Poly (ADP-Ribose) Polymerase as a Novel Therapeutic Target in Cancer

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Abstract

Cancer chemotherapy exploits limitations in repairing DNA damage in order to kill proliferating malignant cells. Recent evidence suggests that cancers within and across tissue types have specific defects in DNA repair pathways, and that these defects may predispose for sensitivity and resistance to various classes of cytotoxic agents. Poly (ADP-ribose) polymerase (PARP) and BRCA proteins are central to the repair of DNA strand breaks and, when defective, lead to the accumulation of mutations introduced by error-prone DNA repair. Breast, ovarian, and other cancers develop in the setting of BRCA deficiency, and these cancers may be more sensitive to cytotoxic agents that induce DNA strand breaks, as well as inhibitors of PARP activity. A series of recent clinical trials has tested whether PARP inhibitors can achieve synthetic lethality in BRCA-pathway-deficient tumors. Future studies must seek to identify sporadic cancers that harbor genomic instability, rendering susceptibility to agents that induce additional and lethal DNA damage.

DNA sustains damage through exposure to chemicals, UV light, ionizing radiation, chemotherapy, or products of cellular and oxidative metabolism (1). Damaged DNA triggers cell-cycle arrest and transcriptional activation. Single-strand breaks are faithfully repaired through base excision (BER), nucleotide excision (NER), or mismatch repair (MMR; Fig. 1; ref. 2). Double-strand breaks are repaired by homologous recombination (HR), a process that restores the original nucleotide sequence, or by nonhomologous end-joining (NHEJ) or single-strand annealing (SSA), processes that lack fidelity to the germline DNA sequence (2, 3). Use of DNA repair pathways that are prone to error generates new erroneous sequences. Carcinogenesis occurs when DNA is repaired with errors causing mutations that activate oncogenes and/or inactivate tumor-suppressor genes, and are propagated in a permissive environment.

Subclasses of chemotherapeutic agents cause specific molecular events that damage DNA (4). Methylating agents (e.g., temozolomide) add a methyl group to the O6 position of guanine nucleotides. This lesion causes mispairing of guanine to thymine, and must be repaired by the enzyme O6-methylguanine-DNA methyltransferase (MGMT) using the MMR pathway. Alkylating agents (e.g., melphalan) induce formation of alkylated nucleotide bases that must be repaired by the BER pathway. Chloroethyllating agents (e.g., carmustine) result in the formation of O6-chloroethylguanine, which, if not repaired by MGMT, will result in interstrand DNA cross links. Platinum agents (e.g., cisplatin) form both inter- and intrastrand DNA cross-links that must be repaired by NER and HR pathways. The mechanism of anthracycline-induced DNA damage is less straightforward, but likely is due to the anthracyclines’ inhibition of topoisomerase II (5), as well as to their ability to form adducts with DNA at the amino group of guanine residues (6). Repair of anthracycline-induced DNA damage uses both the NER and the HR pathways (7).

Activation of these DNA repair mechanisms triggers cellular signaling pathways that block gene transcription, arrest the cell cycle, and halt replication forks during mitosis (2, 8–10). At replication forks, un repaired single-strand breaks or cross-links are converted to double-strand breaks by nucleases in the DNA polymerase machinery. Double-strand breaks in DNA are predominantly repaired by HR or NHEJ (8). If DNA is damaged to such an extent that it cannot be repaired by any mechanism, then cells cannot transcribe genes or replicate chromosomes. In these instances, collapsed replication forks trigger apoptosis via p53-dependent or -independent mechanisms (4, 11).

