Abstract

Prostate cancer is the second leading cause of cancer-associated deaths among men in the western countries. Here, we report that human RecQL4 helicase, which is implicated in the pathogenesis of a subset of cancer-prone Rothmund-Thomson syndrome, is highly elevated in metastatic prostate cancer cell lines. Increased RecQL4 expression was also detected in human prostate tumor tissues as a function of tumor grade with the highest expression level in metastatic tumor samples, suggesting that RecQL4 may be a potential prognostic factor for advanced stage of prostate cancer.Transient and stable suppression of RecQL4 by small interfering RNA and short hairpin RNA vectors drastically reduced the growth and survival of metastatic prostate cancer cells, indicating that RecQL4 is a prosurvival factor for prostate cancer cells. RecQL4 suppression led to increased poly(ADP-ribose) polymerase (PARP) synthesis and RecQL4-suppressed prostate cancer cells underwent an extensive apoptotic death in a PARP-1-dependent manner. Most notably, RecQL4 knockdown in metastatic prostate cancer cells drastically reduced their cell invasiveness in vitro and tumorigenicity in vivo, showing that RecQL4 is essential for prostate cancer promotion. Observation of a direct interaction of retinoblastoma (Rb) and E2F1 proteins with RecQL4 promoter suggests that Rb-E2F1 pathway may regulate RecQL4 expression. Collectively, our study shows that RecQL4 is an essential factor for prostate carcinogenesis.

Cancer Res; 70(22); 9207–17. ©2010 AACR.

Introduction

The prototypical *Escherichia coli* RecQ protein acts as a suppressor of illegitimate recombination and RecQ mutants exhibit genomic instability due to improper resolution of DNA secondary and tertiary structures arising during replication and recombination (1). Human cells express five distinct RecQ homologues, three of which are associated with autosomal recessive diseases characterized by cancer susceptibility (2, 3): Bloom syndrome, Werner syndrome, and Rothmund-Thomson syndrome (RTS). Mutations in *RecQ* are linked to three autosomal recessive disorders (RTS, RAPADILINO, and Boller-Gerold syndrome; ref. 4), and the RecQL4 protein shares 40.8% homology with the *E. coli* RecQ protein (see ref. 5 and references therein).

Human RecQ helicases play diverse roles in DNA metabolism (3, 6). RecQL1 promotes chromosome stability and integrity (7). WRN and BLM participate in organizing the DNA replication complex (8), and WRN helicase plays a role in RNA polymerase II–mediated basal transcription in human cells (9). Although the precise biological role of RecQL4 awaits further investigations, some studies implicate RecQL4 in DNA replication and DNA repair (10, 11). Interaction of RecQL4 with proteins involved in genome surveillance was recently reported (12). Xu and Liu (13) provided initial evidence for the DNA unwinding activity of RecQL4 in vitro in the presence of ssDNA, and the activity has been subsequently shown for both ssDNA and dsDNA (14). It is currently unclear whether or not cancer cells exhibit deregulation of RecQ helicases, and if so whether deregulated RecQ helicase activities initiate and promote cancer development processes.

Prostate cancer incidence is increasing at an alarming rate, now representing 33% of new cases of male cancers. Gene deletion and amplification events involving chromosome 8q are intimately associated with prostate carcinogenesis (15–19), although the critical genes in this region have not been extensively characterized. Chromosome 8q amplification was reported in metastatic and recurrent prostate cancers (20–22). Chromosome 8q (11–24) gain is not only observed in prostate cancers but also in cervical cancer (23, 24), breast cancer (25), and colorectal cancer (26), indicating its intimate association with tumorigenesis. RecQL4 imbalance in expression has recently been reported in 18 sporadic osteoblastoma samples (27). Here, we report that
human RecQL4 helicase localized at 8q24.3 is highly expressed in metastatic human prostate cancer cells and tumor tissues. RecQL4-suppressed PC3 cells displayed drastically reduced cell invasion potential in vitro and tumorigenic potential in vivo. These findings suggest that RecQL4 may play a critical role in prostate carcinogenesis. Furthermore, the high level of RecQL4 expression in metastatic prostate cancer samples suggests that RecQL4 may be a novel biomarker for advanced stage of prostate cancer.

