

SHORT REPORTS

Elevated expression of *PCGEM1*, a prostate-specific gene with cell growth-promoting function, is associated with high-risk prostate cancer patientsGyorgy Petrovics*¹, Wei Zhang², Mazen Makarem¹, Jesse P Street¹, Roger Connelly¹, Leon Sun¹, Isabell A Sesterhenn², Vasantha Srikantan¹, Judd W Moul^{1,3} and Shiv Srivastava¹¹Department of Surgery, Center for Prostate Disease Research (CPDR), US Military Cancer Institute, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799, USA; ²Department of Genitourinary Pathology, Armed Forces Institute of Pathology, Washington, DC 20306-6000, USA; ³Urology Service, Walter Reed Army Medical Center, Washington, DC 20307-5001, USA

***PCGEM1* is a novel, highly prostate tissue-specific, androgen-regulated gene. Here, we demonstrate that *PCGEM1* expression is significantly higher in prostate cancer (CaP) cells of African-American men than in Caucasian-American men ($P=0.0002$). Further, increased *PCGEM1* expression associates with normal prostate epithelial cells of CaP patients with a family history of CaP ($P=0.0400$). *PCGEM1* overexpression in LNCaP and in NIH3T3 cells promotes cell proliferation and a dramatic increase in colony formation, suggesting a biological role of *PCGEM1* in cell growth regulation. Taken together, the cell proliferation/colony formation-promoting functions of *PCGEM1* and the association of its increased expression with high-risk CaP patients suggest the potential roles of *PCGEM1* in CaP onset/progression, especially in these high-risk groups.**

Oncogene (2004) 23, 605–611. doi:10.1038/sj.onc.1207069

Keywords: *PCGEM1*; prostate-specific gene; prostate cancer; high-risk patients; cell proliferation

Introduction

Prostate cancer (CaP) is a complex, multifactorial disease with heterogeneous tumor types, making prediction of the clinical course for individual CaP patients a difficult challenge (Small, 1998; Isaacs *et al.*, 2002). Traditional prognostic markers, such as Gleason grade, clinical stage, and pretreatment prostate-specific antigen (PSA) levels have only limited prognostic value for an individual patient (Small, 1998). The established risk factors for CaP are patient age, ethnic origin, and CaP family history (Isaacs *et al.*, 2002). The molecular mechanisms of CaP susceptibility are being currently defined (Isaacs *et al.*, 2002; Xu *et al.*, 2002).

Among several approaches to define CaP-specific genetic alterations, comparison of global gene expression profiles in cancer cells and corresponding normal cells is emerging as a successful strategy, revealing consistent overexpression of *HEPSIN* (Dhanasekaran *et al.*, 2001; Magee *et al.*, 2001; Welsh *et al.*, 2001; Luo *et al.*, 2002) and *AMACR* (Rubin *et al.*, 2002) in CaP, and *EZH2* (Varambally *et al.*, 2002) in metastatic CaP. Recently, the expression pattern of a group of five genes was reported to correlate with CaP progression (Singh *et al.*, 2002). Gene expression alterations in tumor cells may also predispose to cancer (Yan *et al.*, 2002). CaP-specific global gene expression analyses in our laboratory have defined *PCGEM1* as a highly prostate-specific, androgen-regulated gene with cancer-associated overexpression (Srikantan *et al.*, 2000).

PCGEM1 appears to be a noncoding functional RNA gene (Srikantan *et al.*, 2000). *PCGEM1* is similar to *DD3* (Bussemakers *et al.*, 1999) in that these genes are highly prostate-specific, nonprotein-coding genes. However, no sequence homology exists between *PCGEM1* and *DD3*. Recent reviews of the literature (Szymanski and Barciszewski, 2002) and a database of noncoding RNAs demonstrate (<http://biobases.ibch.poznan.pl/ncRNA/>) that an increasing number of noncoding RNA genes are being discovered that may have biological functions in diverse cellular processes. *H19*, *His-1*, and *Bic* represent examples of noncoding RNAs implicated in tumorigenesis (Szymanski and Barciszewski, 2002). In this regard, *PCGEM1* and *DD3* may represent a new class of prostate-specific genes. Therefore, our laboratory is pursuing an in-depth evaluation of the biological functions of *PCGEM1* and features of its tumor-associated expression.

In order to gain an insight into the cell biologic function of *PCGEM1*, NIH3T3 and LNCaP cells overexpressing *PCGEM1* were generated. Cells were transfected with *PCGEM1* cDNA cloned into a eucaryotic expression vector (pEAK8). Puromycin-resistant transfectants were selected and pooled cell lines stably overexpressing *PCGEM1* have been established. The strong expression of *PCGEM1* RNA in both

*Correspondence: G Petrovics, Department of Surgery, Center for Prostate Disease Research, Uniformed Services University of the Health Sciences, 1530 East Jefferson Street, Rockville, MD 20852, USA; E-mail: gpetrovics@cpdr.org

Received 25 May 2003; revised 29 July 2003; accepted 30 July 2003

the LNCaP and the NIH3T3 transfectants was detectable by Northern blot analysis (Figures 1a and 2a Inset). Control cells were transfected with the vector without the *PCGEM1* insert.

