Low Levels of Circulating Estrogen Sensitize PTEN-Null Endometrial Tumors to PARP Inhibition In Vivo

Deanna M. Janzen, Daniel Y. Paik, Miguel A. Rosales, Brian Yep, Donghui Cheng, Owen N. Witte, Huseyin Kayadibi, Christopher M. Ryan, Michael E. Jung, Kym Faull, and Sanaz Memarzadeh

Abstract

Earlier in vitro work demonstrated that PARP inhibition induces cell death in PTEN-null endometrial cancer cell lines, but the in vivo therapeutic efficacy of these agents against endometrial cancer remains unknown. Here, we test the efficacy of AZD2281 (olaparib), an oral PARP inhibitor, in the therapy of PTEN-null endometrial tumors in a preclinical endometrial cancer mouse model. Primary endometrial tumors were generated by epithelial loss of PTEN using an in vivo model. This model recapitulates epithelial-specific loss of PTEN seen in human tumors, and histologically resembles endometrioid carcinomas, the predominant subtype of human endometrial cancers. Olaparib was administered orally to tumor-bearing mice in two hormonal extremes: high or low estrogen. In striking contrast, no response to olaparib was seen in tumors exposed to high levels of estrogen. Two key observations were made when estrogen levels were dropped: (i) the serum concentration of olaparib was significantly increased, resulting in sustained PARP inhibition at the tumor bed; and (ii) the homologous recombination pathway was compromised, as evidenced by decreased Rad51 protein expression and function. These two mechanisms may account for the sensitization of PTEN-null tumors to olaparib with estrogen deprivation. Results of this preclinical trial suggest that orally administered PARP inhibitors in a low estrogenic hormonal milieu can effectively target PTEN-null endometrial tumors. Extension of this work to clinical trials could personalize the therapy of women afflicted with advanced endometrial cancer using well-tolerated orally administered therapeutic agents.

Introduction

A major challenge in advanced recurrent and metastatic endometrial cancer is the limited benefit of current treatments (1). Clinical trials comparing the efficacy of chemotherapy using platinums, taxanes, and anthracyclines have not shown any clear benefit for women with advanced disease (2). Instead, the focus is shifting toward the development of drugs targeting specific molecular pathways involved in endometrial carcinogenesis (3). The mechanism responsible for initiation of most endometrial cancers involves activation of the phosphoinositide 3-kinase (PI3K) pathway resulting from the loss of the PTEN function (4). PTEN mutations have been identified in up to 80% of sporadic endometrioid cancers, the most common subtype of endometrial tumors (5, 6). Given the prevalence of this mutation, strategies exploiting cellular defects associated with loss of PTEN function in endometrial tumor cells could have broad clinical application. Previous studies demonstrated that PTEN-null endometrial cancer cell lines can be targeted with PARP inhibitors in vitro (7). However, to date, the in vivo antitumor effects of these agents in the therapy of endometrial tumors have not been tested.

PARP inhibitors have emerged as targeted cancer therapeutic agents (8). Although PARP inhibitors have proven efficacious in small trials (9, 10), their therapeutic utility remains limited on the basis of less promising results in larger clinical series (11). Classically, these drugs cause cell death through synthetic lethality, a concept in which a substance not inherently detrimental to cells becomes cytotoxic when combined with another specific defect (12, 13). As a single agent, PARP inhibition blocks the base excision repair pathway (8). This leads to the accumulation of single-strand DNA breaks, which are converted to double-strand breaks during DNA replication. In normal cells, double-strand DNA breaks are repaired by the homologous recombination (HR) DNA repair pathway.
pathway. However in cells with compromised HR, death ensues with PARP inhibition (8). As an example, PARP inhibitors exhibit antitumor activity in patients with BRCA-mutant cancers (9, 10). Because BRCA proteins are essential for homologous recombination, BRCA-deficient tumors regress because of their inability to repair double-strand DNA breaks induced by PARP inhibition (12, 13).

To extend the use of PARP inhibitors, nontraditional pathways that may interfere with HR have been investigated. Inactivation of PTEN in mouse embryonic fibroblasts induced chromosome instability due to defective Rad51-mediated HR DNA repair (14). Rad51 is a critical component of the HR pathway, functioning in the homology search and strand-exchange phases of DNA repair (15).

