PARP-1 regulates epithelial–mesenchymal transition (EMT) in prostate tumorigenesis

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Poly (ADP-ribose) polymerase (PARP) is involved in key cellular processes such as DNA replication and repair, gene transcription, cell proliferation and apoptosis. The role of PARP-1 in prostate cancer development and progression is not fully understood. The present study investigated the function of PARP-1 in prostate growth and tumorigenesis in vivo. Functional inactivation of PAR-1 by gene-targeted deletion led to a significant reduction in the prostate gland size in young PARP-1−/− mice (6 weeks) compared with wild-type (WT) littermates. To determine the effect of PARP-1 functional loss on prostate cancer onset, PARP-1−/− mice were crossed with the transgenic adeno-carcinoma of the mouse prostate (TRAMP) mice. Pathological assessment of prostate tumors revealed that TRAMP+/−, PARP-1+/− mice exhibited higher grade prostate tumors compared with TRAMP+/+ mice (16–28 weeks) that was associated with a significantly increased proliferative index and decreased apoptosis among the epithelial cells in TRAMP+/+. PARP-1−/− prostate tumors. Furthermore tumors harboring PARP-1 loss, exhibited a downregulation of nuclear androgen receptor. Impairing PARP-1 led to increased levels of transforming growth factor-β (TGF-β) and Smads that correlated with induction of epithelial–mesenchymal transition (EMT), as established by loss of E-cadherin and β-catenin and upregulation of N-cadherin and ZEB-1. Our findings suggest that impaired PARP-1 function promotes prostate tumorigenesis in vivo via TGF-β-induced EMT. Defining the EMT control by PARP-1 during prostate cancer progression is of translational significance for optimizing PARP-1 therapeutic targeting and predicting response in metastatic castration-resistant prostate cancer.

Introduction

PARP-1 is the major isoform of poly-ADP-ribose polymerases (PARP); it is a nuclear protein with an ADP-ribose transferase catalytic domain with multiple cellular functions including transcriptional regulation and DNA damage repair (1,2). PARP-1 catalyzes the polymerization of ADP-ribose units from donor nicotinamide adenine dinucleotide (NAD+) molecules on DNA strand breaks (1,3). During DNA damage, cells employ multiple types of DNA repair mechanisms: base excision repair, nucleic acid excision repair, homologous recombination, single strand annealing, mismatch repair and non-homologous end joining to repair these damages (3). Under low levels of DNA damage, PARP-1 acts as a survival factor specifically involved in DNA repair while, in the presence of extensive DNA damage, PARP-1 promotes apoptosis (2).

The current understanding that DNA damage-responsive PARPs are the most likely mediators of the therapeutic effect by PARP inhibitors, defines PARP-1 as the primary responder to DNA damage. Targeting PARP-1 has considerable therapeutic value against human cancer. PARP-1 inhibitors are exciting new agents that have shown promise in their toxicity profile, efficacy and resistance mechanism for BRCA-mutated ovarian and breast cancer (4,5). The clinical activity of different PARP inhibitors against tumors from BRCA mutation carriers is established (5,6); more recent studies indicate selective targeting of PARP allosteric regulator to enhance the antitumor therapeutic response (7). Emerging evidence also supports the use of PARP-1 inhibitors as an effective treatment modality in other human cancers harboring BRCA mutations including prostate tumors (8,9).

Prostate cancer development and early onset disease is driven by an aberrant androgen signaling via the androgen receptor (AR) activity for growth promotion and apoptosis inhibition (10,11). Development and progression to advanced metastatic prostate cancer is a consequence of activation of survival processes in direct link with the tumor microenvironment such as apoptosis suppression, resistance to anoikis and epithelial–mesenchymal transition (EMT) (12,13). In preclinical models of prostate cancer EMT endows epithelial cells with invasive properties, induces stem cell properties and prevents apoptosis, thus facilitating cancer metastasis (14–17). Surgical or medical androgen deprivation therapy represents a first line therapy for the treatment of locally advanced prostate tumors (11,18). Despite the initial success, the majority of patients will relapse and develop castration-resistant prostate cancer (CRPC) and these prostate tumors continue to be dependent on androgen/AR signaling despite being in the presence of castrate androgen levels (18–20). Persistently active AR signaling in CRPC is documented by the antitumor activity exhibited by the new androgen/AR axis inhibitors, such abiraterone and enzalutamide (21,22). Yet the emerging therapeutic resistance to these promising agents (23), underscores the significant challenge of developing novel effective strategies beyond AR-targeted activity, or optimizing its targeting by combination strategies. An additional role for PARP-1 inhibitors has recently been documented (besides its DNA damage repair functionality) to regulate AR transcriptional activity in models of CRPC, thus identifying a new therapeutic targeting value for PARP-1 in advanced disease (24).