Materials and Methods

Cell lines and prostate tumor tissues
Human primary prostate epithelial cells (PrEC) were procured from Lonza, Inc. Immortalized (RWPE1) and metastatic prostate cancer cell lines (CWR22, DU145, LNCaP, PC3, and RWPE2) were procured from American Type Culture Collection. All the cell lines were authenticated based on viability, recovery, growth, and morphology by the suppliers. Human prostate tissue panel array slides (PR951 and PR751) were procured from US Biomax, Inc.

Fluorescence in situ hybridization and immunohistochemistry
Procedures for metaphase chromosome preparation, fluorescence in situ hybridization (FISH), and multicolor FISH were essentially the same as described before (28). FISH was performed using multicolor-band probe specific for chromosome 8 and a spectrum orange-labeled bacterial artificial chromosome (BAC) probe (RP11-374B7, Open Biosystems) corresponding to the proximal region of chromosome 8q24.3 locus. Human M-band and M-FISH probes were obtained from MetaSystems.

Rabbit polyclonal antibodies specific for NH2 terminal and COOH terminal regions of RecQL4 were generously provided by Dr. Igor Stagljar (University of Toronto). RecQL4 expression in the prostate tumor tissue arrays was analyzed by immunohistochemistry using a COOH terminal antibody. Detailed procedures for immunostaining, image acquisition, and quantification are given in Supplementary Data.

Suppression of RecQL4 in prostate cancer cells
Immortalized normal prostate epithelial cells (RWPE1) and metastatic prostate cancer cell lines (DU145, LNCaP, and PC3) in exponential growth phase were transfected with 25 to 100 nmol/L of either control scrambled small interfering RNA (siRNA; Santa Cruz Biotechnology) or RecQL4-specific siRNA (Santa Cruz Biotechnology). Cells were transfected using Lipofectamine RNAi Max (Invitrogen). RecQL4 expression in the total cellular proteins isolated 72 hours after transfection was monitored by Western blot using either COOH terminal or NH2 terminal antibody. Empty vector–transfected clone C5, and two RecQL4 shRNA vector–transfected clones (C6 and C4) were injected into nude mice. At least five mice were used for each treatment. The entire experimental animal protocol and procedure were in compliance with the guidelines of Institutional Animal Care and Use Committee of Columbia University Medical Center. Tumor growth and size (mm3) were monitored for up to 4 weeks.

Effect of histone deacetylase inhibitor Trichostatin A on RecQL4 expression
Immortalized prostate epithelial cells (RWPE1) and the three metastatic prostate cancer cell lines (DU145, LNCaP, and PC3) in exponential growth phase were treated with 0.5 and 1 μmol/L of Trichostatin A (TSA; Sigma-Aldrich) for 48 hours. RecQL4 expression was monitored at the mRNA level by reverse transcription-PCR (RT-PCR), quantitative real-time PCR, and Western blot analyses. Forward and reverse primer sequences for RecQL4 are given in Supplementary Data. Antibodies were procured from Santa Cruz Biotechnology [cyclin A, cyclin E, Cdk2, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-actin, and α-tubulin], Millipore (γ-H2AX), Cell Signaling Technology [phospho-retinoblastoma (Rb) Ser807/811], and Invitrogen (phospho-Rb T222).

Chromatin immunoprecipitation assay
The chromatin immunoprecipitation (ChIP) assay was performed in HeLa cells using a standard protocol essentially as described before (30). Antibodies for Rb and E2F1 were procured from Santa Cruz Biotechnology and Novus Biologicals, respectively.

Results

Human RecQL4 helicase expression is elevated in human prostate cancer cell lines
RecQL4 expression was assessed by RT-PCR and quantitative real-time PCR in primary (PrEC) and immortalized (RWPE1) human prostate epithelial cell lines, as well as in four human prostate cancer cell lines (CWR22, DU145, LNCaP, and PC3). RecQL4 expression was markedly increased in all the metastatic prostate cancer cell lines compared with primary (PrEC) and immortalized human prostate epithelial cells...
RecQL4 and Prostate Cancer

(RWPE1; Fig. 1A) in an androgen-independent manner (Fig. 1A). RecQL4 transcript detected by real-time PCR (Fig. 1A) correlated well with RecQL4 protein in the prostate cancer cells (Fig. 1B). To examine whether or not elevated RecQL4 expression is linked with malignant transformation process, RecQL4 expression was analyzed in the moderately tumorigenic RWPE2 cells that were derived from the parental nontumorigenic RWPE1 cells by transformation with Ki-ras using the Kirsten murine sarcoma virus (31). Strikingly, RecQL4 expression was ∼15-fold higher in tumorigenic RWPE2 cells than RWPE1 cells (Fig. 1C and D).