Hereditary Defects in Homologous Recombination

The BRCA1 gene was identified in 1990 as a cause of hereditary breast cancer. It was sequenced in 1994 and has since been shown to play a pivotal role in the complex HR pathway of DNA repair (12). Women carrying a

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heterozygous deleterious germline mutation in the BRCA1 gene carry a 57% cumulative risk of developing breast cancer by the age of 70 years and a 40% risk of developing ovarian cancer (13). In the case of a deleterious germline BRCA2 mutation, women have a 49% risk of developing breast cancer and an 18% risk of developing ovarian cancer by age 70. Tumors that arise in BRCA mutation carriers have lost the wild-type allele of the BRCA gene and express only mutated and/or truncated alleles. Therefore, the tumor cells are unable to repair DNA through BRCA-dependent mechanisms.

BRCA1 and BRCA2 regulate repair of damaged DNA through HR (Fig. 2; ref. 12). Active BRCA1 promotes cell-cycle arrest in conjunction with p53 and associates with DNA double-strand breaks. The first steps in HR involve degradation of one strand of the DNA at the site of double-strand damage, creating a stretch of single-stranded DNA (14). RAD51 molecules bind to the single-stranded DNA and form filamentous structures. These RAD51 foci promote recognition of homologous sequences on the sister chromatid, and catalyze pairing between the complementary bases in the intact chromosome. Ataxia telangiectasia mutated (ATM) and BRCA2 accumulate with BRCA1 and RAD51 at the sites of double-strand DNA damage, and DNA polymerase proceeds using the sister chromatid as the template for repair (15).

In cells lacking functional BRCA1 or BRCA2, HR is deficient, and DNA repair proceeds through more error-prone repair pathways such as NHEJ (12). Repair triggered in the NHEJ pathway typically removes the region of damaged DNA and joins the truncated DNA fragment with intact DNA (8). The joined DNA may be on the same chromosome, resulting in a deleted portion of the genome. Further genomic instability occurs, however, when the fragmented DNA is joined to intact DNA on another chromosome, resulting in chromosomal translocations that are propagated to cellular progeny when the cell cycle resumes (16). Such chromosome instability in the BRCA1-mutated HR-deficient cells sets the stage for oncogene activation and tumor-suppressor deletion, and is reflected in the increased aneuploidy and loss of heterozygosity common to malignant cells.

It is unclear why breast and ovarian epithelial cells are more susceptible to the oncogenic outcome of BRCA deficiency. One hypothesis is that these estrogen-responsive tissues undergo higher rates of proliferation during peak times of estrogenic stimulation (17). Heightened proliferation increases the accumulation of mutations in the estrogen-responsive tissues. Simultaneously, estradiol itself can form adducts with DNA by directly binding to adenine and guanine bases in stretches of uncoiled chromatin (18). The estradiol-bound nucleosides are subsequently cleaved from the DNA, and the damaged DNA backbone is at a higher risk of erroneous repair in HR-deficient cells. Progesterone may also contribute to the development of breast cancer in BRCA1-germline mutation carriers. Normal mammary epithelial cells from women carrying BRCA1 mutation show higher levels of the progesterone receptor, perhaps amplifying the growth stimulatory effect of this hormone (19). Loss of BRCA1 stabilized the progesterone receptor, leading to increased proliferation of mammary ducts in a mouse model of BRCA1-deficient breast cancer (20). Progesterone antagonists were shown to inhibit mammary tumorigenesis in this BRCA1-null murine cancer model.

In addition to BRCA1- and BRCA2-germline mutations, other inherited molecular defects may prevent effective HR DNA repair in breast cancer (Table 1). Germinal mutations in two proteins known to interact directly with the
BRCA complex have been linked to hereditary causes of breast cancer. Partner and localizer of BRCA2 (PALB2) physically connects the BRCA1 and BRCA2 proteins at sites of double-stranded DNA damage (Fig. 2; ref. 21). Mutations in PALB2 have been linked to hereditary breast cancers not associated with mutations in BRCA genes (22). Additionally, the PALB2 promoter may be hypermethylated, downregulating PALB2 expression in both hereditary and sporadic breast cancers (23). BRCA1 interacting protein C-terminal helicase 1 (BRIP1) is a RecQ helicase that interacts directly with the C-terminal end of BRCA1 (Fig. 2; ref. 24). Its interaction with BRCA1 is required for maintaining genomic stability through its helicase activity during DNA replication, specifically in GC-rich regions of DNA (25). Depletion of either BRIP1 or BRCA1 causes genomic instability (24), and germline mutations in BRIP1 confer a twofold relative risk of breast cancer (22).

