniques. Antigen retrieval was performed in a high-pressure cooker using citrate buffer (pH = 6.5) for XPF, FANCD2, PAR, PARP1, pMK2, MLH1, and Ki67. For p53, antigen retrieval was performed in a high-pressure cooker, using Tris-EDTA (pH = 7.8). Antibody incubations were overnight for XPF (1:1,200), p53 (1:400), and FANCD2 (1:100). Two-hour antibody incubations were performed for PAR (1:6,400), PARP1 (1:10,000), pMK2 (1:100), MLH1 (1:100), and Ki67 (1:100). Renaissance TSA (Tyramide Signal Amplification) Biotin System (Perkin Elmer) was used for the
detection of XPF and FANCD2. Super Sensitive IHC Detection System (BioGenex) was used for the detection of MLH1, PARP1, PAR, pMK2, and Ki67. Envision + System-HRP (Dako) was used for the detection of p53. Antibody dilution was optimized such that the antibody level was appropriate to explore a dynamic range.

Scoring

Microscope slides were scanned using a digital pathology platform (Aperio). A nuclear score was reported for FANCD2, XPF, PAR, MLH1, PARP1, p53, and Ki67 each. Image analysis macros were developed by modifying the Aperio IHC nuclear macro and were used to identify tumor nuclei for the nuclear staining markers. In addition, the percentage of nuclei staining at 4 different intensities (high, moderate, low, and negative) was outputted and used to create an H-score (0–300 scale) for XPF, PAR, PARP1, FANCD2, p53, and MLH1 (26). For Ki67, the score was based on the standard method of percentage of positively staining nuclei. Nuclear and cytoplasmic scores were separately compiled for pMK2, using a modified Aperio colocalization macro. Cytoplasmic staining was additionally scored for pMK2 on the basis of the prior observation that kinase activation is coupled with nuclear export (27). The tumor area was manually annotated. The presence of hematoxylin was used to define the nuclear area, whereas its absence is used to define the cytoplasmic area. Not all patients had IHC scoring data generated for each of the antibodies because of technical reasons or lack of tumor tissue on specific TMA slides.

Statistical analysis

IHC scoring to measure expression levels of DNA repair proteins was analyzed with clinical data to assess for correlation with clinical outcome. Time to recurrence (TTR) was defined as the time from diagnosis until the time of first recurrence. Patients without evidence of recurrence were censored at last follow-up. The 112 patients available for analysis were randomly assigned to training (n = 68) and test (n = 44) sets; the test set was withheld for final verification at the end of the modeling process. The training and test sets were generated to ensure an equal fraction of recurrences in each group, with a primary consideration to provide adequate numbers in the training set to facilitate discovery. We estimated that 6 to 8 samples would be required per model parameter; this allows 8 independent parameters to be explored (at the high end) with 64 patients in the training set. Ensuring equal outcome fractions in the training and test sets led to the actual numbers of 68 and 44, respectively. Each antibody IHC score was examined by univariate analysis and its ability to discriminate patient outcomes was assessed. Antibodies that showed significant discriminatory ability were moved forward to further multivariate analysis.

To assess the ability of the antibody scores to segregate patients into high and low recurrence risk groups, thresholds for each were determined that separated patients into 2 groups with maximally different survival curves. These optimal divisions were selected by choosing the threshold that generated minimum recurrence-free survival curve P value and had at least 5 samples per group.

A multiantibody model was developed to determine whether combinations of antibodies from different repair pathways were more informative than was any individual antibody from a single pathway. To determine which combinations of antibody scores enhanced performance, all pairs were analyzed using a single-node multivariate decision tree, with the impurity of the partition given by the survival curve P value (28). The optimal scoring thresholds for any 2 antibodies divide the samples into 4 groups; the group with the largest proportion of recurrent samples was labeled as the “high-risk” group and the remaining 3 groups together form the “low-risk” group. Combinations that significantly segregated low- versus high-risk groups were used for development of a 4-antibody model. The optimal scoring thresholds for the 4 antibodies were determined simultaneously by dividing the range of each antibody score into 20 equal intervals and establishing a grid of thresholds to be analyzed. The set of thresholds that generated the minimum survival curve P value were determined to be the optimal set. The number of patients available for each marker combination varied—not all patients had data available for every marker.

