Priority Report

PCAT-1, a Long Noncoding RNA, Regulates BRCA2 and Controls Homologous Recombination in Cancer

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Louis L. Pisters9, Ashutosh K. Tewari10, Christine E. Canman5, Karen E. Knudsen12, Naoki Kitabayashi11, Mark A. Rubin11, Francesca Demichelis11,13, Theodore S. Lawrence3, Arul M. Chinnaiyan1,2,6,7,8, and Felix Y. Feng1,3,7

Abstract
Impairment of double-stranded DNA break (DSB) repair is essential to many cancers. However, although mutations in DSB repair proteins are common in hereditary cancers, mechanisms of impaired DSB repair in sporadic cancers remain incompletely understood. Here, we describe the first role for a long noncoding RNA (lncRNA) in DSB repair in prostate cancer. We identify PCAT-1, a prostate cancer outlier lncRNA, which regulates cell response to genotoxic stress. PCAT-1 expression produces a functional deficiency in homologous recombination through its repression of the BRCA2 tumor suppressor, which, in turn, imparts a high sensitivity to small-molecule inhibitors of PARP1. These effects reflected a postranscriptional repression of the BRCA2 3′UTR by PCAT-1. Our observations thus offer a novel mechanism of "BRCAness" in sporadic cancers. Cancer Res; 74(6); 1651–60. © 2014 AACR.

Introduction
The uncontrolled accumulation of double-stranded DNA breaks (DSB) represents a putative Achilles heel for cancer cells, because these lesions are toxic and their repair requires religation of disrupted genetic material (1–3). Several mechanisms, such as nonhomologous end joining (NHEJ), microhomology-mediated end joining (MMEJ), and homologous recombination (HR), contribute to DSB repair and are employed variously during the cell cycle depending on whether a specific DSB harbors either large, small, or no stretches (NHEJ, MMEJ, and HR, respectively) of complementary DNA sequences on the two fragments of broken DNA (4). In particular, the lethality of excess DSBs has been exploited for the therapeutic treatment of hereditary breast and ovarian cancers harboring BRCA1/2 mutations, which leads to defective HR and increased DSBs (5). These cancers exhibit synthetic lethality when treated with small-molecule inhibitors of the PARP1 DNA repair enzyme, whose inhibition prevents a second method of DNA repair and leads to gross collapse of cellular DNA maintenance (6–8).

Recently, long noncoding RNAs (lncRNA) have emerged as new layer of cell biology (9), contributing to diverse biologic processes. In cancer, aberrant expression of lncRNAs is associated with cancer progression (9, 10), and overexpression of oncogenic lncRNAs can promote tumor cell proliferation and metastasis through transcriptional regulation of target genes (11–13). Recent studies have also identified lncRNAs induced by genotoxic stress as well as involved in the repair of DNA damage (14, 15); however, the role of lncRNAs in the regulation of DSB repair remains unclear.

Here, we report the characterization of PCAT-1 as a prostate cancer lncRNA implicated in the regulation of DSB repair. We find that PCAT-1 represses the BRCA2 tumor suppressor gene, leading to downstream impairment of HR. Importantly, PCAT-1–expressing cells exhibit a BRCA-like phenotype, resulting in cell sensitization to PARP1 inhibitors. In human prostate cancer tissues, high PCAT-1 expression predicts for low BRCA2 expression, supporting our observations in model systems. To our knowledge, this report is the first to demonstrate a role for...
lncRNAs in the regulation of DSBs in prostate cancer and suggests a new mechanistic basis for impaired HR in this disease.

Materials and Methods

For full details on methodology, please refer to the Supplementary Information for a complete Materials and Methods section.

Patient samples

For the University of Michigan patient samples, prostate tissues were obtained from the radical prostatectomy series and Rapid Autopsy Program at the University of Michigan tissue core. These programs are part of the University of Michigan Prostate Cancer Specialized Program of Research Excellence (SPORE). All tissue samples were collected with informed consent under an Institutional Review Board (IRB) approved protocol at the University of Michigan [SPORE in Prostate Cancer (Tissue/Serum/Urine) Bank IRB # 1994-0481]. For the Weill Cornell Medical College patient samples, prostate tissues were collected as part of an IRB-approved protocol at Weill Cornell Medical College (New York, NY).