A highly evolutionarily conserved complex of MRE11, RAD50, and NBS1 (the MRN complex) also interacts with BRCA1 during HR DNA repair. Loss of function of each of these genes has been associated with the development of breast cancer (26). Germline mutations affecting RAD50 were found to confer an odds ratio of 4.3 in Finnish families predisposed to breast cancer (22). More recently, sequencing of these three genes from breast cancer families revealed two with germline mutations in MRE11 (27).

ATM may account for up to 3% of familial breast cancers that do not have mutations in the BRCA1/2 genes (22). ATM functions in DNA repair by phosphorylating and activating proteins involved in HR including p53, histone H2AX, NBS1, and BRCA1 (4). Dysfunction of ATM has been discovered in both hematologic malignancies and solid tumors (22). Patients with ataxia telangiectasia have a 30% incidence of lymphoid malignancies, especially acute lymphoblastic leukemia and diffuse large B-cell lymphoma (28). The ATM gene may be somatically mutated in epithelial cancers as well, including lung, breast, and ovarian cancers. BRCA-mutant or sporadic triple-negative breast cancers (TNBC) have been found to express lower levels of ATM protein compared with estrogen receptor (ER) or HER2-positive breast cancers (29).

A case-control study of women at high risk for breast cancer, from the UCLA Family Cancer Registry, also found associations of single-nucleotide polymorphisms (SNP) in eight genes involved in HR DNA repair. Among these were NBS1, RAD51, and BRIP1 (30). Additionally, specific SNPs in XRCC4 and RAD21, also involved in HR DNA repair pathways, were associated with the development of breast cancer in high-risk kindreds, in addition to known BRCA1/2 mutations. These pathway-associated SNPs indicate that defects throughout the double-strand DNA repair process can predispose to familial breast cancer.

The Role of Poly (ADP-Ribose) Polymerase in DNA Repair

Poly (ADP-ribose) polymerase (PARP) is a highly abundant nuclear protein that is activated when DNA is damaged (31). As the name implies, PARP enzymes...
modify DNA-associated proteins by adding chains of ADP ribose units. To date, 17 members of the PARP family have been identified on the basis of sequence homology (31). Only six PARP enzymes, however, have confirmed or are likely to have true PARP activity. These enzymes functionally group into three main categories. PARPs 1, 2, and 3 share sequence homology; PARP4 is structurally distinct and may serve as a tumor suppressor, although its function is less well defined; and TNKS and TNKS2 are tankyrase enzymes that regulate telomeres during mitosis (31). Genomic deletion of the PARP family enzymes in mice revealed that PARPs 1 and 2 are required for genomic stability in mice, because the PARP1- or 2-deficient mice accumulated DNA single-strand breaks and displayed hypersensitivity to DNA-damaging agents (31). Knock-out of the TNKS, however, resulted in growth retardation but no defects in telomere maintenance, suggesting redundancy in this function within the mouse genome.

PARP1 is the most extensively studied of all the PARP family proteins. The action of PARP1 is essential for repair of single-strand DNA breaks, predominantly through the BER mechanism (31). Chemotherapy agents, UV light, and products of cellular metabolism typically cause single-strand DNA damage that must be identified and repaired by PARP pathway activation. With continuous PARP inhibition, these single-strand breaks are converted to double-strand breaks during DNA replication, in which replication forks stall at the point of DNA damage (8–10). Repair of double-strand DNA breaks predominantly depends on the HR pathway of DNA repair to maintain chromosomal integrity (10). Because of this dependence, HR-deficient cells are excessively sensitive to killing by PARP inhibition (32). When the single-strand repair capacity of PARP is inhibited in cells that lack effective HR, double-strand DNA breaks accumulate and overwhelm the less efficient mechanisms of DNA repair, such as the NHEJ pathway. PARP also contributes to repair double-strand breaks through NHEJ, which is further impaired when PARP activity is inhibited (10). Irreparable DNA damage triggers apoptotic cell death in cells with or without intact p53 (4). Therefore, inhibiting PARP has been undertaken as a strategy to selectively kill cells with dysfunctional HR (32).