PTEN-null tumor cells were hypothesized to be sensitive to PARP inhibition as a result of Rad51 downregulation and or loss of function. Two in vitro studies reported cytotoxic effects of PARP inhibition in PTEN-null cancer cell lines associated with altered Rad51 function (7, 16), but the in vivo capacity of these drugs to resolve PTEN-null tumors, particularly endometrial cancer, has not been fully investigated. The existence of Rad51 defects in PTEN-null tumors has been controversial as recent studies did not demonstrate defective Rad51 function in PTEN-null prostate cancer cell lines (17). Although PTEN status may not predict response to PARP inhibition, Rad51 function may define sensitivity to this therapy (ref. 18 and reviewed in ref. 19). Decreased expression of Rad51 promoted sensitivity to PARP inhibitors in vitro (17). Conversely, increased Rad51 levels have been implicated in the resistance to PARP inhibitors (20, 21).

Overall, mechanisms accounting for sensitivity or resistance to PARP inhibitors remain debated. To test the efficacy of PARP inhibitors in the therapy of endometrioid carcinomas driven by loss of PTEN, an in vivo mouse model established by our group was utilized (22). In this model, combinations of PTEN-null epithelia and wild-type (WT) stroma regenerate, giving rise to tumors that closely resemble human endometrial cancers based on two major criteria. In human endometrioid tumors, PTEN expression is lost in the tumor epithelia but preserved in the stroma (23). Unlike existing PTEN-null mouse models (24–26) but similar to human endometrial cancers, tumors in our model harbor epithelial-specific loss of PTEN but maintain its expression in the tumor stroma. The histology of tumors established in this model recapitulates the histopathology of endometrioid carcinomas, the major subtype of human endometrial cancers. In this preclinical study, olaparib (AZD2281), an orally bioavailable PARP inhibitor tested in human trials (http://clinicaltrials.gov/show/NCT01237067), was administered to mice bearing PTEN-null endometrial tumors. The efficiency of this therapeutic agent was tested under two hormonal conditions, high or low estrogen. These hormonal milieus were examined as many patients with endometrial cancer have a hyperestrogenic state due to obesity (27) that can be reduced pharmacologically.

Using this model, we demonstrate that PTEN-null endometrial tumors are sensitive to synthetic lethal effects of PARP inhibitors, but this response hinges on the hormonal milieu. When estrogen levels were high, a measurable response to PARP inhibition was not detected. In striking contrast, low levels of circulating estrogen sensitized these same tumors to PARP inhibition demonstrated by a reduction in tumor burden. Here, we dissect cellular mechanisms that account for this dichotomous response. Our results show that the endogenous estrogen levels impact: (i) levels of circulating olaparib modulating PARP inhibition at the tumor bed and (ii) levels and function of Rad51, a critical protein involved in homologous recombination. These results suggest that PARP inhibition should be tested in conjunction with hormonal ablation in clinical trials treating women with PTEN-null endometrial tumors.

Materials and Methods

Animals

Mouse strains Bl6 (C57BL/6), PTENloxPloxP (C;129S4-Pten<sup>tm1Hvau</sup> /J), and CB17Scid/Scid were from Jackson Laboratories. Mice were maintained in accordance with University of California Los Angeles (UCLA) Division of Laboratory Animal Medicine guidelines. All animal experiments were approved by the UCLA Animal Research Committee.

Isolation of PTEN-null endometrial epithelia and assays

(i) Preparation of PTEN-null epithelia: Trop1<sup>+</sup> CD90<sup>−</sup> CD45<sup>−</sup> CD31<sup>−</sup> Ter119<sup>−</sup> epithelia were isolated by fluorescence-activated cell sorting (FACS) from the uteri of PTEN<sup>loxPloxP</sup> adult female mice (22). Epithelia were infected with Cre-RFP (22) to delete PTEN. (ii) In vivo tumor generation: PTEN-null tumors were prepared (22), and details are available in Supplementary Materials. Cellular combinations were grown for 8 weeks to establish tumors prior to initiation of therapy. (iii) In vitro generation of PTEN-null spheres: Cre-infected PTEN<sup>loxPloxP</sup> epithelia were plated in Matrigel (BD Bioscience) and PrEGM media (Lonza) as previously described (28). WT mouse uterine stroma was added to each well. Cells were cultured for 2 weeks. PTEN deletion was confirmed by PCR as described previously (29).