Cell lines

All cell lines were obtained from the American Type Culture Collection (ATCC). Cell lines were maintained using standard media and conditions. Du145-derived cell lines were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% FBS (Invitrogen) and 1% penicillin-streptomycin (Invitrogen) in a 5% CO2 cell culture incubator. RWPE-derived cell lines were maintained in keratinocyte serum-free (Invitrogen) supplemented with bovine pituitary extract, EGF, and 1% penicillin-streptomycin in a 5% CO2 cell culture incubator. LNCaP-derived and PC3-derived cell lines were maintained in RPMI-1640 (Invitrogen) supplemented with 10% FBS and 1% penicillin-streptomycin in a 5% CO2 cell culture incubator. PC3 cells containing the GFP HR assay construct were generated as described previously (16, 17).

PCAT-1 or control-expressing cell lines were generated by cloning PCAT-1 or control LacZ into the pLenti6 vector (Invitrogen). After confirmation of the insert sequence, lentiviruses were generated at the University of Michigan Vector Core and transduced into RWPE or Du145 cells. Stably transfected cells were selected using blasticidin (Invitrogen).

For LNCaP cells with stable knockdown of PCAT-1, cells were seeded at 50%–60% confluency, incubated overnight, and transfected with PCAT-1 or non-targeting short hairpin RNA (shRNA) lentiviral constructs for 48 hours. GFP+ cells were drug-selected using 1 μg/mL puromycin. PCAT-1 shRNAs were custom generated by Systems Biosciences using the following sequences: shRNA 1 GCAGAAACACCAAUGGAUAUU; shRNA 2 AUACAUAAAGACCAUGGAUAU.

To ensure cell identity, all cell lines were used for less than 6 months after resuscitation and confirmed by genotyping after resuscitation. DNA samples were diluted to 0.10 ng/μL and ten genotyping loci (D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, FGA, vWA, and the Amelogenin locus) were analyzed by the University of Michigan DNA Sequencing Core using the Profiler Plus PCR Amplification Kit (Applied Biosystems).

Cell line assays

LNCaP, Du145, PC3, and RWPE cell lines were obtained from the ATCC and maintained in standard conditions. Stable overexpression and knockdown cell lines were generated with lentiviral constructs with blasticidin or puromycin selection as appropriate. RNA isolation and cDNA synthesis were performed according to standard protocols. Quantitative PCR was performed with Power SYBR Green Mastermix on an Applied Biosystems 7900HT Real-Time PCR system. Chemosensitivity assays were performed on 5,000 cells plated per well in 96-well plates and treated with a single dose of olaparib or ABT-888 as indicated for 72 hours. WST assays (Roche) were performed according to the manufacturer’s instructions. Immunofluorescence experiments were performed with 1 × 104 cells in 12-well plates according to standard protocols; RAD51 and γ-H2AX staining was performed 6 hours or 24 hours after treatment, respectively.

Luciferase assays

The indicated cell lines were transfected with full-length BRCA2 luciferase constructs as well as pRL-TK vector as internal control for luciferase activity. After 2 days of incubation, the cells were lysed and luciferase assays conducted using the dual luciferase assay system (Promega). Each experiment was performed in quadruplicate.

Immunoblot analysis

Cells were lysed in radioimmunoprecipitation assay lysis buffer (Sigma) and briefly sonicated for homogenization. Aliquots of each protein extract were boiled in sample buffer, size fractionated by SDS-PAGE at 4°C, and transferred onto polyvinylidene difluoride membrane (GE Healthcare). The membrane was then incubated at room temperature for 1 to 2 hours in blocking buffer [Tris-buffered saline, 0.1% Tween (TBS-T), 5% nonfat dry milk] and incubated at 4°C with the appropriate antibody. Following incubation, the blot was washed 4 times with TBS-T and incubated with horseradish peroxidase-conjugated secondary antibody. The blot was then washed 4 times with TBS-T and twice with TBS and the signals visualized by enhanced chemiluminescence system as described by the manufacturer (GE Healthcare).

The following antibodies were used for immunoblot analysis: BRCA2 (EMD, OP95), BRCA1 (Cell Signaling Technology, #9025S), XRCC1 (Abcam, ab1838), XRCC3 (Abcam, ab97390), XRCC4 (GeneTex, GTX83406), Ku70 (BD Biosciences, #614892), Ku80 (Cell Signaling Technology, #2180S), γ-H2AX (Cell Signaling Technology, #9718) and β-actin (Sigma, A5441).
For immunoblot densitometry, the densitometric scan of the immunoblots was performed using ImageJ. Three replicate experiments were quantified for the final analysis.