### Poly-ADP Ribose Polymerase Inhibitors in Clinical Development

Small molecule inhibitors of PARP activity began development as sensitizers to DNA-damaging chemotherapy or ionizing radiation (Fig. 3; ref. 33). BRCA-deficient cells are 1,000 fold more sensitive to single-agent PARP inhibition than are wild-type BRCA1 and 2 cells (15, 34). The concept of synthetic lethality, in which functional inhibition of two proteins leads to cell death, but blockade of either alone does not, is well illustrated by the striking cytotoxicity of PARP inhibitors in BRCA-deficient cancers. Loss of both functions is incompatible with life (35, 36). All cells in a person who has inherited a BRCA1- or 2-germline mutation contain one functional and one nonfunctional allele. Inhibiting PARP should not affect nonmalignant cells that contain one functional copy of BRCA, as HR remains intact by the presence of the normal allele. Cancer cells in BRCA mutation carriers have lost the only functional copy of BRCA1 or 2, and blockade of PARP activity in these BRCA-null cancer cells was hypothesized to be selectively synthetically lethal. Preclinical studies supported these concepts in vitro and in mouse models of BRCA-deficient tumors, thus prompting the clinical development of PARP inhibitors in patients with BRCA1- or 2-germline mutation-associated cancers (15, 32, 34).

More than half a dozen highly potent and specific PARP inhibitors are currently undergoing clinical development in cancer populations (Table 2; http://clinicaltrials.gov). Iniparib (BSI201, BiPar Sciences Inc.) is being evaluated in a phase III trial in TNBC patients in combination with gemcitabine and carboplatin. Three agents, olaparib (AZD 2281, AstraZeneca), veliparib (ABT888, Abbott Laboratories), and AG014699 (Pfizer Inc.), are being studied in phase II clinical trials as single agents or in combination with chemotherapy. Two PARP inhibitors are undergoing phase I evaluation: MK4827 (Merck & Co. Inc.) and CEP9722 (Cephalon, Inc.). Two additional agents entered clinical development but have not been pursued: GPI 21016 (Sanofi-aventis) and INO-1001 (Genentech, Inc.).

A recent phase I clinical trial identified the maximum tolerated dose of olaparib in patients with solid tumors and then evaluated antitumor activity in an expansion cohort of 20 patients with hereditary BRCA-deficient tumors (37). Of the 19 BRCA-deficient patients evaluable, 12 (63%) showed evidence of clinical benefit as defined by radiographic or tumor marker response, or stabilization of disease for 4 months or longer. PARP inhibition was measured in tumor cells at doses as low as 40 mg twice daily, and seemed to plateau at 100 mg twice daily. No