Tumor generation

Endometrial tumor generation was performed as previously described (22). Combinations of WT stroma and Cre-infected PTEN<sup>loxPloxP</sup> epithelia were suspended in collagen, implanted under the renal capsule of oophorectomized CB17Scid /Scid mice with a time-release estrogen pellet (60-day time release, 0.72-mg β-estradiol/pellet; SE-121 Innovative Research of America) and regenerated for 6 to 8 weeks. Details are outlined in Supplementary Methods.

Drug preparation and administration

(i) In vivo: Olaparib stocks were prepared as previously described (12, 13). Details are outlined in Supplementary Materials. Olaparib at a dose of 50 mg/kg or vehicle was
orally administered to mice once daily for 3 weeks. Drug doses and schedules were selected on the basis of published data (12, 13). (ii) In vitro: Olaparib (10 μmol/L) or equal volume of vehicle were added to each well.

**Measurement of drug levels and activity**

The in vitro efficacy of olaparib was confirmed by examining drug-induced DNA damage measured by formation of γH2AX foci as previously described (7). Details are outlined in Supplementary Materials. PARP enzymatic activity in tumors was determined by measuring poly(ADP-ribose) levels using the PARP PDA II ELISA assay (cat no. 4520-096-K; Trevigen). Serum estrogen concentrations were determined using the estradiol EIA kit with associated controls according to the manufacturer’s instructions (Cayman Chemical). Serum olaparib concentrations were determined by high-performance liquid chromatography (HPLC)/mass spectrometry (MS). Details are given in Supplementary Materials. Three independent measurements were made for each time point.

**Measurement of Rad51 foci formation**

In vitro cocultures of Cre-infected PTENloxP/loxP epithelia with WT stroma were treated with 10 μmol/L olaparib or vehicle for 3 days. Epithelial spheres were released from Matrigel as previously described (28). RFP-positive spheres were picked, dissociated to single cells (28), and transferred to slides by cytospin. Cells were fixed and costained for γH2AX and Rad51 (antibodies in Supplementary Table S1). Cells were visualized using a Zeiss Axiovert with Apotome, EXFO X-Cite series 120Q source, Zeiss McR camera, and Axiosvision software. Nuclear γH2AX foci with or without Rad51 were scored on five random high-powered fields of view per sample.

**Statistical analysis**

Results are expressed as the mean of three or more measurements with standard errors. Normal distributions were verified using normal quantile plots. To determine if differences between two groups were significant, P values were calculated using the unpaired Student t test. For comparisons between three or more groups, one-way ANOVA was performed where all pairwise mean comparisons were assessed using the Tukey HSD criterion. ANOVA analysis of serum estrogen and CYP3A41 transcript levels was performed on log-transformed values. All statistical calculations were performed using GraphPad Prism software.

**Results**

**PTEN-deficient endometrial tumors were resistant to olaparib in a high estrogenic hormonal milieu**

Many patients with endometrial cancer also suffer from obesity (27). In obese patients, aromatase enzyme in adipocytes converts androgen to estradiol, resulting in high circulating estrogen levels (30). Therefore, in the clinic, many patients with endometrial cancers also have high serum estrogen. Based on in vitro studies (7, 16), we hypothesized that endometrial tumors initiated by epithelial loss of PTEN would respond to PARP inhibition in vivo. To mimic the high estrogen levels in patients with endometrial cancer, the response of PTEN-null tumors to PARP inhibition was initially examined in a high estrogenic milieu.