Therapeutic targeting of PARP-1 via pharmacologic inhibition has promising applications in the clinic, either alone or in combination with radiation therapy, for the treatment of advanced prostate cancer (8,25,26). Dissecting the mechanisms driving resistance to PARP-1 inhibitors will enable the identification of specific conditions to sensitize tumor cells to PARP-1 inhibition and development of effective combination approaches. In this study we investigated the consequences of physiological impairing of PARP-1 function in vivo, on normal and tumorigenic prostate growth. We found that functional inactivation of PARP-1 by gene-targeted deletion, against a transgenic adeno-carcinoma of the mouse prostate (TRAMP) background, results in high-grade prostate tumors due to enhanced proliferative capacity, loss of apoptosis and EMT induction potentially via transforming growth factor-β (TGF-β) signaling.

Materials and methods

Transgenic mouse models

Transgenic and knockout male mice were maintained under environmentally controlled conditions and subject to a 12 h light/dark cycle with food and water ad libitum. The TRAMP mouse model (C57BL/6-Tg-TRAMP-8247N/J; Jackson Laboratories, Bar Harbor, ME). (Stock #: 003135) is a well-characterized model of prostate cancer progression to metastasis. The TRAMP transgene is in the C57BL/6J genetic background. PARP-1−/− mouse model (Jackson Laboratories, # 002779) is from 129Sv background. PARP-1−/− mice harbor an inactive PARP-1 protein via the ADPRT mutation (27) that does not affect protein expression levels (Supplementary Figure S2, available
at *Carcinogenesis* Online). By crossing the original PARP-1−/− female mice (background 129Sv) with wild-type (WT) C57BL/6d male mice for six generations, PARP-1−/− in the C57BL/6d background mice were generated. PARP-1−/− males in the C57BL/6d background were crossed to TRAMP heterozygous females. After genotyping, TRAMP+/− PARP-1−/− females were crossed to TRAMP wild-type (WT) males. Animals (3–6 mice/group) were divided into the following experimental groups: (i) TRAMP−/−, PARP-1+/−; (ii) TRAMP−/−, PARP-1+/−; (iii) TRAMP−/−, PARP-1+/+; (iv) TRAMP−/−, PARP-1−/−; (v) TRAMP−/−, PARP-1−/−; (vi) PARP-1−/− mice.

**Ethics statement**

Studies involving animals: For all the studies with mice the institutional and national guidelines for the care and use of animals were followed according to a protocol approved by the University of Kentucky Institutional Review Committee.

**Immunohistochemical analysis**

Tissue specimens are fixed in 10% (vol/vol) formalin (Sigma–Aldrich, St Louis, MO), and formalin-fixed paraffin-embedded tissue specimens are sectioned (6 μm) using a Finesse microtome (Thermo Shandon, Pittsburgh, PA). Paraffin sections (6 μm) were deparaffinized, rehydrated and stained with hematoxylin and eosin and were subjected to pathologic evaluation by the pathologist (C.H.). Formalin-fixed paraffin-embedded sections of mouse prostate tissues were deparaffinized and subjected to antigen retrieval in citrate buffer (pH 6; Dako, Glostrup, Denmark). The following antibodies were used for detection of specific proteins: rabbit polyclonal antibody against nuclear antigen Ki-67 (Abcam, Cambridge, MA), as a marker of cell proliferation; rabbit polyclonal antibody against E-cadherin (Cell Signaling Technology, Danvers, MA), rabbit polyclonal antibody against the AR and mouse monoclonal antibody against N-cadherin (Santa Cruz Biotechnology, Santa Cruz, CA); the rabbit monoclonal antibody against Smad3 was from Epitomics (Burlingame, CA) and the ZEB-1 antibody was a generous gift from Dr Darlington (University of Louisville, KY). Negative controls consisted of staining with rabbit and mouse IgG (Santa Cruz Biotechnology).