**Xenograft assays**

Xenograft experiments were performed according to University of Michigan-approved protocols and conform to their relevant regulatory standards. Five-week-old male severe combined immunodeficient (SCID) mice (CB.17. SCID) were purchased from Charles River, Inc. (Charles River Laboratory). A total of 1 × 10⁶ DU145-control or DU145-PCAT-1 stable cells were resuspended in 100 μL of saline with 50% Matrigel (BD Biosciences) and were implanted subcutaneously into the left and right flank regions of the mice. Mice were anesthetized using a cocktail of xylazine (80–120 mg/kg, i.p.) and ketamine (10 mg/kg, i.p.) for chemical restraint before tumor implantation. All tumors were staged for 2 weeks before starting the drug treatment. At the beginning of the third week, mice with tumors (10 tumors/treatment group, average size 150–200 mm³) were treated with olaparib (100 mg/kg, i.p. twice daily) or an equal volume of dimethyl sulfoxide (DMSO) control. Growth in tumor volume was recorded weekly by using digital calipers.

**Statistical analyses**

All data are presented as means ± SD or SEM, as indicated. All experimental assays were performed in duplicate or triplicate. Statistical analyses shown in figures represent Fisher exact tests or Student t tests, as indicated.

**Results**

**PCAT-1 regulates BRCA2 levels and HR**

We previously reported the systematic nomination of lncRNAs associated with prostate cancer, termed Prostate Cancer Associated Transcripts (PCAT) ref. 10. Among these, we noted that PCAT-1 expression was a prostate cancer outlier associated with low levels of BRCA2. We therefore hypothesized that PCAT-1 mediated the repression of BRCA2, and thus PCAT-1 may be implicated in the dysregulation of HR upon genotoxic stress. To pursue this hypothesis, we generated a panel of three in vitro cell culture model systems: PCAT-1 overexpression in DU145 prostate cancer cells (which lack endogenous expression of this lncRNA), PCAT-1 overexpression in RWPE benign prostate cells (which lack endogenous expression of this lncRNA), and stable knockdown of PCAT-1 in LNCaP prostate cancer cells (which harbor high endogenous levels of PCAT-1 expression; Fig. 1A, left).

Western blot analysis of these three isogenic models uniformly revealed strong downregulation of BRCA2 protein levels in RWPE and DU145 prostate cells and upregulation of BRCA2 in LNCaP sh-PCAT-1 cells (Fig. 1A, right). To ensure that these observations were not restricted to cell line-based studies, we further confirmed an inverse relationship between PCAT-1 and BRCA2 in two independent cohorts of human prostate cancer samples. Using 58 prostate cancer tissues and 20 prostate cancer xenografts derived from human specimens, we found that increasing PCAT-1 expression correlated with decreased BRCA2 expression (Fig. 1B and Supplementary Fig. S1A). Together, these data suggest that PCAT-1 expression antagonizes BRCA2 expression.

Importantly, BRCA2 inactivation impairs HR of DSBs and serves as a predictive biomarker for response to treatment with inhibitors of the PARP1 DNA repair enzyme through synthetic lethality that results from joint inactivation of two DNA repair pathways (HR via BRCA2 inactivation, and base excision repair via PARP1 inhibition). Accordingly, treatment of our isogenic cell lines with either a PARP1 inhibitor (olaparib or ABT-888) or radiation resulted in modulation of RAD51 foci formation, which is a component of the HR pathway and a marker for engagement of the HR machinery (18). Specifically, PCAT-1 overexpression decreased RAD51 foci formation after therapy and PCAT-1 knockdown increased RAD51 foci formation after therapy in prostate cells (Fig. 1C and Supplementary Fig. S1B–S1D). We further used a well-characterized HR assay, in which cells employ HR to recombine an I-SceI-cut plasmid to produce GFP signaling (16), to evaluate the function of PCAT-1 on HR directly. We found that transient overexpression of PCAT-1 in PC3 prostate cancer cells resulted in a significant inhibition of GFP signaling following I-SceI–induced HR in addition to decreased RAD51 foci (Fig. 1D and Supplementary Fig. S2A–S2D). Of note, PCAT-1 expression does not show substantial change following induction of DNA damage via radiation (Supplementary Fig. S2E).