The antibodies and optimal thresholds determined from the training data set were then applied to the test data set. Survival curves for the low- and high-risk groups were compared using Kaplan–Meier models, and the P value was reported. In addition, the apparent error rate (AER), receiver operating characteristic (ROC) curve, and area under curve (AUC; for univariate analyses), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and relative risk were computed and reported.

The correlation of additional clinical and pathologic data with outcome was examined with Cox models, using all data from test and training sets to provide maximal statistical power. Clinical data in addition to the single multiantibody model was examined to determine whether there was any correlation with outcome using univariate and multivariate analyses to control for known prognostic factors.

Results

Patient cohort

Primary treatment and follow-up data were available for 112 patients, with a median follow-up of 61.5 months (Table 1). Median age for the cohort was 49.2 years. Sixty-four patients were treated with breast conservation therapy and 47 with mastectomy, 17 of which received postmastectomy radiation. Treatment details were unavailable for 1 patient. There were 110 grade 3 tumors, 1 grade 2 tumor, and 1 grade unknown. Lymphovascular invasion (LVI) was present in 48 (43%) of tumors and there was nodal involvement in 50 (47%) cases. One hundred eight patients received chemotherapy as part of their treatment: 41 with anthracycline/cyclophosphamide, 49 with anthracycline/cyclophosphamide/taxane, 15 with cyclophosphamide/Doxorubicin/taxane, 4 with cyclophosphamide/etoposide/cisplatin, and 10 with cyclophosphamide/etoposide/cisplatin/taxane.
methotrexate/5-fluorouracil–based regimens, and 3 with other regimens. Eighteen patients had BRCA1 mutations and 5 had unknown variants; for 11 patients, BRCA1 status was unknown. Testing for BRCA2 mutations was not performed. There were 37 recurrences classified by site of first recurrence: 19 were distant, 12 local, and 7 were local and distant simultaneously. The median TTR was 16.9 months (range = 2.3–109.2 months).

IHC scoring
Antibody staining from a single patient is shown in Figure 1. To demonstrate that the variability of scoring

| Table 1. Patient distribution within the cohort |
|------------------|--|---|---|
| Age | Treatment |
| <40 | 25 | 12 | 13 |
| ≤50 | 34 | 26 | 8 |
| >50 | 53 | 30 | 23 |
| T stage | Chemotherapy |
| 0 | 1 | 0 | 1 |
| 1 | 60 | 38 | 22 |
| 2 | 38 | 22 | 16 |
| 3 | 8 | 5 | 3 |
| 4 | 5 | 3 | 2 |
| Nodes positive | BRCA1 mutation status |
| 0 | 62 | 38 | 24 |
| 1–3 | 32 | 21 | 11 |
| ≥4 | 18 | 9 | 9 |
| M stage | Unknown variant |
| 0 | 110 | 66 | 44 |
| 1 | 2 | 2 | 0 |
| Grade | Neoadjuvant chemotherapy |
| Unknown | Yes | 7 | 4 |
| 1 | No | 105 | 64 |
| 2 | LVI | 57 | 37 |
| 3 | – | 48 | 27 |
| | + | 37 | 20 |
| | Unknown | 7 | 4 |

Fig. 1. Immunohistochemical patterns for the several DNA repair antibodies in a triple-negative breast tumor.
among patients was greater than the scoring within the triplicate cores of a single patient, the triplicate variation was determined and scored against the patient rank value for each antibody. For the 8 DNA repair and proliferation antibodies tested, the average rank error was a low percentage of the total (8.8%–11.1% DNA repair, 11.1% Ki67). The observed variations between triplicate specimen cores per patient did not significantly change the patient rank order for any of the antibodies tested, so the antibody score assigned by averaging cores was therefore a robust measure of expression. The distribution of each antibody score with intrapatient variation among cores is shown graphically in Figure 2.

Association of DNA repair IHC scoring with recurrence of cancer

Eight antibodies were analyzed for association with recurrence. P53 protein expression ($P = 0.02$), low XPF ($P = 0.005$), pMK2.C ($P = 0.01$), MLH ($P = 0.007$), and FANCD2 ($P = 0.001$) were each associated with shorter TTR on univariate analysis in the training cohort (Table 2). Scoring for PAR, PARP1, and Ki67 was not significantly correlated with TTR.