The effects of olaparib were tested in vivo using an endometrial cancer model developed by our group (22). Endometrial epithelia FACS isolated from PTENloxP/loxP uteri were infected with a Cre-expressing lentivirus, resulting in epithelial-specific deletion of PTEN. Combinations of PTEN-null epithelia and WT stroma were placed under the kidney capsule of immunocompromised mice to prevent rejection of grafted tissue (22). Concurrent with cell implantation, mice were oophorectomized and supplemented with a 60-day time-release estradiol pellet to establish high and steady circulating estrogen levels. Within 8 weeks, cell combinations gave rise to endometrial tumors in the context of cell-autonomous loss of PTEN evidenced by presence of tumor attached to the kidney (Fig. 1A and Supplementary Fig. S1A). This mouse tumor model is highly reproducible, resulting in the formation of PTEN-null tumors that share histologic features and molecular markers of human endometrioid endometrial cancer with 100% efficiency (Supplementary Fig S1A; ref. 22). Sixteen independent tumor-bearing mice were supplemented with fresh estrogen pellets to maintain a high estrogen milieu then separated into two groups. The first eight mice were treated daily with olaparib at 50 mg/kg, whereas the remaining eight mice were treated on the same schedule with 10% dimethyl sulfoxide (DMSO) vehicle for 3 weeks (Fig. 1B). All treatments were delivered by oral gavage to replicate the oral route of administration in patients. Prior to the initiation of therapy, drug efficacy was verified in vitro (Supplementary Fig. S1B). After 3 weeks of treatment, tumors were harvested and analyzed. Olaparib treatment had no effect on tumor size or mass in this hormonal milieu (Fig. 1C). Tumors treated with olaparib or vehicle had similar histology, characterized by infiltrating glands mostly with lumens containing atypical and prominent nucleoli (Fig. 1D, a and b vs. d and e). In these tumors, only the epithelia was PTEN-null whereas the stroma expressed PTEN (Fig. 1D, c and f and Supplementary Fig. S1C). The expression of estrogen receptor alpha (ERα) was similar in olaparib- and vehicle-treated controls (Supplementary Fig. S1D). To validate our results, this experiment was performed on an additional cohort of mice confirming that olaparib treatment had no significant effect on the size of PTEN-null tumors in a high estrogenic hormonal milieu (Supplementary Fig S1E).

These findings suggest that olaparib was ineffective in treating PTEN-null tumors exposed to high levels of estrogen. One possibility is that PARP inhibition is not effective against PTEN-null tumors in an in vivo setting. Alternatively, high levels of estrogen may induce resistance to PARP inhibition.
Low levels of circulating estrogen sensitized PTEN-deficient endometrial cancers to the antitumor effects of olaparib

Our results demonstrate that PTEN-null endometrial tumors are resistant to PARP inhibition in a high estrogenic milieu. Clinically, endogenous estrogen levels can be reduced by surgical removal of ovaries and coadministration of aromatase inhibitors. Aromatase inhibitors block the conversion of adrenal androgens to estrogen in adipose tissue (31). Given the feasibility of this clinical approach, we wanted to test the efficacy of PARP inhibitors in achieving tumor response in a low estrogen state. We hypothesized that reduced of estrogen levels might sensitize PTEN-null endometrial tumors to the synthetic lethal effects of PARP inhibitors.

PTEN-null endometrial tumors were established in 17 independent oophorectomized, estrogen-supplemented mice as outlined in the previous experiment (Fig. 2A). Tumor establishment was confirmed by sacrificing one random mouse (Fig 2A and Supplementary Fig. S2A). At this point, estrogen pellets were surgically removed from all mice after endometrial tumors were established (Fig. 2A). Prior to the initiation of olaparib therapy, mice were rested 1 week to clear circulating estrogen (Fig. 2A). After establishing a low estrogen milieu, tumor-bearing mice were separated into equal groups. Half were treated with olaparib (50 mg/kg daily) orally,
whereas remaining mice received vehicle (Fig. 2B). After 3 weeks of therapy, all tumors were harvested and analyzed. Estrogen deprivation was confirmed in this experimental cohort as compared with estrogen-supplemented mice (Supplementary Fig. S2B). The concentration of estrogen in our hormone-depleted mice was within the range of plasma estradiol concentrations reported in postmenopausal women with or without aromatase therapy (32). Olaparib treatment resulted in a 6-fold decrease in tumor mass when administered under low levels of circulating estrogen (Fig. 2C). Despite the efficacy of this treatment in reducing tumor size, olaparib did not eradicate the PTEN-null tumors demonstrated by evidence of residual cancer in these tissues (Fig. 2D, a and d) that were pankeratin positive (b and e) but PTEN negative (c and f). Scale bars, 5 mm in A and C and 100 μm in D.

Figure 2. PARP inhibition decreased tumor mass when administered under low levels of circulating estrogen. A, strategy for therapy of PTEN-null tumors in a low estrogen milieu. B, treatment design. C, PARP inhibition with low circulating estrogen levels significantly decreased tumor mass compared with vehicle-treated controls (n = 8 per group, P < 0.0001). D, both olaparib- and vehicle-treated tumors contained neoplastic epithelial glands with lumens (a and d) that were pankeratin positive (b and e) but PTEN negative (c and f). Scale bars, 5 mm in A and C and 100 μm in D.
would impact response to this combined therapy (olaparib + estrogen depletion), the number of implanted epithelia and stroma were reduced approximately 2.5-fold. Results of this experiment confirmed previous findings by showing olaparib resistance in a high estrogenic milieu (Fig 3B). In this experiment, complete resolution of PTEN-null tumors treated with olaparib in a low estrogenic milieu was found (Fig 3B), suggesting that the initial tumor burden may impact response to this dual therapy.