### Table 1. Inherited defects in homologous recombination

<table>
<thead>
<tr>
<th>Gene mutated</th>
<th>Function(s) affected</th>
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</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>Cell-cycle arrest; recruitment of HR repair complex</td>
</tr>
<tr>
<td>BRCA2</td>
<td>HR repair complex assembly</td>
</tr>
<tr>
<td>PALB2</td>
<td>HR repair complex assembly</td>
</tr>
<tr>
<td>BRIP1</td>
<td>DNA helicase activity</td>
</tr>
<tr>
<td>MRE11</td>
<td>Assembly and activation of HR and NHEJ repair complexes</td>
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<tr>
<td>RAD50</td>
<td>Assembly and activation of HR and NHEJ repair complexes</td>
</tr>
<tr>
<td>NBS1</td>
<td>Assembly and activation of HR and NHEJ repair complexes</td>
</tr>
<tr>
<td>ATM</td>
<td>Activation of HR repair complex</td>
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antitumor activity was seen in the 37 patients who were not known to have BRCA-deficient cancers, and no clinical activity was seen at doses lower than 100 mg twice daily. The trial was subsequently expanded to include 50 patients with BRCA-associated ovarian, fallopian tube, or primary peritoneal cancer treated with olaparib 200 mg twice daily (38). Post hoc analyses detected a significant correlation between length of platinum-free interval and depth of measurable response to olaparib. More women with platinum-sensitive ovarian cancer (>6 months from platinum therapy to relapse) achieved an objective response to olaparib therapy than women with platinum resistant (0 to 6 months to relapse) or platinum-refractory (tumor progression on platinum therapy) ovarian cancer (P = 0.001).

These results prompted phase II trials to extend the evaluation of efficacy specifically in patients with BRCA-deficient ovarian or breast cancer. Early results of these phase II trials were presented at the American Society of Clinical Oncology (ASCO) 2009 Annual Meeting. The phase II trial of the oral PARP inhibitor olaparib in hereditary BRCA-deficient advanced breast cancer enrolled two sequential cohorts of patients (39). The first 27 patients received olaparib at 400 mg twice daily, and the second 27 patients received olaparib 100 mg twice daily, because the pharmacodynamic results from the phase I trial showed maximal PARP inhibition in peripheral blood cells at the 100 mg dose. The therapy was well tolerated, and investigators reported a 41% overall response rate in the patients of the 400 mg cohort, with a median progression-free survival of 5.7 months; the 27 patients receiving 100 mg twice daily achieved a 22% overall response rate with a median progression-free survival of 3.8 months at the time of initial reporting. The 400 mg dose caused mild adverse events of grade 1 to 2 nausea, fatigue, and anemia in one third or fewer patients, and more severe grade 3 fatigue, nausea, and anemia in five (19%) of patients.

The second clinical trial similarly tested two sequential dose cohorts in women with hereditary BRCA-deficient advanced ovarian cancer (40). At the time of the preliminary report, 33 patients had been treated at the 400 mg twice-daily
dose, with clinical benefit (objective radiologic response or greater than 50% decline in CA125) observed in 57.6% of patients and with a median progression-free survival of 5.8 months. In 24 other patients who were treated at the 100 mg dose, 16.7% had achieved a clinical benefit, with a progression-free survival of 1.9 months. Although not statistically powered to evaluate differences between the two dose cohorts, these preliminary results suggest that the pharmacodynamic saturation of PARP inhibition, as tested in peripheral blood cells of the patients in the phase I trial, may not have adequately reflected the dose of olaparib that inhibits PARP optimally in the patients' cancers. Seven other ongoing phase I and II clinical trials are evaluating the efficacy of PARP inhibitors as single agents in patients with known BRCA-mutated cancers (http://clinicaltrials.gov).

PARP inhibitors are also under investigation in patients without BRCA germline mutations. TNBC, lacking expression of ER, progesterone receptor, and HER2, is an aggressive breast cancer subtype that shares molecular and pathologic features with BRCA1-related breast cancers. The majority of BRCA1-deficient breast cancers occur as the triple-negative phenotype. Recent molecular evidence suggests that functional BRCA1 protein is necessary to induce expression of ER in breast cell progenitors (41, 42). Sporadic TNBC occurring in nonmutation carriers may harbor epigenetic silencing of BRCA1 and ER by aberrant methylation of these genes' promoters or may have other defects in the HR pathway such as overexpression of ID4, loss of PTEN, or dysfunction of PALB2 or BRIP (43). Sporadic basal TNBC also displays defective repair of oxidative DNA damage through the BER pathway, a characteristic shared by BRCA1-mutated breast cancers (44).