Development of a multiple DNA repair antibody panel that distinguishes recurrence groups

Pairwise combinations of various markers to maximally segregate low- versus high-risk groups in the training set yielded 4 proteins—XPF, FANCD2, pMK2.C, and PAR—that were more discriminatory when used in combination than individually. Not all patients had data available for every antibody; only patients with all 4 antibody values were included in the analysis. The training set had 50 patients with scoring available for XPF, FANCD2, PAR, and pMK2.C, inclusive (down from 68 patients in the randomized set), whereas the test set had 35 patients (down from 44). The thresholds that best segregated the samples into outcome groups were as follows: XPF = 229, FANCD2 = 42, PAR = 56, pMK2.C =

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**Table 2.** Univariate and partition analysis biomarker output data from training cohort

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>$P^a$</th>
<th>AER</th>
<th>Relative risk</th>
<th>Odds ratio</th>
<th>Positive power</th>
<th>Negative power</th>
<th>AUC$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FANCD2</td>
<td>0.001</td>
<td>0.3</td>
<td>3.83</td>
<td>7.52</td>
<td>0.57</td>
<td>0.85</td>
<td>0.71</td>
</tr>
<tr>
<td>XPF</td>
<td>0.005</td>
<td>0.3</td>
<td>2.66</td>
<td>4.77</td>
<td>0.56</td>
<td>0.79</td>
<td>0.67</td>
</tr>
<tr>
<td>PAR</td>
<td>0.293</td>
<td>0.35</td>
<td>1.64</td>
<td>2.29</td>
<td>0.5</td>
<td>0.7</td>
<td>0.54</td>
</tr>
<tr>
<td>pMK2</td>
<td>0.012</td>
<td>0.42</td>
<td>3.02</td>
<td>4.68</td>
<td>0.45</td>
<td>0.85</td>
<td>0.65</td>
</tr>
<tr>
<td>PARP1</td>
<td>0.259</td>
<td>0.41</td>
<td>1.5</td>
<td>1.88</td>
<td>0.43</td>
<td>0.71</td>
<td>0.53</td>
</tr>
<tr>
<td>MLH1</td>
<td>0.017</td>
<td>0.37</td>
<td>2.34</td>
<td>3.61</td>
<td>0.48</td>
<td>0.79</td>
<td>0.61</td>
</tr>
<tr>
<td>p53</td>
<td>0.024</td>
<td>0.35</td>
<td>2.06</td>
<td>3.11</td>
<td>0.5</td>
<td>0.76</td>
<td>0.6</td>
</tr>
<tr>
<td>Ki67$^c$</td>
<td>0.070</td>
<td>0.39</td>
<td>2.36</td>
<td>3.5</td>
<td>0.45</td>
<td>0.81</td>
<td>0.59</td>
</tr>
<tr>
<td>4-marker$^d$</td>
<td>9.05E-07</td>
<td>0.22</td>
<td>3.52</td>
<td>16.11</td>
<td>0.83</td>
<td>0.76</td>
<td>NA</td>
</tr>
</tbody>
</table>

$^a$ $P$ value for separation of early recurrence from late recurrence groups.

$^b$ From receiver operator characteristics.

$^c$ Ki67 quantity score, weighting used is 0111 for 0, 1+, 2+, 3+ bins.

$^d$ Multimarker model containing FANCD2, XPF, PAR, and pMK2.

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Fig. 2. Inter- and intrapatient immunohistochemical (IHC) scoring variations. For each antibody, the x-axis represents the sorted patient ranks according to mean score among the triplicate core specimens whereas the y-axis represents the IHC score. Error bars display the intrapatient variability between each of the 3 cores for each individual sample.
0.36 corresponding to the 0.39, 0.61, 0.71, and 0.62 quantiles of the scoring data, respectively.

In the training data set, the high-risk group defined by the 4-antibody model contained 12 patients with 10 recurrences whereas the low-risk group contained 38 patients with 9 recurrences. The difference in mean TTR (28 vs. 103 months) was highly significant ($P = 9.05E-07$; Fig. 3). Using thresholds obtained from the training data set to assign patients in the test set to recurrence risk groups yielded a high-risk group with 5 patients and 4 recurrences and a low-risk group containing 30 patients with 9 recurrences. The difference in mean TTR (31 vs. 129 months) in the test set was also statistically significant ($P = 0.0186$; Fig. 4). To verify that the high- and low-risk groups in both data sets are similar, the TTR curves were compared. There was no difference between the high-risk groups from test and training data sets ($P = 0.625$) or between the 2 low-risk groups ($P = 0.606$). There was also no statistically significant difference in the distribution of antibody scores between the training and test sets.