Our results suggest that modulation of the hormonal milieu can sensitize PTEN-deficient endometrial carcinomas to olaparib therapy. No response to olaparib was noted when estrogen levels were high. The same dose of olaparib, in an estrogen-deprived milieu, resulted in significant decreases in tumor size. A number of factors could underpin this dichotomous response, including effects of estrogen on drug metabolism or components of the HR DNA repair pathway.

Figure 3. Levels of estrogen modulate the sensitivity of PTEN-null endometrial tumors to PARP inhibition in vivo. A, schema for generation and treatment of tumors. B, with estrogen supplementation tumors were resistant to PARP inhibition based on histology and tumor weight. Estrogen deprivation sensitized tumors to PARP inhibition, as demonstrated by reduction in graft size and resolution of tumor tissue.
Estrogen depletion maximized PARP inhibition at the tumor bed concomitant with an increase in circulating levels of olaparib

To assess the bioavailability and activity of olaparib in a high and low estrogenic milieu, we measured serum levels of olaparib in experimental mice and its efficacy in inhibiting PARP activity in the tumors. Effects of olaparib on tumor cell proliferation and apoptosis-induced cell death were also examined in each hormonal condition.

To measure the kinetics of drug response, serum and tumors were collected from tumor-bearing mice sacrificed at 30 minutes, and 2, 6, and 24 hours after olaparib administration. Serum olaparib concentrations were measured using mass spectrometry. To assess inhibition of PARP enzyme, poly-ADP ribose levels, a surrogate marker for PARP activity (33), were measured in tumor extracts. In mice supplemented with estrogen, serum olaparib concentrations peaked at 2 hours coincident with maximal PARP inhibition in the tumor (Fig. 4A, a). No differences were detected in the epithelial tumor proliferation (Fig. 4A, b) or apoptosis (Fig. 4A, c). After 2 hours, serum levels of olaparib steadily declined, concomitant with decreased PARP inhibition in the tumor (Fig. 4A, a). The serum olaparib concentration achieved with the dose in our study (50 mg/kg daily) was 5.2 μg/mL at peak concentration. This is comparable with serum concentrations of 5.9 μg/mL in patients treated with a commonly administered dose of olaparib (400 mg twice/d; ref. 34). In tumor-bearing mice subjected to low estrogen levels, serum olaparib concentrations peaked earlier at 30 minutes and were 20-fold higher than in estrogen-supplemented mice (Fig. 4B, a vs. 4A, a). An olaparib-induced increase in tumor apoptotic cell death (Fig. 4B, c) was detected in this hormonal milieu without a change in the proliferation (Fig. 4B, b). In this low estrogenic milieu, a profound and sustained inhibition of PARP enzyme activity was detected in the tumor bed (Fig. 4B, a), likely resulting in increased cell death and decreased tumor size.

CYP3A4, the primary enzyme that metabolizes olaparib in humans (35, 36), is reported to be regulated by estrogen (37). To account for difference in circulating levels of olaparib in each hormonal milieu, we examined the expression of CYP3A4, a mouse homolog of CYP3A4, in the livers of experimental mice. Significantly higher levels of CYP3A4 transcript was detected in estrogen-treated compared with estrogen-depleted mice (Supplementary Fig S3A). These findings were confirmed on Western blot analysis (Supplementary Fig S3B). This may explain why concentrations of olaparib were lower in estradiol-supplemented mice. Definitive testing of this hypothesis would require measurement of PARP metabolites in each hormonal condition, an approach that was not feasible as specific metabolites for olaparib are not published or disclosed.

Results here demonstrate that the bioavailability and activity of olaparib were enhanced in a low estrogenic hormonal milieu in our in vivo model. This may be one mechanism sensitizing estrogen-deprived PTEN-null endometrial tumors to olaparib therapy. However, olaparib resistance was noted despite measurable inhibition of PARP enzyme activity in estrogen-supplemented mice. Failure of olaparib therapy in this cohort may be due to an intact HR pathway repairing olaparib-induced DNA damage.