Preclinical studies have suggested that TNBC cell lines undergo a greater degree of cell death compared with non-TNBC cells, upon treatment with the DNA-damaging agents cisplatin and/or gemcitabine or with a PARP inhibitor (45). This concept has been applied and tested clinically. A randomized phase II trial, presented at the ASCO 2009 Annual Meeting, evaluated the efficacy of gemcitabine plus carboplatin, with or without the intravenous PARP inhibitor iniparib, in patients with metastatic TNBC (46). Patients were randomly assigned to gemcitabine plus carboplatin alone or gemcitabine plus carboplatin with iniparib. Endpoints were clinical benefit rate (CBR; i.e., complete response plus partial response plus stable disease of at least 6 months), and time to progression (TTP). The early results from this trial showed that iniparib improved CBR from 21 to 62% (P = 0.0002), increased TTP from...
PARP Inhibitors in Cancer

3.3 to 6.9 months (P < 0.0001), and extended overall survival from 5.7 to 9.2 months (P = 0.0005) in these patients. An updated analysis from the San Antonio Breast Cancer Symposium 2009 showed a median overall survival of 7.7 months with gemcitabine plus carboplatin versus 12.5 months with gemcitabine plus carboplatin and iniparib (hazard ratio 0.5, P = 0.005; ref. 47). The final analysis of this trial is ongoing, and a follow-up phase III trial, which recapitulates the question asked in the phase II, has completed enrollment.

Biomarkers of Sensitivity to Poly-ADP Ribose Polymerase Inhibition

TNBC is a heterogeneous disease, and it is not known whether all TNBC have defects in HR that would predict for sensitivity to PARP inhibition. Most TNBC express a basal gene signature, but some within the histologic category of TNBC express the molecular profiles of other subtypes identified to date (luminal A, luminal B, HER2-driven, or claudin low), and therefore may not share the pathologic DNA repair defects that permit synthetic lethality upon PARP inhibition (48). A BRCA-like gene-expression signature, developed from germline BRCA mutation carriers, could aid in detecting sporadic breast cancers with HR DNA repair defects that make cells vulnerable to inhibition of PARP (49). A DNA repair signature, based on a previously published BRCA1-associated gene-expression pattern (50), was able to predict patient benefit from DNA-damaging chemotherapy agents when applied to sporadic TNBC samples (51). In this analysis, BRCA1-like sporadic TNBCs overexpressed DNA repair genes, including PARP1, RAD51, CHEK1, and FANCA, and comprised approximately 50% of the sporadic TNBCs. Patients with BRCA1-like sporadic TNBC had a higher pathologic complete response rate with alkylator- and/or anthracycline-based preoperative chemotherapy than did patients with non-BRCA1–like TNBC. In a similar study, patients with metastatic breast cancer, whose sporadic cancers expressed a BRCA1-like array CGH profile, had a greater likelihood of achieving prolonged progression-free survival with intensive high-dose cyclophosphamide, thiopeta, carboplatin (CTC) chemotherapy than did patients with sporadic, non-BRCA1–like profiles (52).

Platinum sensitivity of a tumor may indicate defects in HR (53–55). Cancers that are treated routinely with platinum-based regimens as initial therapy include ovarian cancer, testicular cancer, lung cancer, and head and neck cancers, among others. The inherent vulnerability to cell death with the single-strand DNA breaks caused by platinum agents could indicate underlying defects in HR in these tumors, leading to reliance on PARP. Tumors that are sensitive to cisplatin may depend more highly on functional PARP activity, and high doses of cisplatin may overcome the ability of PARP to repair the cisplatin-induced DNA breaks, leading to cell death in the setting of dysfunctional HR. Alternatively, massive activation of PARP following platinum treatment may deplete NAD+, leading to necrotic cell death (33). A recent clinical study of BRCA1-mutated TNBC patients (n = 25) showed a 72% pathologic complete response with preoperative cisplatin (56). Similarly, the pathologic complete response rate of 22%, in a cohort of 28 TNBC patients treated with four cycles of preoperative cisplatin, was associated with low BRCA1 mRNA expression and BRCA1 promoter methylation (57). Conversely, several BRCA1/2-germline mutation-associated metastatic breast cancer patients did not respond to treatment with the PARP inhibitor olaparib if they had relapsed early after treatment with cisplatin, whereas the majority of cisplatin-naïve BRCA patients did benefit from PARP inhibition (37, 38). This finding prompts the hypothesis that cancers sensitive to killing by cisplatin may also be more responsive to PARP inhibition.