**PCAT-1 expression impairs DNA damage repair**

Because PCAT-1 impairs HR, genotoxic stress of PCAT-1–expressing cells should lead to an accumulation of DSBs, which can be visualized using γ-H2AX foci, a marker of DSBs that have not been repaired (4). To test this, we treated our isogenic DU145 and LNCaP cell line models with olaparib, ABT-888, or radiation. As predicted, PCAT-1 overexpression in DU145 led to an increase in γ-H2AX foci under stress conditions (Fig. 2A and B), indicating that PCAT-1 impairs DSB repair in these cells. Similarly, LNCaP cells with PCAT-1 knockdown displayed decreased levels of γ-H2AX foci (Fig. 2A and B). Immunoblot analysis of γ-H2AX protein abundance in these cells following genotoxic stress confirmed a downregulation of γ-H2AX with knockdown of PCAT-1 and upregulation of γ-H2AX with overexpression of PCAT-1 (Supplementary Fig. S3).

Finally, we also evaluated the ability for our isogenic cell lines to sustain growth in clonogenic survival assays, a gold-standard assay for cell viability following genotoxic stress, after treatment of cells with PARP1 inhibition or radiation. We found that PCAT-1 expression led to decreased cell...
survival in Du145 and RWPE cells, whereas PCAT-1 knockdown increased LNCaP cell survival, in these assays (Supplementary Fig. S4). To exclude a regulatory relationship between PCAT-1 and other major actors in DNA damage, we performed analysis of XRCC1 (base excision repair pathway), XRCC3 (HR), XRCC4 (NHEJ), Ku70 (NHEJ), Ku80 (NHEJ), and BRCA1 (multiple pathways) in our in vitro models, which showed no change in protein abundance upon modulation of PCAT-1 (Supplementary Fig. S5A). Together, these data indicate that PCAT-1 expression may impart cell sensitivity to genotoxic stress by decreasing the HR response through downregulation of BRCA2.

**PCAT-1 expression leads to increased cell death following genotoxic stress**

Because PCAT-1–expressing cells exhibit reduced HR efficiency when challenged, we investigated whether PARP1 inhibition selectively killed PCAT-1–expressing cells. Following treatment with two PARP1 inhibitors (olaparib or ABT-888), we observed that knockdown of PCAT-1 in LNCaP cells prevented cell death, whereas overexpression of PCAT-1 in Du145 and RWPE prostate cells increased cell death in response to PARP inhibition (Fig. 3A, left and Supplementary Fig. S5B–S5D). This change in cell sensitivity to PARP1 inhibitors was striking, with a five-fold change in the IC$_{50}$ for LNCaP and Du145 cells (Fig. 3A, right and Supplementary Fig. S6). Similar results were observed in RWPE cells overexpressing PCAT-1 (Supplementary Fig. S7).

To ensure that these effects were dependent on BRCA2, we undertook rescue experiments by performing knockdown of BRCA2 in LNCaP shPCAT-1 cells (which have increased levels of BRCA2). These experiments demonstrated a corresponding increase in the sensitivity of these cells to PARP1 inhibition in a
dose-dependent manner according to the efficiency of the BRCA2 knockdown (Fig. 3B). We further observed reduced RAD51 foci after treatment following BRCA2 knockdown in LNCaP shPCAT-1 cells as well (Supplementary Fig. S8). To exclude a role for altered cell-cycle distributions in these phenotypes, we performed flow cytometry, which demonstrated no change in cell cycle in our model systems (Supplementary Fig. S9).

PCAT-1 expression leads to decreased in vivo tumor growth following PARP inhibition

To evaluate the contribution of PCAT-1 to PARP inhibitor response in vivo, we generated xenografts of Du145 cells expressing either empty vector control or PCAT-1. We observed that Du145-PCAT-1 cells grew significantly more rapidly in SCID mice, consistent with our previous findings that PCAT-1 accelerates prostate cell proliferation in vitro (Fig. 3C; ref. 10). Moreover, Du145-PCAT-1 xenografts showed marked susceptibility and tumor regression following intraperitoneal administration of olaparib, whereas Du145-control cells showed only a subtle change in growth while the drug was administered, indicating that the background effect of olaparib therapy, possibly due to its effects on other members of the PARP family (19), is small (Fig. 3C). Mice in all groups of treatment maintained their body weights and showed no evidence of weight loss (Supplementary Fig. S10A).