Rad51 function in a high estrogen milieu may promote resistance of PTEN-null endometrial tumors to PARP inhibition

Homologous recombination requires the action of a large complement of proteins that normally safeguard the genome (15). If the HR is defective, cells become susceptible to synthetic lethal effects of PARP inhibitors. Previous work suggests that estrogen may regulate the expression of key components of the HR pathway (38–40). Given that the expression and function of Rad51 has been implicated as a potential biomarker of response to PARP inhibition (17, 18, 41), we first examined the expression of Rad51 protein in PTEN-null tumors propagated in low and high estrogen environments.

Western blotting was performed to measure levels of Rad51 in tumor lysates from three independent mice treated with olaparib or vehicle in low or high estrogenic milieu. In the presence of estrogen, significant amounts of tumor Rad51 were detected (Fig. 5A). Conversely, when estrogen was withdrawn a dramatic reduction in tumor Rad51 expression was noted (Fig. 5A). To test whether estrogen deprivation altered the expression of other HR proteins, levels of BRCA1 were also measured in tumor lysates (Fig. 5A). Similar to Rad51, higher levels of BRCA1 were detected in estrogen-supplemented versus estrogen-deprived PTEN-null tumors (Fig. 5A). Our findings demonstrate that the estrogenic hormonal milieu may affect expression of HR proteins in PTEN-null endometrial tumors.

To test the functional implications of reduced Rad51 levels, we examined recruitment of Rad51 to sites of DNA damage in olaparib- or vehicle-treated PTEN-null endometrial epithelial cells exposed to high and low levels of estrogen. Addressing this question requires clear visualization of Rad51 and γH2AX colocalization in nuclei of olaparib-treated cells. Given that nuclei are tightly packed in the tumor, we chose an alternative in vitro experimental approach to measure this colocalization. Primary endometrial epithelia harvested from PTENloxPloxP mice were infected with a Cre-red fluorescent protein (RFP)-tagged lentivirus and plated in a three-dimensional (3D) sphere assay (28), supplemented with estrogen or placebo, and cocultured with WT endometrial stroma to model the paracrine interactions between epithelium and stroma seen in tumors (Supplementary Fig. S4). Within 2 weeks, spheroid epithelial structures outgrew from single cells, many of which were RFP positive, indicating expression of Cre and deletion of PTEN. Established spheres were treated with olaparib or vehicle for 36 hours. Following treatment, RFP-positive spheres were handpicked and
dissociated into single cells (Supplementary Fig. S4). Loss of PTEN was validated in this cell population (Supplementary Fig. S5A). A 6-fold reduction in the PTEN transcript was detected in PTEN-null epithelia exposed to low levels of estrogen, suggesting estrogen modulation of Rad51 expression in vitro (Supplementary Fig. S5B) as well as in vivo (Fig. 5A). To assess if reduced Rad51 levels led to compromised HR, colocalization of Rad51 to γH2AX foci was examined in all experimental groups. Robust recruitment of Rad51 to sites of DNA damage was detected in the nuclei of olaparib-treated, estrogen-exposed PTEN-null endometrial epithelia (Fig. 5B and Supplementary Fig. S5C). In contrast, in the absence of estrogen, a significant reduction in Rad51 recruitment was
Targeting PTEN-Null Endometrial Tumors with PARP Inhibition

Figure 5. Decreased levels and function of Rad51 may drive the sensitivity of PTEN-null tumors to PARP inhibition in estrogen-depleted conditions. A, Western blot reveals diminished expression of both Rad51 and BRCA1 in lysates of tumors from estrogen-deprived compared with estrogen-supplemented mice regardless of treatment with olaparib or vehicle. 3T3 and HeLa lysates were positive controls for Rad51 and BRCA1, respectively. Erk was used as a loading control. Expression of GAPDH was not altered by estrogen levels, suggesting that reduced proliferation with estrogen deprivation is not responsible for changes in Rad51 or BRCA1 levels. B, Rad51 was efficiently recruited to olaparib-induced sites of DNA damage (marked by γH2AX foci) in PTEN-null endometrial epithelial cells supplemented with estrogen (a–c). In estrogen-deprived cells, low (d–f) or no (g–i) levels of Rad51 incorporation into γH2AX foci were observed compared with that in estrogen-supplemented mice (P < 0.0001). Olaparib-induced γH2AX foci formation was unaffected by estrogen deprivation (P = 0.29). Scale bars, 10 μm.