Biotinylated goat anti-rabbit and goat anti-mouse IgG and horseradish peroxidase-streptavidin conjugate were used (Millipore, Billerica, MA). Color development was performed using a FAST 3,3′-diaminobenzidine-based kit (Sigma–Aldrich) and counterstained with hematoxylin. Images were captured using an Olympus BX51 microscope system (Olympus America, Center Valley, PA). The number of positive cells (for each protein) over the total number of prostate epithelial cells/field (200–300) was counted by two independent observers (three fields per each section). For the nuclear immunohistochemical quantification of AR, targeted areas on each slide were quantified via digital imaging with an Aperio ScanScope XT whole slide scanner, followed by analysis with Aperio Spectrum Version 11.2.0.80 software (Aperio, Vista, CA).

**Apoptosis detection**

The incidence of apoptosis was evaluated in situ using the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay (Millipore). Prostate tissue sections were counterstained with methyl green and TUNEL-positive cells were counted per high power field (13). Numerical values represent as the average number of positive cells counted from three different fields per section.

**Western blot analysis**

Prostate tissue was homogenized in TRIZol Reagent (Life Technologies, Grand Island, NY), NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce Biotechnology, Rockford, IL). Whole tissue protein (see Supplementary Data, available at *Carcinogenesis* Online), cytoplasmic and nuclear protein were extracted following the manufacturer’s instructions. Protein expression was determined by immunoblotting using the following specific antibodies: anti-β-catenin, anti-Snail and Slug (Cell Signaling Technology); anti-E-cadherin, anti-N-cadherin, anti-Smad3 (same as immunohistochemical analysis), Smad4 and AR (Santa Cruz Biotechnology). Whole and cytoplasmic protein levels were normalized to glyceraldehyde 3-phosphate dehydrogenase expression, using the GAPDH antibody (Cell Signaling Technology). Nuclear protein expression was normalized to histone H3 expression, using the histone H3 antibody (Cell Signaling Technology). Protein content was quantified using the Pierce BCA Protein Assay Kits (Thermo Scientific, Rockford, IL) and protein samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to Hybond-C membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Membranes were blocked in 5% milk (2% bovine serum albumin combined 2% goat serum for ZEB-1 antibody) in Tris-buffered saline containing 0.05% Tween 20, and following incubation with the respective primary antibody (overnight at 4°C), membranes were exposed to species-specific horseradish peroxidase-labeled secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). Signal detection was achieved with HyGLO Quick Spray Chemiluminescent HRP Antibody Detection Reagent (Denville scientific, Metuchen, NJ). Fold change was determined based on glyceraldehyde 3-phosphate dehydrogenase and histone H3 expression as loading controls for the cytoplasmic and nuclear protein analyses, respectively.