RecQL4 expression is enhanced in human prostate tumor tissues

RecQL4 expression was next investigated in human prostate panel arrays PR951 and PR751. PR951 array included 8 normal prostate, 36 prostate adenocarcinoma with different Gleason scores, and 4 metastatic adenocarcinoma. PR751 array included 7 benign prostatic hyperplasia (BPH), 4 prostatic intraepithelial neoplasias (PIN), and 64 prostate adenocarcinoma with Gleason scores from 2 to 10. In general, intense RecQL4 protein staining was mainly detected in the nucleoplasmic regions. The integrated absorbance (IA) was used to categorize the staining intensity for RecQL4 as low, medium, and high. Representative images of normal and prostate tumor tissue sections are shown in Fig. 2A and B, and quantitative results are summarized in Table 1 and Fig. 2C. The highest level of RecQL4 expression was detected in all the metastatic prostate adenocarcinoma samples (Fig. 2A, IV–VI), and RecQL4 immunoreactivity somewhat correlated with increasing Gleason scores in the prostate tumor samples. Thirty prostate tumor tissue samples of 48 in the PR951 array had PSA values ranging from 0.5 to 161 ng/mL, but the PSA value was unavailable for the samples in the PR751 array. In general, PSA values of the samples did not seem to correlate with Gleason scores (2–10). However, PSA value and RecQL4 staining intensity correlated for 12 of 30 samples. Two of four PIN samples and two of seven BPH samples showed a medium level of RecQL4 staining intensity correlated for 12 of 30 samples.
RecQL4 gene silencing inhibits proliferation potential of prostate cancer cells

To verify the importance of RecQL4 in prostate cancer cell proliferation and survival, siRNA-mediated RecQL4 gene silencing studies were performed in normal immortalized (RWPE1) and metastatic prostate cancer cell lines. Transfection of RecQL4-specific siRNA reduced the expression of RecQL4 by 70% to 90% in all the cell lines. Representative pictures of RecQL4 knockdown in prostate cancer cell lines after transfection with 50 nmol/L of control and RecQL4-specific siRNA are shown in Fig. 3A and B. Proliferation measured using the CyQuant assay kit (Invitrogen; ref. 29) showed a modest inhibition in proliferation in RWPE1 cells transfected with 10 nm (9.8%) 25 nmol/L (20.2%) of RecQL4-specific siRNA. In contrast, PC3 cells transfected with 10, 25, and 50 nmol/L of RecQL4-specific siRNA showed 42.1%, 48.3%, and 51.4% inhibition in proliferation, respectively (Fig. 3C). These findings suggest that RecQL4 depletion affected the proliferation more profoundly in metastatic prostate cancer cells than in normal prostate epithelial cells.

RecQL4 gene silencing reduces prostate cancer cell viability

The reduced proliferation potential in RecQL4-silenced PC3 cells prompted us to examine the effects of RecQL4 ablation on cell cycle progression and cell death in the three prostate cancer cell lines. For this purpose, cells in exponential growth phase (RWPE1, DU145, LNCaP, and PC3) were transfected with 25 to 100 nmol/L of either control siRNA or RecQL4-specific
siRNA and subjected to cell cycle analysis 72 hours after transfection. Whereas cell cycle progression was hardly affected by RecQL4 knockdown in RWPE1 cells, all the prostate cancer cell lines transfected with RecQL4-specific siRNA showed a dose-dependent increase in the sub-G1 fraction (Fig. 3D). The proportion of G1 cells also decreased as a function of increasing RecQL4 siRNA concentration in all the prostate cancer cell lines but most notably in LNCaP and PC3 cell lines (Fig. 3D). The fraction of G2-M phase cells was also reduced in PC3 cells at the highest dose of RecQL4 siRNA (Fig. 3D). Induction of apoptotic markers [poly(ADP-ribose) polymerase I (PARPI), Bax, and apoptosis inducing factor (AIF)] was next analyzed in RecQL4-suppressed PC3 cells. Consistent with the cell cycle data, cleaved PARP-1, Bax, and AIF proteins showed a dose-dependent enrichment in RecQL4 siRNA-transfected PC3 cells but not in RWPE1 cells (Fig. 4A). Collectively, these results point out that RecQL4 is critical not only for maintaining cell cycle progression but also for survival in prostate cancer cells.