Discussion

PARP inhibitors have been tested in clinical trials as single-agent therapies against hormonally regulated solid tumors (reviewed in ref. 20). Although administration of this targeted therapy has been coupled with chemotherapeutic agents, no studies have examined the efficacy of PARP inhibition in conjunction with hormonal blockade (reviewed in ref. 20). Results of our experiments demonstrate that estrogen levels affect both the availability of olaparib and efficiency of cell death induced by PARP inhibition. When estrogen levels were high, resistance to olaparib was noted in PTEN-null endometrial tumors. In contrast, a measurable tumor response to PARP inhibition was detected with the same dose of olaparib in a low estrogenic milieu.

In clinical trials of patients with breast (42) or ovarian cancer (10), improved outcomes were reported with administration of higher olaparib doses. An unexpected finding in our study was that estrogen significantly impacted serum concentrations of olaparib. Despite the administration of a fixed dose of olaparib in our experiments, higher levels of circulating olaparib coupled with greater and sustained PARP inhibition at the tumor bed was detected upon estrogen depletion. The cytochrome P450 superfamily of enzymes play a role in hepatic drug metabolism (43). A member of this family, CYP3A4, is thought to be the enzyme responsible for olaparib metabolism in humans (35, 36). In our experiments, an upregulation of CYP3A4, a mouse homolog of CYP3A4, was observed with estrogen supplementation. Estrogen-mediated induction of this enzyme could lead to decreased olaparib bioavailability. These findings suggest that the patient’s hormonal status could alter the optimal dosing of olaparib required to achieve a therapeutic response.

Mechanisms that induce resistance or sensitivity to PARP inhibition remain poorly understood. One challenge in interpreting human trials has been the variability of the biologic response (44), which should be carefully taken into noted despite olaparib treatment (Fig. 5B and Supplementary Fig. S5C). In the absence of olaparib, γH2AX foci formation was minimal (Supplementary Fig. S5D). Findings demonstrate that the recruitment of Rad51 is compromised in estrogen-deprived conditions.

Based on our results, PTEN-null endometrial tumors are sensitized to PARP inhibition in a low estrogenic milieu. When estrogen is depleted, increased amounts of DNA damage may occur due to sustained PARP enzyme inhibition at the tumor bed as well as impaired Rad51-mediated HR. High levels of estrogen may induce PARP resistance in PTEN-null endometrial tumors by lowering levels of circulating olaparib in vivo, resulting in less PARP inhibition in the tumor. Simultaneously, robust expression and recruitment of Rad51 in this hormonal milieu may induce efficient tumor DNA repair. The net result is resistance to olaparib therapy when estrogen levels are high (Fig. 6).
is that epithelial inactivation of PTEN is the sole genetic addition to PTEN loss may account for defective HR and (7, 16). In these cell lines, coexisting genetic defects in PTEN-null background have all utilized cancer cell lines studies examining the effects of PARP inhibition in a biomarker for sensitivity to PARP inhibition (41). Prior upon estrogen treatment (45).

Rad51 both human endometrial tissue demonstrated upregulation of array analysis of control- and estrogen-treated normal microarray analysis of control- and estrogen-treated normal human endometrial tissue demonstrated upregulation of both Rad51 (1.5-fold) and BRCA1 (2.2-fold) expression upon estrogen treatment (45).

Loss of PTEN function has been proposed as a potential biomarker for sensitivity to PARP inhibition (41). Prior studies examining the effects of PARP inhibition in a PTEN-null background have all utilized cancer cell lines (7, 16). In these cell lines, coexisting genetic defects in addition to PTEN loss may account for defective HR and sensitivity to PARP inhibition. An advantage of our model is that epithelial inactivation of PTEN is the sole genetic change, allowing for a definitive evaluation of PARP response in PTEN-null tumor cells. In this model, PTEN status was not an independent biomarker of sensitivity to PARP inhibition as endometrial tumors were resistant to olaparib therapy in a high estrogenic milieu. However, a significant response to therapy was observed in PTEN-null endometrial tumors when serum estrogen was decreased. PTEN-null cells are proposed to have compromised HR DNA repair that stems from decreased activity of Rad51 (14), but the association between PTEN deletion and Rad51 function has been controversial (7, 14, 16, 17). Here, we find that Rad51 expression and recruitment to sites of DNA damage was robust in PTEN-null epithelia exposed to high estrogen levels. With estrogen deprivation, Rad51 protein levels were diminished and its function was decreased in PTEN-null epithelia. We suspect that estrogen-mediated regulation of Rad51 in PTEN-null endometrial epithelia is a regulator of PARP sensitivity in our model.