**Real-time PCR analysis**

Real-time reverse transcription–PCR was used to determine messenger RNA (mRNA) expression for transmembrane protease, serine 2 (TMSR2), TGF-β, Smad4, AR, β-catenin, Twist-1, ZEB-1, E-cadherin and N-cadherin genes. Total RNA was extracted from prostate tissue using the TRIzol Reagent (Invitrogen, Life Technologies), and 1 μg was subjected to reverse transcription into complementary DNA using a reverse transcription kit from Promega Corporation (Madison, WI) under the following conditions: 25°C, 10 min; 42°C, 60 min and 95°C, 5 min. Primer pairs and TaqMan probes (Applied Biosystems, Foster City, CA) were used to determine TGF-β, AR, β-catenin, Snail, E-cadherin and N-cadherin gene expression. Primer pairs to assess Smad4, Twist-1 and ZEB-1 mRNA expression were designed using Primer Express software (Applied Biosystems, Branchburg, NJ). The following primers were used: Smad4: forward, GCTTGGGTGATACCTCCCAATG, reverse, TGGTGCACCTGCTTCTCTCA; TMPRSS2: forward, AGGTCTGGTGACACAGACTGT, reverse, AGCT GAT GCA TGTGCTCTGAAG; Twist-1: forward, CCGAGCCCTAGATGCTATGGT, reverse, ATCCAGCTCCAGATGCTTACG; ZEB-1: forward, GAGA CAC AAAA TGACACACAGTTA, reverse, ATGACGTTGTGTGTTTAATGTTCC; Real-time PCR was conducted in an ABI Prism 7300 system (Applied Biosystems), in TaqMan Universal PCR Master Mix and Power SYBR green PCR Master Mix. Numerical data (normalized to 18s mRNA and β-actin levels) indicate mean values ± standard error of the mean, n = 4–6.

**Statistical analysis**

Group I, group II and group III are identified as first littermate pair, second littermate pair and third littermate pair. Each pair of littermates are two offspring (TRAMP−/−, PARP-1−/− and TRAMP−/−, PARP-1+/+) produced by the same pregnancy. Due to different period extracted protein from different littermate pairs, there were large variations from one littermate pair to another littermate pair. Considering this heterogeneity among the transgenic mice, the raw data are included for relevant offsprings comparison among the littermates. The numerical data are analyzed for statistical significance using the unpaired t-test by Graphpad prism4. Values are expressed as the mean ± standard error of the mean. Statistical differences were considered significant at P value of <0.05.

**Results**

**Effect of PARP-1 loss on prostate glandular growth**

To assess the effect of PARP-1 deficiency on prostate development, PARP-1−/− (homozygous), PARP-1−/− (heterozygous) and PARP-1+/+ (WT) mice were generated by intercrossing of PARP-1−/− mice born according to a Mendelian ratio. The different prostate lobes from the PARP-1−/− (homozygous) mice, 6–12 weeks of age, and age-matched PARP-1+/+ littermates, were subjected to histopathological analysis; the histology appeared phenotypically normal (Figure 1A). The prostate glands from PARP-1−/− mice were underdeveloped relative to WT littermates as revealed by the difference in the prostate weight normalized to the body weight in 6 weeks old mice (Figure 1B). There was also a significant decrease in testicular weight in PARP-1−/− mice compared with WT (Supplementary Data, Figure S1, available at *Carcinogenesis* Online). Evaluation of the proliferative capacity based on Ki-67 nuclear antigen immunostaining revealed that prostate glands from PARP-1−/− mice (at 8 and 12 weeks) had a significantly decreased proliferative index compared with PARP-1+/+ mice (Figure 1C and D). Figure 1E reveals the incidence of prostate apoptosis from age-matched mice. There was no significant change in the number of TUNEL-positive prostate epithelial cells in tumors from the PARP-1−/− versus the WT control mice.

**Functional inactivation of PARP-1 accelerates tumor initiation in TRAMP mouse model**

Characterizing the time-dependent events between the initiation of prostate premalignant growth and the progression to metastatic disease is critical to optimizing therapeutic targeting of CRPC. To determine the functional involvement of PARP-1 in prostate tumorigenesis,
PARP-1−/− mice were crossed with the TRAMP mouse model. Male mice were classified as follows after genotyping: TRAMP+/−, PARP-1−/−; TRAMP+/−, PARP-1+/− and TRAMP+/−, PARP-1+/+.