**Stable RecQL4 knockdown increases spontaneous DNA strand break accumulation in metastatic prostate cancer cells**

Effects of RecQL4 suppression on clonogenic survival was subsequently analyzed in PC3 cells through the use of RecQL4 targeting shRNA vectors (TI339521 and TI339524) together with empty (TR20003) and scrambled shRNA (TR30003) control vectors (Origene Technology). Transient RecQL4 suppression by both shRNA vectors reduced the clonogenic survival of PC3 cells (Fig. 4B). To extend these studies, several stably transfected (empty, scrambled, and RecQL4 specific) puromycin-resistant clones were selected. Because complete RecQL4 silencing dramatically increased the apoptotic cell death, two stable clones (TI339521 vector–transfected clone 6 and TI339524 vector–transfected clone 4; hereafter designated as clone 6 and clone 4) with RecQL4 expression level similar to non-tumorigenic RWPE1 cells were selected. These clones showed 80%–90% suppression in RecQL4 protein expression (Fig. 4C). For comparison, clonal cell lines stably expressing empty (Clone 5) and scrambled (Clone 7) vectors were also established.

The increased apoptotic death of RecQL4-suppressed cells is presumably due to a high level of spontaneous DNA damage accumulation. To test this possibility, intranuclear distribution of PARP-1, a marker for DNA single-strand breaks and nicks, was monitored in PC3 cells. In comparison to empty vector–transfected cells, RecQL4-suppressed clonal cell lines showed increased fociization of PARP-1 protein indicating the spontaneous accumulation of DNA single-strand breaks (Fig. 4D). Additionally, increased fociization of a DNA double-strand break (DSB) marker, 53BP1, as well as specific enrichment of yet another DSB marker, phosphorylated histone H2AX (γH2AX), was also detected in RecQL4-suppressed clonal cell lines (Fig. 4D). Consistent with DNA strand break accumulation, elevation of structural and numerical chromosomal aberrations were observed in RecQL4-suppressed clonal cell lines (Supplementary Fig. S2A). Also, RecQL4-suppressed PC3 cells showed elevated apoptotic death after treatment with UVC (Supplementary Fig. S2B) and γ-ray radiation. These findings illustrate that RecQL4 is critical for protecting the prostate cancer cells from exogenous DNA damage.

**RecQL4 suppression reduces invasiveness and tumorigenicity of prostate cancer cells**

To verify whether RecQL4 silencing affects the tumorigenic potential of PC3 cells, an *in vitro* cell invasion assay was performed. The results showed that cell invasion was greatly reduced in C4 and C6 cell lines (Fig. 5A). *In vivo* tumorigenicity of RecQL4-suppressed cell lines was subsequently evaluated in a xenograft mouse model. For this purpose, empty vector (C5) and RecQL4 shRNA-transfected clonal cell lines (C6 and C4) were injected into athymic nude mice. Representative images of the tumor dissected after 4 weeks from the injected nude mice are shown in Fig. 5B. Tumor size (mm³) measured over 4 weeks of postinjection is given in Fig. 5C. Tumors grew rapidly in mice injected with PC3 or C5 (empty vector transfected) cells. In contrast, tumor growth was much slower in mice injected with C6 or C4 cells. In fact, one mouse injected with clone 6 remained tumor-free for 4 weeks. These *in vivo* data strongly support the conclusion that RecQL4 is essential for tumorigenic potential of prostate cancer cells.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Gleason score</th>
<th>RecQL4 staining intensity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>BPH</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PIN</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma 2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma 3</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma 4</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma 5</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Adenocarcinoma 6</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Adenocarcinoma 7</td>
<td>7</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Adenocarcinoma 8</td>
<td>0</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Adenocarcinoma 9</td>
<td>0</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Adenocarcinoma 10</td>
<td>0</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Metastatic</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>49</td>
<td>42</td>
</tr>
</tbody>
</table>