Collectively, our findings suggest that the use of PARP inhibitors in combination with hormonal therapy could provide an exciting novel approach to the treatment of PTEN-null endometrial cancers. Preclinical studies have demonstrated the chemo- and radiosensitizing activity of PARP inhibitors, but despite these positive results, a major limiting factor in translation to clinical trials has been the untoward toxicities of chemo- and radiotherapies (46). Aromatase inhibitors and estrogen-receptor antagonists are well-tolerated medications that can decrease the

![Figure 6. Model for the differential sensitivity of PTEN-null endometrial cells to PARP inhibition in high and low estrogen.](image)
production and block the effects of estrogen (31, 47). Although they have been unsuccessful as monotherapy against endometrial cancer (48, 49), they could be used to sensitize estrogen-dependent tumors to PARP inhibition. Our preclinical findings support this combined approach and merit progression into clinical trials investigating the synergistic effects of PARP inhibitors and hormonal ablation in patients with PTEN-null endometrial cancers. We recognize that there are limitations in extrapolating the data from these experiments to human patients, as the hormonal manipulations in our mouse model do not completely represent the hormonal milieu of patients with endometrial cancer. However, some similarities do exist. Similar to our model, almost all endometrial cancer patients are oophorectomized as part of their treatment. A recent case report showed that the administration of olaparib was efficacious in the therapy of a patient with a PTEN-null endometrial tumor and a predicted low estrogen level due to removal of her ovaries (50). Based on our findings, the combined use of PARP inhibition and hormonal ablation should also be tested in breast and prostate cancer tumor models. We demonstrate that the hormonal milieu can profoundly affect sensitivity to PARP inhibition in this preclinical in vivo endometrial cancer model. Future work by our group will focus on testing this therapeutic strategy in endometrial tumors driven by alternative genetic pathways.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References

Authors' Contributions
Conception and design: D.M. Janzen, D.Y. Paik, O.N. Witte, S. Memarzadeh
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D.M. Janzen, D.Y. Paik, M.A. Rosales, B. Yep, D. Cheng, K. Faull, S. Memarzadeh
Analysis and interpretation of data (e.g., statistical analysis, biosistatics, computational analysis): D.M. Janzen, D.Y. Paik, B. Yep, D. Cheng, S. Memarzadeh
Writing, review, and/or revision of the manuscript: D.M. Janzen, D.Y. Paik, S. Memarzadeh
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.Y. Paik, M.E. Jung, S. Memarzadeh
Study supervision: S. Memarzadeh

Acknowledgments
The authors thank Drs. Jeffery Gornbein, Xianyu Rao, and Yuri Buminovich for helpful discussions of this article.

Grant Support
This work was financially supported by the Concern foundation, CDU/UCLA NIH/NCTI Grant #US4-CA143931, VA CDA-2 Award, Scholars in Translational Medicine Program, Mary Kay Foundation Award, STOP Cancer Award, Broad Stem Cell Research Center Research Award, Sidney Kimmel Foundation award, National Center for Research Resources Grant UL1TR000124, and the University of California Cancer Research Coordinating Committee grant to S. Memarzadeh. O.N. Witte is an Investigator of the Howard Hughes Medical Institute.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 18, 2013; revised September 16, 2013; accepted September 30, 2013; published OnlineFirst November 12, 2013.

www.aacajournals.org
Mol Cancer Ther; 12(12) December 2013 2927

Published OnlineFirst November 12, 2013; DOI: 10.1158/1535-7163.MCT-13-0572

Copyright © 2013 American Association for Cancer Research. mct.aacrjournals.org Downloaded from mct.aacrjournals.org on June 20, 2017. © 2013 American Association for Cancer Research.


Molecular Cancer Therapeutics

Low Levels of Circulating Estrogen Sensitize PTEN-Null Endometrial Tumors to PARP Inhibition  In Vivo

Deanna M. Janzen, Daniel Y. Paik, Miguel A. Rosales, et al.


Updated version Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-13-0572

Supplementary Material Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2013/10/09/1535-7163.MCT-13-0572.DC1

Cited articles This article cites 47 articles, 17 of which you can access for free at:
http://mct.aacrjournals.org/content/12/12/2917.full.html#ref-list-1

Citing articles This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/12/12/2917.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.