Pathologic assessment was conducted using a standard grading scale in TRAMP mice (28) (Table 1) by a pathologist. As summarized on Table 1, prostate tumorigenic lesions from TRAMP+/− PARP-1−/− mice exhibited higher grade compared with lesions in TRAMP+/− PARP-1+/+ for mice 16–28 weeks of age (Figure 2A). Moreover, there was a sharp increase in the size of the tumor compared with same age of TRAMP+/− PARP-1+/+ mice. The differences in the severity and aggressiveness in the tumor grade of the lesions between the TRAMP+/− PARP-1−/− and TRAMP+/− PARP-1+/+ groups were also further validated by increased proliferative capacity among tumor cells as detected by Ki-67 immunoreactivity in serial sections (Figure 2B). A significant increase in the number of Ki-67-positive epithelial cells from prostate tumors from PARP-1−/− mice was detected compared with WT littermates in TRAMP mice (Figure 2B and D). Apoptosis evaluation in serial prostate tumor sections of increasing age revealed a considerable reduction in the number of TUNEL-positive cells in the TRAMP+/− PARP-1−/− derived prostate tumors, compared with WT (Figure 2C). This decrease in apoptosis reached statistical significance for mice at 20 and 28 weeks of age (Figure 2E; P < 0.05).

Loss of PARP-1 impairs AR nuclear localization and activity

The AR is a transcription factor and when inactive, it resides in the cell cytoplasm and upon activation by androgens, it translocates to

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**Table 1**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Prostate Tumor Grade</th>
<th>Differences</th>
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<tbody>
<tr>
<td>TRAMP+/− PARP-1−/−</td>
<td>Higher</td>
<td></td>
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<tr>
<td>TRAMP+/− PARP-1+/+</td>
<td>Lower</td>
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**Figure 1.** Reduction in prostate glands in PARP-1−/− mice due to decreased proliferative activity. (A) Reveals comparative hematoxylin and eosin staining of prostate tissue sections from PARP-1+/+ and PARP-1−/− of mice during development in early age (6, 8 and 12 weeks). (B) Shows the weights of the prostate glands (all lobes included, ventral, lateral, dorsal and anterior lobe) normalized to body weight from PARP+/+ and PARP−/− mice (6 weeks). (C) Indicates the Ki-67 nuclear antigen immunoreactivity in prostate tissue from PARP-1+/+ and PARP-1−/− mice; magnification x400. (D) Represents the quantitative analysis of the data from C. The number of proliferating cells based on Ki-67 positivity was used to determine the proliferative index as described in Materials and methods. Values indicate average from three independent groups ± standard error of the mean. (E) Reveals TUNEL staining for apoptosis detection in prostate tissue from PARP-1+/+ and PARP−/− mice (age 6, 8 and 12 weeks). (F) Indicates the quantitative analysis of TUNEL-positive cells per section with no significant differences between the two groups. Statistical significance (*) for all the data analyses was determined at a value of P < 0.05. Error bars represent standard error of the mean.
PARP-1 in prostate cancer EMT

**Table 1. Impact of PARP-1 on prostate tumor grade and aggressiveness**

<table>
<thead>
<tr>
<th>Duration</th>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 weeks</td>
<td>TRAMP/+ PARP+/+</td>
<td>Grade 2–3: Variable filling of lumen with papillary epithelial in growths</td>
</tr>
<tr>
<td></td>
<td>TRAMP/+ PARP−/−</td>
<td>Grade 3: Extensive papillary protrusions into the lumen, but not yet cribriforming</td>
</tr>
<tr>
<td>20 weeks</td>
<td>TRAMP/+ PARP+/+</td>
<td>Grade 3: Papillary projection in all acini, but no cribriform architecture or filling of the lumen</td>
</tr>
<tr>
<td></td>
<td>TRAMP/+ PARP−/−</td>
<td>Grade 4: Filling of the lumen, with variably distinct masses beginning to form</td>
</tr>
<tr>
<td>24 weeks</td>
<td>TRAMP/+ PARP+/+</td>
<td>Grade 3: Robust papillary hyperplasia with some cribriform architecture, but not yet completely filling the lumen and no complex glandular formation. Also, not yet invasive</td>
</tr>
<tr>
<td></td>
<td>TRAMP/+ PARP−/−</td>
<td>Grade 5: Large mass of complex, disorganized glandular structures, often in back-to-back arrangement, some microscopic foci of possible early invasion</td>
</tr>
<tr>
<td>28 weeks</td>
<td>TRAMP+/− PARP+/+</td>
<td>Grade 3: Robust papillary ingrowths, but not yet completely filling the lumen or expanding the overall acinar structure</td>
</tr>
<tr>
<td></td>
<td>TRAMP+/− PARP−/−</td>
<td>Grade 6: Scattered foci of unequivocally invasive cancer</td>
</tr>
</tbody>
</table>