NOTE: The data obtained from two prostate tumor tissue arrays (PR951 with 48 core samples and PR751 with 75 core samples) were pooled together. Four tumor samples in the arrays without any Gleason score were omitted from the analysis. Out of the four PIN samples, three had Gleason grades I, II, and III, respectively, whereas the fourth sample had Gleason grade of between I and II.
Histone deacetylase inhibitor TSA reduces RecQL4 expression in prostate cancer cells

As p16 methylation and Rb hyperphosphorylation are frequent molecular events during tumorigenesis, expression levels of p16 and phosphorylated Rb (Ser807/811) were monitored in RWPE1 and the three prostate cancer cell lines. Whereas p16 was hardly detectable in DU145, LNCaP, and PC3 cell lines (Fig. 6A), a high level of hyperphosphorylated Rb was observed in the three prostate cancer cell lines. Immunohistochemical studies also showed a positive correlation between Rb hyperphosphorylation and RecQL4 expression in 64 prostate tumor tissues from the PR751 array (Supplementary Fig. S3A).

To reverse p16 methylation and Rb hyperphosphorylation, PC3 cells were treated separately with either a DNA demethylating agent zebularine (200 μmol/L) or a histone deacetylase inhibitor TSA (1 μmol/L) for 48 hours. In both RWPE1 and PC3 cell lines, TSA treatment (1 μmol/L) reduced the proportion of G1 phase cells with a concomitant increase in G2-M phase cells.

Figure 3. RecQL4 suppression leads to proliferation failure and apoptosis in metastatic prostate cancer cells. A and B, analysis of RecQL4 expression in RWPE1 and prostate cancer cell lines 72 h after transfection with control and RecQL4 specific siRNA. C, effect of RecQL4 silencing on proliferation by CyQuant assay 48 h after transfection with indicated concentrations of scrambled and RecQL4-specific siRNA in RWPE1 and PC3 cell lines. Error bars, SD. D, cell cycle analysis of immortalized RWPE1 and three metastatic prostate cancer cell lines (DU145, LNCaP, and PC3) transfected with 25 to 100 nmol/L of either scrambled control or RecQL4-specific siRNA. Cell cycle analysis was performed 72 h after siRNA transfection.
phase cells but the enrichment of G2-M phase cells was higher at a lower TSA concentration (0.5 μmol/L). TSA at 1 μmol/L concentration reduced the proportion of S-phase cells only by 2% to 3% relative to mock control in both cell lines (Supplementary Fig. S3B). Strikingly, the apoptotic sub-G1 cells were more in PC3 cells (23.28%) than in RWPE1 cells (6.42%) after treatment with 1 μmol/L of TSA. Although p16 status remained unaffected, Rb hyperphosphorylation was abolished to a great extent in TSA-treated PC3 cells with a concomitant decline in RecQL4 expression (Fig. 6A). However, RecQL4 expression was reduced by either of the treatments (zebularine and TSA) in RWPE1 cells (Fig. 6A). As cyclin A, cyclin E, and

![Figure 4](https://example.com/figure4.png)

**Figure 4.** RecQL4 suppression leads to PARP-1–mediated apoptotic death in prostate cancer cells. A, analysis of cleaved PARP-1, Bax, and AIF proteins in RWPE1 and PC3 cells after 72 h of transfection with control and RecQL4 siRNA. B, PC3 cells transfected with RecQL4 shRNA targeting vectors (TI339521 and TI339524) showed reduced survival. C, analysis of RecQL4 expression in empty vector (clone 5), scrambled vector (clone 7), and RecQL4 shRNA (clones 6 and 4) transfected clonal cell lines of PC3. β-Actin was used as a loading control. D, RecQL4-suppressed clonal cell lines (C6 and C4) showed increased fociization of PARP-1 and 53BP1. Western blot analysis showed increased γ-H2AX level in RecQL4-suppressed cells. Scale bar, 10 μm.