Importantly, Du145 xenografts retained both PCAT-1 expression and BRCA2 repression (Fig. 3D). To investigate PCAT-1 signaling under control-treated (DMSO) and
Figure 3. PCAT-1 expression results in prostate cell sensitivity to PARP inhibition in vitro and in vivo. A, left, LNCaP cells with PCAT-1 knockdown exhibit enhanced cell survival 72 hours after treatment with olaparib. Right, Du145 cells with PCAT-1 overexpression exhibit reduced cell survival 72 hours after treatment with olaparib. Cell survival is determined via WST assays. B, BRCA2 knockdown in LNCaP shPCAT-1 cells rescues cell sensitivity to olaparib. Right, Western blot showing efficiency of BRCA2 knockdown. C, tumor growth curves for Du145-control and Du145-PCAT-1 xenografts following initiation of treatment with DMSO control or 25 μmol/L olaparib. Tumor volumes are normalized to 100, and time = 0 represents the start of treatment administration. Treatment was initiated 3 weeks after xenograft engraftment. D, expression level of PCAT-1 and BRCA2 protein in Du145-PCAT-1 xenografts. Error bars, ± SEM.
olaparib-treated conditions, we also observed in vivo upregulation of PCAT-1–induced target genes (TOP2A, E2F8, BRC5, and KIF15; Supplementary Fig. S10B) defined by previous microarray profiling of LNCaP cells with PCAT-1 siRNAs and confirmed in RWPE-PCAT-1–overexpressing cells (Supplementary Fig. S10C; ref. 10). These data suggest that PCAT-1 is mechanistically linked to increased prostate cell sensitivity to PARP1 inhibitors via its repression of BRCA2 both in vitro and in vivo.

PCAT-1 does not operate via traditional IncRNA-mediated mechanisms

Although many IncRNAs are noted to regulate gene transcription through epigenetic mechanisms (11, 13, 20), we did not observe evidence for this possibility with PCAT-1. Although PCAT-1 regulated BRCA2 mRNA in vitro (Supplementary Fig. S11A), treatment of RWPE-LacZ and RWPE-PCAT-1 cells with the DNA methylation inhibitor 5-azacytidine (5-aza), the histone deacetylase inhibitor TSA, or both, did not reveal enhanced epigenetic regulation of BRCA2 mRNA in PCAT-1–expressing cells (Supplementary Fig. S11B), although there was a baseline regulation of BRCA2 in both cell lines when 5-aza and TSA were combined. Furthermore, bisulfite sequencing of the BRCA2 promoter in our isogenic LNCaP and RWPE model systems demonstrated minimal Cpg island methylation in all cell lines (Supplementary Fig. S11C). These results suggest that epigenetic repression of BRCA2 is not the primary mechanism of PCAT-1. Moreover, IncRNAs containing Alu elements in their transcript sequence may utilize these repetitive sequences to regulate target gene mRNAs via STAU1-dependent degradation (21). Although PCAT-1 harbors an Alu element from bps 1103–1402, knockdown of STAU1 in LNCaP or VCaP cells, which endogenously harbor PCAT-1, did not alter BRCA2 levels (Supplementary Fig. S11D).

PCAT-1 regulates BRCA2 post-transcriptionally

To determine whether PCAT-1 may function in a manner more analogous to microRNAs, which regulate mRNA levels post-transcriptionally (22), we generated a luciferase construct of the BRCA2 3′UTR, which is 902 bp in length (Fig. 4A). Surprisingly, we found that RWPE-PCAT-1 cells, but not control RWPE-LacZ cells, were able to directly repress the activity of the wild-type BRCA2 3′UTR construct (Fig. 4A). Supporting these data, we found that PCAT-1 was localized to the cell cytoplasm (Supplementary Fig. S12A) and overexpression of PCAT-1 in Du145 cells significantly reduced the stability of endogenous BRCA2 mRNA, consistent with a posttranscriptional mechanism (Supplementary Fig. S12B and S12C).

To map a region of PCAT-1 required for repression of the BRCA2 3′UTR, we additionally generated a series of PCAT-1 deletion constructs and overexpressed these in RWPE cells (Fig. 4B and Supplementary Fig. S13A). We generated these constructs to establish whether the 5′ end of PCAT-1, which contains portions of ancestral transposase and Alu repeat elements (Fig. 4B; ref. 10), or the 5′ end of PCAT-1, which consists of nonrepetitive DNA sequences, was required for BRCA2 repression. We observed that the 5′ end of PCAT-1 was sufficient to downregulate the BRCA2 3′UTR luciferase signal as well as endogenous BRCA2 transcript levels (Fig. 4B and C), and for this regulation, the first 250 bp of the PCAT-1 gene were required. In contrast, the 3′ end of PCAT-1 was expendable. Importantly, the 5′ end of PCAT-1 was similarly sufficient to sensitize RWPE cells to olaparib treatment in vitro (Fig. 4D). To rule out the possibility that RNA instability was responsible for the inactivity of the PCAT-1 constructs, we performed RNA stability assays, which demonstrated equivalent rates of RNA decay between full-length PCAT-1 and the inactive PCAT-1 deletion constructs in RWPE cells (Supplementary Fig. S13B). Together, these results indicate that PCAT-1 overexpression is able to directly repress the activity of the BRCA2 3′UTR and that this repression required the 5′ end of PCAT-1.