We also determined whether the 4-antibody model improved the assignment of patients into the expected recurrence groups on the basis of several statistical criteria. The area under ROC curves (AUC values) for the 4 individual antibody scores were as follows: FANCD2, 0.71; pMK2, 0.65; XPF, 0.67; and PAR, 0.54 (Table 2). However, using a probability analysis for the 4-antibody model, a significantly higher AUC value of 0.77 was calculated. Both the PPV (0.45–0.57) and NPV (0.7–0.85) of the individual antibodies were inferior to the PPV (0.83) and NPV (0.76) of the same 4-antibody model. The AER, the fraction of samples incorrectly classified, for the 4-antibody model was lower (0.22) than the individual antibodies (0.30–0.42) or other markers such as p53 (0.35) and Ki67 (0.39). The 4-antibody model yielded sensitivities of 53% and 31% and specificities of 94% and 95% in the training and test sets, respectively.

DNA repair IHC scoring in addition to clinical variables

To compare the discriminatory ability of the 4-antibody model to available clinical data, univariate and multivariate Cox proportional hazard models were constructed. Univariate results presented in Table 3 indicate that of the clinical parameters available, higher T stage, N stage, and positive LVI were each associated with increased risk of recurrence. The 4-antibody model is more highly associated with increased risk of recurrence than any single clinical variable ($P = 1.5e-06$).

All clinical variables and the 4-antibody model score (0 = low recurrence risk or 1 = high recurrence risk) were combined into a single multivariate Cox model to isolate the effect of the DNA repair markers from other clinical data. The 4-antibody model was highly significant ($P = 0.007$), whereas only T stage (0.038) retained a statistically significant association with recurrence (Table 3).

Discussion

Both normal cellular processes and exogenous agents contribute to the accumulation of DNA damage for which eukaryotic cells have evolved complex and redundant repair mechanisms to ensure stability and high-fidelity replication of the genetic material (23, 24, 29, 30). There are 6 identified major DNA repair pathways: BER; NER; MMR; HR/FA; nonhomologous end joining; and translesion synthesis (23, 24, 31). The 6 DNA repair pathways may also operate in cell survival and chemotherapy responses in coordinated and concerted ways (23, 24, 31, 32). Cancer cells frequently exhibit dysfunctional DNA repair, with genome instability a hallmark of many cancers (31). Dysfunctional DNA repair can lead to a “mutator” phenotype in which cells accumulate damage at an accelerated rate, leading to oncogenesis, tumor aggressiveness, and an enhanced ability to become resistant to therapy (23, 33, 34). Assessment of the cellular DNA repair machinery may therefore have prognostic
DNA repair proteins from distinct pathways provided additional prognostic information than any 1 protein alone. Analysis of proteins in multiple pathways may provide independent information, given the complexity of DNA repair processes. For example, repair of cisplatin-induced intrastrand cross-links involves not only NER but also the HR/FA pathway and integral in the repair of DNA double-strand breaks [DSBs; (4)].

In this study, we hypothesized that IHC scoring of critical proteins in various DNA repair pathways could be used in TNBCs to identify subsets of patients with worse clinical outcome. We showed that basal expression of DNA repair proteins was variable in TNBCs, that the pattern of variability was antibody dependent, and that DNA repair protein expression from different pathways was related in some cases. For example, no tumors with low XPF staining had high FANCD2 staining (data not shown). This finding is consistent with recent cell line data showing that XPF–ERCC1–deficient cells show dramatically reduced levels of chromatin-bound FANCD2 and interstrand cross-link–induced FANCD2 foci (19).