The expression of PARP itself was recently associated with improved clinical response rates with neoadjuvant chemotherapy across multiple breast cancer subtypes (58). High levels of PARP protein expression were found in a proportion of all subtypes of breast cancer, but more frequently occurred in hormone receptor-negative tumors with or without HER2 expression. Patients were treated with docetaxel, adriamycin, and cyclophosphamide (TAC) chemotherapy, with or without sequential vinorelbine and capetitabine. The odds ratio for obtaining a pathologic complete response was 5.3 in patients whose cancers had high PARP expression, compared with those with low PARP expression. These data suggest that elevated PARP protein may predict for sensitivity to DNA-damaging chemotherapy, and suggest that PARP inhibition might be of clinical benefit in patients whose breast cancers have high PARP expression.

Increased measures of genomic instability in cancer cells may also indicate defective HR DNA repair, and thus identify tumors susceptible to PARP inhibition. Genomic profiling of breast cancers by array CGH patterns identified a BRCA profile that was 91% accurate in distinguishing BRCA-mutated cancers from sporadic, nonhereditary cancers (59). In this study, 2 of 48 hereditary, but non-BRCA-mutated, cancers were found to be BRCA-like based on the 191 discriminatory features. In one of these two cases, the BRCA1 gene was found to be methylated. These investigators have subsequently developed a BRCA2 classifier using array CGH that showed 89% sensitivity and 84% specificity in 89 cases of hereditary, non-BRCA–associated cancers (60). A similar approach used array CGH to identify four distinct groups of patients: simple-profile, BRCA1-related, BRCA2-related, and Genomic Instability-high Group 3 (GII-high-III) subgroups (61). The BRCA1- and BRCA2-related cancers showed many large regions of genomic gains or deletions on array CGH. Sporadic breast cancers within the GII-high-III group similarly had many regions of high-level amplifications or deletions, but the individual regions of change on the chromosomes were notably shorter in length. The prominence of the genomic aberrations suggests that these sporadic breast cancers may harbor defects in HR that could render them sensitive to PARP.
inhibitor therapy. The BRCA2-mutated cancers and the sporadic GII-high-III cases were predominantly luminal in gene expression, whereas the BRCA1-mutated tumors expressed nonluminal patterns [basal, 5-negative (ER, progesterone receptor [PR], HER2, cytokeratin 5/6, epidermal growth factor receptor), or HER2]. Interestingly, the simple profile tumors with a low degree of genomic instability were equally distributed between luminal and nonluminal gene-expression categories. This study suggests that array CGH can identify sporadic breast cancers with genomes as highly unstable as BRCA1- or 2-mutated cancers, and that these sporadic cancers may harbor HR defects predictive of sensitivity to PARP inhibition.