![Figure 5](https://example.com/figure5.png)

**Figure 5.** RecQL4 suppression drastically reduces cell invasiveness in vitro and tumorigenic growth in vivo. A, RecQL4-suppressed clonal cell lines (C4 and C6) showed reduced cell invasion capacity. B and C, suppression of RecQL4 expression in prostate cancer cells reduces tumorigenicity in nude mice. Images of tumors dissected out from the sacrificed mice in B. The tumor size (mm³) versus days of postinjection in C. Difference in relative tumor volume observed at 4 wk between empty vector and RecQL4 shRNA-transfected cells was found to be statistically significant. *, P < 0.01.
Cdk2 mediate Rb phosphorylation, their expression levels were monitored in PC3 and RWPE1 cells (Fig. 6A). The results showed that cyclin A, cyclin E, and Cdk2 levels were noticeably reduced in PC3 cells (Fig. 6A) but were essentially unchanged by TSA treatment in RWPE1 cells. These results suggest that Rb hyperphosphorylation may be regulated by different mechanisms in nontumorigenic and tumorigenic cells. In addition to phospho-Rb Ser807/811, expression of phospho-Rb (T821) was also monitored in RWPE1 and the three prostate cancer cell lines. Similar to phospho-Rb (Ser807/811), phospho-Rb (T821) expression was also reduced in all the cell lines examined (Fig. 6B).

RT-PCR analysis showed that TSA treatment for 48 hours caused a dose-dependent reduction in RecQL4 mRNA in DU145, LNCaP, and PC3 cell lines (Supplementary Fig. S3C). We next verified whether the reduced RecQL4...
expression after TSA treatment is due to reduced E2F1 expression. We found that E2F1 protein expression was considerably reduced only in TSA-treated prostate cancer cell lines but not in BWPE1 cells (Fig. 6B). Also, TSA reduced the expression of both RecQL4 and E2F1 mRNAs in a dose-dependent manner in DU145 cells (Fig. 6C) as well as in LNCaP and PC3 cell lines (data not shown). Collectively, these studies suggest that elevation of RecQL4 expression in prostate cancer cells and prostate tumor tissues is likely due to deregulation of Rb-E2F1 pathway.

**Rb and E2F1 proteins physically interact with RecQL4 promoter in vivo**

The nucleotide sequence of the RecQL4 promoter with potential target sequence for E2F1 binding is shown in Fig. 6D. To test whether Rb and E2F1 proteins directly regulate transcription of RecQL4 via these sites, ChIP assay was performed to detect E2F1 and Rb binding to the RecQL4 promoter in HeLa cells (Fig. 6D). RT-PCR analysis of immunoprecipitated HeLa chromatin DNA detected the RecQL4 promoter bound to Rb and E2F1 proteins (Fig. 6D). The RecQL4 promoter was undetectable in the negative control without any antibody, whereas a positive control using RNA polymerase II antibody detected a specific enrichment of the RecQL4 promoter. Quantification of the RecQL4 promoter bound to Rb and E2F1 proteins was analyzed by real-time PCR (Fig. 6D). These results suggest the possibility that Rb and E2F1 regulate RecQL4 expression in prostate cancer cells.

**Discussion**

Here, we report the novel finding that RecQL4 helicase, which maps to 8q24.3, is upregulated in prostate cancer cells and tissues. The salient findings of this study are (a) RecQL4 expression is elevated in metastatic prostate cancer cells and tumor tissues, (b) RecQL4 depletion causes proliferation failure and apoptosis in prostate cancer cells lines, (c) RecQL4 protects the genomic integrity of prostate cancer cells from endogenous and exogenous DNA damage, and (d) RecQL4 suppression in metastatic PC3 cells drastically reduced their tumorigenic potential both in vitro and in vivo. These data support our hypothesis that RecQL4 is a critical factor for prostate carcinogenesis. Based on our study, we propose that elevated RecQL4 expression confers survival advantage to prostate cancer cells by protecting their genomic integrity from endogenous and exogenous DNA damage. In support, several earlier studies have shown a direct or indirect participation of RecQL4 in diverse DNA repair pathways (10, 32–36). Collectively, our findings show that RecQL4 is a critical factor for prostate cancer cell growth and viability.

Unlimited replicative potential is a hallmark of immortalized and metastatic prostate cancer cells. Recent lines of evidence indicate an essential role for RecQL4 in DNA replication initiation (11, 13, 40–42). Furthermore, the NH2 terminal domain of RecQL4 shares extensive homology to an essential DNA replication protein, Sld2 (40). Recent studies have established a direct interaction of RecQL4 with factors involved in replisome assembly (43, 44). A direct interaction of RecQL4 with minimal chromosome maintenance complex (MCM 10 and MCM 2–7 helicase), CDC45, and GINS was recently documented (44). A more direct role of RecQL1 and RecQL4 in DNA replication initiation was also recently established (45). Therefore, reduced proliferation observed in RecQL4-suppressed prostate cancer cells is probably due to deficiencies in DNA replication initiation.