Considering the critical function of TGF-β signaling pathway as a driver of EMT (29,30), we subsequently examined the expression and cellular distribution of TGF-β effectors Smad3 and Smad4. A striking increase in Smad3 immunoreactivity and β-catenin nuclear localization was detected in prostate tumors from the PARP-1−/− compared with WT controls (Figure 5A) (Supplementary Figure S4, available at Carcinogenesis Online). Protein levels for both Smad3 and Smad4 were markedly elevated in prostate tumors from TRAMP+/− PARP−/− mice (Figure 5B, C). These changes in the Smad signaling effectors were associated with an increase in TGF-β ligand expression in tumors in TRAMP mice harboring PARP-1 loss (Figure 5B and C). There was also an upregulation in Snail protein levels in prostate tumors consequential to PARP loss (Figure 5B and C).

**Discussion**

The role of PARP-1 in prostate cancer has not been fully identified despite the use of PARP inhibitors to impair advanced disease. The present study demonstrates that functional inactivation of PARP-1 by gene-targeted deletion, in an in vivo model of prostate cancer progression, leads to EMT induction toward high-grade prostate tumors. The association of the clinical emergence of CRPC with increased PARP-1 activity during disease progression (24,31) and the documented therapeutic efficacy of PARP-1 inhibitors in the treatment of patients with advanced disease (8), implicate a significant involvement of PARP-1 in prostate cancer development and progression. Mounting mechanistic evidence supports a critical role for PARP-1 in EMT control via the ability of PARP-1 to transcriptionally regulate functionally relevant genes, in direct accord with our findings. Specifically PARP-1 directly regulates Smad-mediated transcriptional activation during TGF-β-induced EMT in cancer cells (29). PARP-1 has also been shown to bind to Smad promoter, integrin-linked kinase responsive element (SIRE) to modulate Smad expression (32); in addition, PARP-1 is required for Smad cooperative interaction with LSD1 to functionally repress PTEN (33). Moreover, PARP-1 can transcriptionally activate fibronectin gene expression (34) and regulate Snail1 protein stability (35). A V762A (valine to alanine) polymorphism in the proline rich PPXXP domain-PARP-1 activation domain has been linked to prostate cancer (31).

As a corollary to the consequences of PARP-1 loss on promoting prostate tumorigenesis, we observed the impact of PARP-1 loss on EMT and the potentiation of its key characteristic features, such as E-cadherin loss and ZEB-1 transcription factor nuclear accumulation and enhanced expression of mesenchymal features such as N-cadherin. Recruitment of mesenchymal stem cells into prostate tumors, promotes their conversion into cancer-associated fibroblasts that facilitate metastasis by establishing a supportive tumor microenvironment (13,36,37). The EMT phenotype was identified in the prostates of TRAMP+/−, PARP-1−/− mice as manifested by loss of E-cadherin and β-catenin and upregulation of N-cadherin (mesenchymal marker) and ZEB-1 and Snail transcriptional regulators of EMT. TGF-β induces prostate cancer EMT via signaling through downstream Smad-dependent signaling (38). Smad-mediated TGF-β
signaling events induce context-dependent expression of ZEB1, mediated in part by signaling interactions with effectors such as the activated R-Smads and the Snail (SNAI1) transcription factor (39,40). This rapidly growing evidence designates a prospective role for ZEB1 as an attractive target for inhibiting EMT (41), toward impairing metastatic disease and overcoming therapeutic resistance in CRPC.

Complete reversion of malignant cells to an epithelial-like phenotype might not be achieved by targeting ZEB transcription factors alone (42), but engaging additional players such as specific microRNAs might be required (43).