Low expression of 4 proteins—XPF, FANCD2, MLH1, and pMK2—was associated with shorter recurrence-free survival on univariate analysis. These findings are consistent with the idea that tumors with reduced DNA repair capacity will have a higher degree of genomic instability and therefore behave more aggressively (23, 31, 33–35). The association of low expression of DNA repair proteins with more aggressive behavior is similar to the results of another study that used immunohistochemistry to assess DNA repair. In a scientific correlate to the International Adjuvant Lung Trial (IALT Bio), low ERCC1 expression, while predictive of response to cisplatin, was also associated with worse overall outcomes (36). Potentially, tumors that have reduced DNA repair capacity may be more susceptible to DNA-damaging agents (leading to a predictive effect) but may also be able to develop more mutations that lead to aggressiveness and eventual resistance to therapy. We were unable to test this hypothesis in this study, which was not derived from a randomized trial in which specific treatment regimens were compared. The IALT Bio study could show this relationship because the parent trial was a randomized study with no therapy in one arm and a uniform DNA-damaging agent (cisplatin) in the second experimental arm. Patients with low levels of ERCC1, a critical protein involved in the repair of cisplatin-induced intrastrand cross-links through the NER pathway (40), comprised the subset that benefited from adjuvant cisplatin (36). Antibody panels such as ours should be further examined in similar, large data sets.

In the present study, we found that the assessing multiple DNA repair proteins from distinct pathways provided additional prognostic information than any 1 protein alone. Analysis of proteins in multiple pathways may provide independent information, given the complexity of DNA repair processes. For example, repair of cisplatin-induced intrastrand cross-links involves not only NER but...
also coordination with other pathways such as the HR/FA pathway (15, 40–43). In addition to NER-related sensitivity, defective HR/FA has been shown to be associated with platinum sensitivity (44, 45) and restoration of HR/FA is one mechanism of acquired platinum resistance (46–48). Preclinical models have further shown that deficiencies in a number of DNA repair genes are associated with sensitivity to platinum (49–53). Therefore, deficiencies in either pathway might be associated with platinum sensitivity, necessitating analysis of both.

A potential utility of IHC assessment of DNA repair pathways is as a biomarker of response to novel therapeutics. Targeting DNA repair pathways with such therapeutics offers the potential for synthetic lethality. One example is the use of PARP inhibitors in recurrent BRCA-deficient breast and ovarian cancers and in unselected metastatic TNBCs (9, 10, 12, 54). This strategy relies on the fact that base damage unrepaired by BER is converted to the more lethal DNA DSBs, which is repaired by HR/FA. The synthetic lethality with PARP inhibitors not only is limited to BRCA1/2-deficient cells but also extends to cells with deficits in a variety of proteins involved in HR (11). PARP inhibition is currently being evaluated in clinical trials of patients with BRCA mutations [12, 55, 56], but profiling of the HR/FA pathway, as we have done in this study using immunohistochemistry, might identify other subsets of patients who might benefit from PARP inhibitors.

Limitations of this study are several. The study is retrospective and the relatively small sample size permits only exploratory assessments. In addition, the cohort is a convenience sample and follow-up was therefore not uniform. The breast tumor tissue in this cohort was sampled prior to treatment with DNA-damaging agents, so analysis was an assessment of basal levels of protein expression rather than quantification of response to DNA damage. For some proteins (such as FANCD2), assessment under normal conditions can illustrate pathway integrity (4). But for others, the inducible level of protein in response to DNA damage measured in posttreatment tissue might provide additional information, albeit not as useful clinically. Finally, a common concern with IHC is the target sensitivity and specificity. By using antibodies that were well characterized in other published work and/or by testing antibodies in FFPE gene-deficient and -complemented cell lines (pMK2, XPF, MLH1, and FANCDD; data not shown), we sought to mitigate this limitation. Further work in this area is ongoing.

In summary, we hypothesized that TNBCs have a high degree of associated DNA repair defects, that we could use antibodies against critical proteins involved in DNA repair pathways to characterize these defects, and that the IHC scoring of those antibodies could be used as prognostic markers. We were able to show that IHC scoring of DNA repair protein provides variability among a cohort of TNBCs and that this variability correlated with clinical outcome. Expressions of DNA repair proteins were not independent of each other, and combinations of antibodies showed more explanatory power than individual antibodies. The results of this study may reflect an underlying biology of these tumors and suggest a potential utility in identifying subsets of patients that might be sensitive to DNA-damaging agents or novel therapeutics targeting DNA repair. The findings require further confirmation in larger data sets.

Disclosure of Potential Conflicts of Interest

D. Weaver, X. Wang, K. Sprott, A. Farrow, employed and/or have financial interest in On-Q-ity, Inc. On-Q-ity was responsible for the immunohistochemical analysis through collaboration with other authors.

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