PTEN has been shown to have an essential role in maintaining genome stability by promoting the transcription of RAD51 (62). PTEN physically associates with the chromatin surrounding the RAD51 promoter, and increases RAD51 gene transcription by an as yet unknown mechanism. Conversely, PTEN deficiency has been linked to impaired HR in tumors of lung and colon origin, and correlates with markedly increased sensitivity to PARP inhibitors in cancer cell lines (63). Within the same pathway, Aurora A overexpression inhibits RAD51 recruitment to the DNA repair complex, resulting in a similar increase in PARP inhibitor sensitivity (64). Some breast cancers display activation of phosphoinositide 3-kinase (PI3K) signaling downstream of genomic alterations, which lead to loss of PTEN function (65). The loss of either PTEN or retinoblastoma protein (RB) function has been shown to be higher in TNBC compared with ER+PR+, or HER2+ breast cancers (66). Aberrations leading to PTEN and RB dysfunction are also associated with increased genomic instability, as measured by a gene signature of instability (66, 67). It is interesting to speculate that PTEN-null TNBCs, therefore, may have heightened sensitivity to PARP inhibitor–induced cytotoxicity.

Is it possible that cancers defective in HR might be identified at diagnosis, prior to treatment, by quantifying the number of chromosomal breakpoints that exist in the tumor cells? An estimate of allelic imbalance was measured on the basis of evaluation of 42,000 SNPs in 21 TNBC patients treated with cisplatin in the neoadjuvant setting (68). These analyses accurately classified 20 of 21 patients according to their degree of responsiveness to neoadjuvant cisplatin at the time of surgery. In addition to sensitivity to DNA-damaging cytotoxic agents, cancers defective in HR may be hypersensitive to the DNA-damaging effects of radiation therapy. A pilot study tested the feasibility of irradiating fresh biopsy specimens from breast cancers in order to determine if the cancer’s ability to form DNA repair foci was intact (69). The investigators quantified the foci by measuring BRCA1, FANCD2, and RAD51 in the specimens before and after ex vivo application of 8 Gy X-irradiation in the laboratory. Lack of induction of DNA repair foci in some of the tumors was proposed as a functional indicator of defective DNA repair. Further studies are indicated to evaluate failure of foci formation as a predictor of sensitivity to PARP inhibitors, DNA-damaging agents, and to irradiation. Conversely, cancers with robust focus formation may be more resistant to DNA-damaging therapies.

Future Directions

Clinical trials have provided proof of principle that synthetic lethality with PARP inhibition can be achieved in the setting of BRCA-deficient human cancers. BRCA-deficient cancers typically show heightened sensitivity to DNA-damaging cytotoxic agents because unrepaired DNA breaks in proliferating cells result in double-strand breaks that rely on HR repair to maintain genomic stability (12). Ongoing clinical trials at the National Cancer Institute and elsewhere are testing the safety and efficacy of administering PARP inhibitors in combination with chemotherapeutic agents that damage DNA, including platinum agents, topotecan, cyclophosphamide, and temozolomide. These trials are testing combination therapies in patients carrying BRCA1- or BRCA2-germline mutations as well as in patients with sporadic cancers. Whether synthetic lethality with PARP inhibition can occur in the setting of wild-type BRCA1 or -2 is unknown. It is likely that bone marrow suppression with each of these chemotherapies will be heightened by the concomitant administration of PARP inhibitors, but also possible that synthetic lethality in the tumor may be achieved at lower doses of each agent, thus sparing nontumor tissues from damage. Indeed, recent studies have suggested that simultaneous interruption of DNA integrity (i.e., with an intrastrand cross-linking agent) and blockade of DNA repair with a PARP inhibitor may produce synergistic DNA damage even in BRCA wild-type cancers.3 Brody and colleagues have disrupted the DNA-binding domain of PARP through exogenous overexpression of a mutated PARP-1 gene and then have treated BRCA-proficient cancer cells with DNA-damaging agents such as gemcitabine, resulting in synergistic antitumor activity.4 Ongoing clinical trials are testing this hypothesis in sporadic cancers by combining standard-of-care DNA-damaging agents with PARP inhibitors in patients with non–small cell lung or ovarian cancers. Results of these trials will clarify the role of PARP inhibition in sporadic, genomically unstable cancers and will provide insights into tumor biomarkers that predict for sensitivity to PARP inhibition.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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3 J.R. Brody, personal communications.
4 J.R. Brody and J. Pascal, unpublished observations.


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