Significantly enough, the germline BRCA2 mutation confers the highest genetic risk of prostate cancer at 8.6-fold among younger men.
Patients with prostate tumors harboring gBRCAm are more aggressive, with a higher likelihood of distant metastasis and poor survival outcomes (45). The focused pursuit of the PARP family of enzymes has identified only few factors other than damaged DNA to activate PARP-1-mediated protein modification (3). The action of PARP inhibitors in prostate cancer cells with other DNA repair abnormalities besides BRCA mutations, is governed by PTEN defects and ETS gene fusions (45,46). The TMPRSS2-ERG fusion gene, observed in ~50% of human prostate cancers, leads to an androgen-induced ERG expression and promotes tumorigenesis. Preclinical evidence has established that PARP1 directly interacts with ERG to inhibit ETS gene fusion activity and inhibiting PARP1 reduces ETS-positive prostate cancer growth, thus enabling a mechanistic rationale for PARP-1 inhibition in ETS gene fusion-positive prostate cancer similar to that of BRCA1/2 deficiency (46). More recent studies demonstrated preferential targeted radiosensitization with PARP-1 inhibition of TMPRSS2-ERG fusion-gene-positive prostate cancer cells (26), especially those that are PTEN-deficient (47). ERG positivity may therefore have a potential predictive biomarker value for sensitivity to PARP inhibitors in CRPC. However, in our experimental model of prostate cancer...

**Fig. 3.** Loss of PARP-1 reduces AR nuclear localization and activity. (A) Profiling of AR protein localization and immunoreactivity in prostate tissues from TRAMP+/− PARP+/+ and TRAMP+/− PARP-1−/− mice (20 weeks); magnification ×400. (B) Quantitative analysis of data from A, reveals a significant decrease in nuclear AR in prostate tissue from TRAMP+/− PARP-1−/− mice compared with controls (P < 0.05). (C) Western blot analysis of cytoplasmic and nuclear fractions from prostate tissue lysates. The blots shown represent the actual results from three independent groups of mice (I, II and III, each group was littermate: TRAMP+/− PARP-1+/+ and TRAMP+/− PARP-1−/−). (D) The barographs on the right indicate the protein band intensity for cytoplasmic and nuclear AR as determined by densitometry and expressed relative to the loading control protein (for each fraction). (E) Shows the numerical data from the reverse transcription–PCR analysis of mRNA expression of AR and its target gene TMPRSS2 in prostate tumors from TRAMP+/− PARP-1−/− mice compared with WT control mice. Values represent average from 4–6 mice per group ± standard error of the mean.
progression, there were no significant differences in PTEN protein and TMPRSS2 mRNA expression in the prostate tumors from TRAMP+/− PARP-1+/+ versus TRAMP+/− PARP-1−/− mice (Supplementary Figures S2 and S3, available at Carcinogenesis Online).

Mechanistic evidence supporting that the transcriptional dynamics triggered by TGF-β are predominantly governed by the Smad nuclear localization rather than activity (48), challenges the conventional view of the TGF-β signaling in control of complex key

![Fig. 4. PARP-1 deficiency yields acquisition of EMT phenotype during prostate tumorigenesis.](image-url)