Discussion

To our knowledge, this is the first report of an IncRNA being involved in the DSB repair process in prostate cancer (Supplementary Fig. S14). These data are supported by a striking inverse correlation between PCAT-1 and BRCA2 expression in human prostate cancer samples. Our results expand the potential roles for IncRNAs in cancer biology and contrast strikingly with previous reports that IncRNAs operate epigenetically through chromatin-modifying complexes (23, 24). Indeed, epigenetic regulation likely represents only one of numerous mechanisms for mRNA function (12, 21, 25, 26). Supporting this notion, we do not observe compelling evidence that PCAT-1 functions in an epigenetic manner, but rather it may exhibit posttranscriptional regulation of its target genes.

Importantly, PCAT-1 is also predominantly cytoplasmic, and thus our work describes the first cytoplasmic prostate IncRNA to be associated with therapeutic response. Cytoplasmic IncRNAs are also less well explored than their nuclear counterparts, and our work sheds light onto the complex mechanistic regulation of cellular processes via cytoplasmic IncRNAs. However, PCAT-1 does exhibit a smaller degree of nuclear expression (see Supplementary Fig. S12A), which may account for our previous observation that PCAT-1 may associate with the nuclear Polycomb Repressive Complex 2 (PRC2). Although our data directly support a role for PCAT-1 in the posttranscriptional regulation of BRCA2, we cannot fully exclude the possibility of additional regulation of BRCA2 at the transcriptional level at this time.

In addition, while the mechanism underlying PCAT-1 function remains incompletely understood, we were intrigued that the 5′ portion of the PCAT-1 RNA, which is comprised of fully unique sequences, was critical for its regulation of BRCA2 mRNA whereas the embedded Alu element was not. Although we did not identify a specific microRNA with high-confidence 7-mer complementary base pair matching to both this region of PCAT-1 and BRCA2 (data not shown), we speculate that alternative mechanisms of mRNA-like mismatch base pairing
may contribute to PCAT-1-mediated regulation in a manner similar to the recently described networks of competing endogenous RNAs (27).

Together, our data suggest that lncRNAs may have a more widespread role in mammalian genome maintenance and DNA repair than previously appreciated. In support of this, a role for small RNAs in human DNA damage repair in human cells has been recently reported and shown to be dependent upon the microRNA biogenesis machinery (28). Of note, Adamson and colleagues nominated the RNA-binding protein RBMX as a novel component of the HR pathway (16), suggesting that RNA–protein interactions may be integral to this process.

This work sheds insight onto potential mechanisms of impaired DSB repair in cancers lacking an inactivating mutation in canonical DSB repair proteins. Thus, our studies have uncovered a novel mechanism of "BRCAness"—the clinical observation that many cancers lacking BRCA1/BRCA2 mutations exhibit the clinical features of impaired DSB repair (2, 29, 30). We hypothesize that other cancers with a BRCA-like phenotype may harbor lncRNAs involved in the regulation and execution of proper HR and other forms of DSB repair. Finally, future clinical trials examining the efficacy of PARP1 inhibitors in prostate cancer will provide critical information as to whether PCAT-1 may serve as a predictive biomarker for patient response to PARP1 inhibitor therapy.

**Disclosure of Potential Conflicts of Interest**

J.R. Prensner has ownership interest (including patents) in patent on PCAT1 and lncRNAs in prostate cancer. A.K. Tewari has honoraria from speakers’ bureau from Intuitive Surgical, has ownership interest (including patents) in the US Patents US 6004267 A—Method for diagnosing and staging prostate cancer and US 8241310 B2—Urethral catheterless radical prostatectomy. A.M. Chinnaiyan has ownership interest (including patents) in Gen-Probe and Wafergen and is a consultant/advisory board member of Wafergen. No potential conflicts of interests were disclosed by the other authors.

**Authors’ Contributions**

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References


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