Liu and colleagues (37) showed that disruption of the Rb and E2F pathway leads to the enhanced expression of RecQ helicases using the mouse model systems deficient in these pathways. The present study shows that Rb hyperphosphorylation and RecQL4 expression correlated with each other in both prostate cancer cells and tissues. Consistent with this, TSA treatment resulted in the abolition of Rb hyperphosphorylation and reduced expression of both E2F1 and RecQL4. Loss of Rb hyperphosphorylation was accompanied by the reduction in the levels of Cdk2, cyclin A, and cyclin E in PC3 cells. Collectively, these data suggest that RecQL4 expression may be regulated by the Rb-E2F1 pathway in prostate cancer cells. Other mechanisms may also regulate RecQL4 expression in prostate cancer cells. For example, p53-mediated transcriptional repression of RecQL4 has been documented in the literature (38) in immortalized human fibroblasts by wild-type p53 but not by tumor-derived mutant p53 forms. However, in this study, elevated RecQL4 expression was found both in p53 mutated (DU145 and PC3) and p53 wild-type (LNCaP) prostate cancer cell lines (39). Thus, different mechanisms may regulate RecQL4 expression in immortalized fibroblasts and metastatic prostate cancer cells.

Among the five RecQ helicases, RecQL4 alone showed an elevated expression consistently in all the six prostate cancer cells lines examined by us. In contrast, BLM helicase was highly expressed only in DU145 cells, whereas the expression levels of other human RecQ helicases (RecQL1, RecQL5, and WRN) were grossly similar in immortalized prostate epithelial and prostate cancer cell lines (Supplementary Fig. S4). On this basis, we propose that elevated RecQL4 expression has two well-defined biological functions in prostate carcinogenesis (a) to confer infinite proliferation potential to prostate cancer cells through its direct participation in DNA replication and (b) to protect the genomic integrity of prostate cancer cells from endogenous and exogenous DNA damage by regulating the efficiency of diverse DNA repair pathways. Observations of the reduced proliferation, clonogenic survival, cell invasion, and in vivo tumorigenicity of RecQL4-suppressed metastatic prostate cancer cells convincingly favor the aforementioned functions of RecQL4 in prostate cancer cells. Specific elevation of RecQL4 expression in the metastatic prostate cancer cells and tumor tissues indicate that RecQL4 may be a novel biomarker for the advanced stage of prostate cancer. Additionally, RecQL4 suppression by TSA raises a potential possibility for development of new
therapeutic strategies for RecQL4 targeting in prostate cancer cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Igor Stagljar (University of Toronto) for the generous gift of RecQL4 antibodies, Dr. V.V. Murty (Columbia University Medical Center) for the generous gift of fluorescence-labeled BAC and centromeric DNA probes, Dr. David J. Brenner (Center for Radiological Research, Columbia University Medical Center) for allowing us to use ISIS MetaSystems software for the image analysis, and Dr. Howard Lieberman for critical comments and suggestions.

Grant Support

U.S. Department of Energy Office of Science Biological and Environmental Research grant DE-FG02-05ER64055 (A.S.B.) and NIH National Cancer Institute grant CA127120 (Y.L.).

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 05/20/2010; revised 08/11/2010; accepted 09/06/2010; published OnlineFirst 11/02/2010.
Human RecQL4 Helicase Plays Critical Roles in Prostate Carcinogenesis

Yanrong Su, Jarah A. Meador, Gloria M. Calaf, et al.

Cancer Res 2010;70:9207-9217. Published OnlineFirst November 2, 2010.

Updated version Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-10-1743

Supplementary Material Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2010/11/12/0008-5472.CAN-10-1743.DC1

Cited articles This article cites 45 articles, 14 of which you can access for free at: http://cancerres.aacrjournals.org/content/70/22/9207.full.html#ref-list-1

Citing articles This article has been cited by 6 HighWire-hosted articles. Access the articles at: /content/70/22/9207.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.