- **Fig. 4.** PARP-1 deficiency yields acquisition of EMT phenotype during prostate tumorigenesis. (A) Expression profile of E-cadherin and N-cadherin in prostate tissue from TRAMP+/− PARP-1+/+ and TRAMP+/− PARP-1−/− mice (28 weeks); magnification x200. (B) Reveals immunoreactivity pattern for ZEB1 in prostate tumor sections from TRAMP+/− PARP-1+/+ and TRAMP+/− PARP-1−/− mice. Magnification, x200 and x1000 (middle and right panels, respectively, ZEB-1 staining). Serial sections were stained with hematoxylin and eosin (left), revealing high grade, poorly differentiated prostate tumor from TRAMP+/− PARP-1−/− mice. (C) Shows western blot analysis of EMT regulators ZEB-1, E-cadherin, N-cadherin, β-catenin expression in prostate tissue from three different groups of TRAMP+/− PARP-1+/+ and TRAMP+/− PARP-1−/− mice (16 weeks). The three blots are representative of three independent groups of mice (groups I, II and III). (D) The barographs indicate the relative band intensity (from C) as determined by densitometry and expressed relative to glyceraldehyde 3-phosphate dehydrogenase (loading control). (E) Shows mRNA profile by reverse transcription–PCR analysis of E-cadherin, β-catenin, N-cadherin, ZEB-1 and Twist gene expression in TRAMP+/− PARP-1+/+ versus TRAMP+/− PARP-1−/− mice (16 weeks). Statistical significance (*) was determined at a value of \( P < 0.05 \). Error bars represent standard error of the mean from the three groups of littermate pairs.
Fig. 5. Dysfunctional PARP-1 induces TGF-β-mediated EMT in prostate tumors. Analysis of expression of TGF-β signaling effectors by immunostaining and western blotting in prostate tissue from TRAMP mice harboring PARP-1 functional loss compared with TRAMP+/− PARP-1+/+ mice (A and B, respectively). (A) Reveals intense nuclear immunoreactivity for the TGF-β intracellular effector, Smad3 protein in prostate tumor cells in tissue specimens from TRAMP+/− PARP-1−/− mice, compared with age-matched TRAMP+/− PARP-1+/+ mice; magnification, ×400. (B) Comparative protein profiling by western blot analysis of prostate tumor lysates for TGF-β (ligand) and its main intracellular effectors Smad 3 and Smad4 in TRAMP+/− PARP-1−/− prostate tumors versus WT mice, and Snail, EMT regulator, were also detected by western blotting. Results are shown for each of the three independent groups of mice analyzed (for each genotype) are shown (groups I, II and III). Molecular weights are shown on the right (kDa) for each specific protein. (C) Indicates the results of densitometric analysis from B, with the barographs showing the average value of band intensity relative to the loading control protein.
processes in cancer cells. Moreover, molecular studies have identified PARP-1 as a Smad-interacting partner and its ability to regulate Smad transcriptional events and responses to TGF-β, including EMT via ADP ribosylation of Smad 3 and 4 (by causing dissociation of nuclear Smads from Smad binging elements) (29). Such a regulatory effect by PARP-1 on nuclear Smad function, is further supported by our findings indicating a significant increase in nuclear Smad3 upon PARP-1 functional loss that is associated with EMT induction in prostate tumors. We also found a marked decrease in nuclear AR localization and cellular content in prostate tumors from TRAMP+/− PARP-1−/− mice (20 weeks), reflecting a reduced AR activity. This is in accordance with two lines of recent preclinical evidence: (i) linking PARP-1 function with the AR transcriptional activity in tumor progression to CRPC (25), and (ii) indicating a requirement of a threshold of AR activity to induce prostate cancer cell EMT and invasive behavior (17). Thus, the emerging EMT landscape in prostate cancer involves the governing by PARP-1 via TGF-β signaling and potential AR contribution toward aggressive disease and therapeutic resistance (Figure 6). Our study gains support from the reported clinical association between prostate tumors with a transcript profile consistent with EMT with early relapse after surgical resection, as well as biochemical recurrence in patients with bone metastasis (49,50).

In summary, our findings demonstrate the ability of PARP-1 to regulate Smad-dependent responses to TGF-β signaling, and potential AR activity, directing both toward EMT in prostate cancer progression (schematically shown on Figure 6). Navigating the EMT–MET cycles via selective inhibition of PARP-1 (under the control of androgen/AR axis and TGF-β signaling) may represent a powerful combination strategy for effectively overcoming resistance and understanding side effects in tissue where PARP-1 is rendered dysfunctional. PARP-1 inhibitors may now have to be optimized for further clinical utility in PARP-1 selective targeting for the treatment of metastatic CRPC, via actions beyond DNA repair deficiencies, by disrupting the EMT cellular process in a TGF-β/AR co-dependent manner. In the pursuit of biomarkers to identify tumors with sensitivity to PARP-1 inhibitors, EMT governing during prostate tumor progression might provide new lead candidates.

**Supplementary material**

Supplementary Table S1 and Figures S1–S4 can be found at http://carcin.oxfordjournals.org/

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