The effects of *PCGEM1* overexpression on cell growth/proliferation and cell cycle were evaluated. NIH 3T3 and LNCaP transfectants overexpressing *PCGEM1* were plated at low density in 96-well plates. Cell growth was followed by either counting the cell number or by a colorimetric assay. Both LNCaP cells (Figure 1a) and NIH3T3 cells (Figure 2a) overexpressing *PCGEM1* exhibited a highly significant increase in proliferation compared to the vector-control-transfected cells ($P < 0.001$, calculated by *t*-test). Further, in colony-forming assays with both LNCaP cells (Figure 1b) and NIH3T3 cells (Figure 2b), overexpression of *PCGEM1* led to a dramatic increase in colony formation compared to the vector control.

The effect of *PCGEM1* overexpression on cell cycle was analysed using a panel of phosphorylation-specific antibodies raised against key cell-cycle-related proteins in Western blot experiments (CDC2, CDC25, p53, Rb Ser780, Rb Ser795, Rb Ser807/811, cyclin D1, and Chk1). In both LNCaP (Figure 1c) and NIH 3T3 (Figure 2c) cells overexpressing *PCGEM1*, a significant increase in Rb phosphorylation (Ser807/811) was detected, indicating that *PCGEM1* overexpression may affect cell proliferation through Rb phosphorylation. Rb Ser807/811 is often phosphorylated in uveal melanoma, the most common malignancy of the eye, and Rb is functionally inactivated by this phosphorylation (Brantley and Harbour, 2000). Rb Ser807/811 phosphorylation has been shown to disrupt Rb binding to the protooncogene *c-abl* (Knudsen and Wang, 1996). Further experiments are needed to reveal the signal transduction pathway involving *PCGEM1* and Rb Ser807/811.

The cell proliferation/colony formation-promoting effects of *PCGEM1* overexpression in the LNCaP prostate cell line suggest that it may have a functional role in cell growth regulation in human CaP cells. Sequence analysis of mouse and rat genome sequences revealed a 131 bp region of strong homology to human *PCGEM1* (83% to mouse, 85% to rat), which weakens (about 60%) with several gaps in the surrounding regions. The homology exists only at the DNA/RNA level; no possible peptides appear to be conserved between the different species. The *PCGEM1* genomic sequence homology is between corresponding regions of human chromosome 2, mouse chromosome 1, and rat chromosome 9. RNA secondary structure prediction analysis (Mfold) indicated that *PCGEM1* RNA has a lower free energy state than coding genes of the same size, or randomized versions of the *PCGEM1* sequence. Within the 1.6 kb *PCGEM1* cDNA, the first half (1–800 bp) had much lower predicted free energy (–221) than the second half (–163), indicating that the presence of a low-energy, stable secondary RNA structure is more likely in the first 800 bp region of *PCGEM1*. Mutational analysis of *PCGEM1* as well as efforts to identify intracellular molecules binding to *PCGEM1* RNA are in progress in our laboratory.

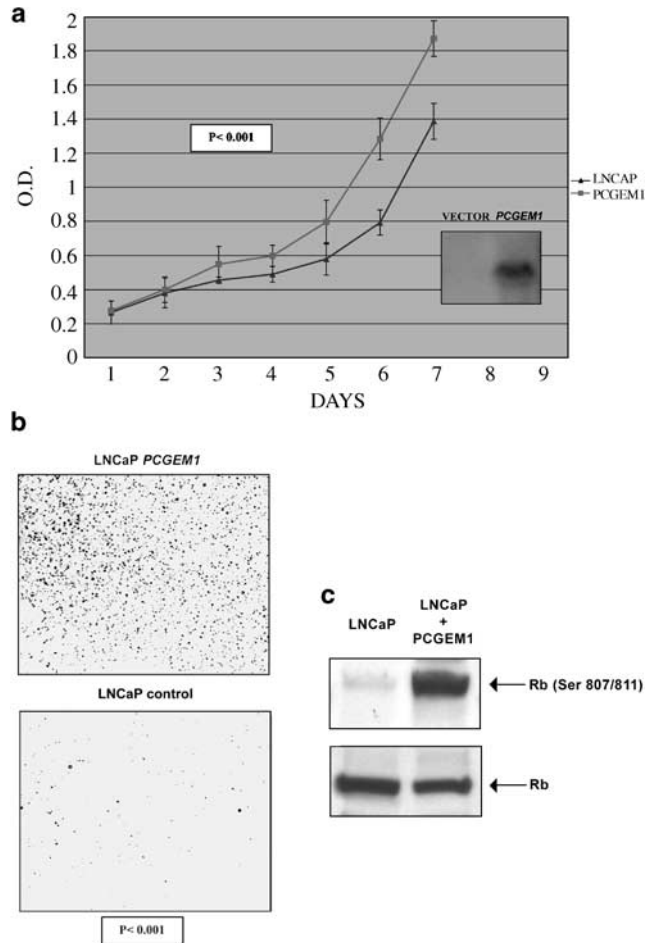


Figure 1 Increased cell proliferation and colony formation by LNCaP cells overexpressing *PCGEM1*. **(a)** Cell proliferation (plotted as optical density, measured by CellTiter Proliferation Kit, Promega) and **(b)** colony formation of LNCaP cells overexpressing *PCGEM1*. Inset: Detection of *PCGEM1* RNA by Northern blot in LNCaP *PCGEM1* transfectants. A total of 1000 cells/well were plated in 96-well plates, and cell proliferation was measured each day for 7 days in triplicate for each cell line. *P*-values for data-point average pairs were determined by the *t*-test. The $P < 0.0001$ value (shown in the figure) is reached by Day 6 and Day 7. For the colony formation assay, 3000 cells were plated in 100 mm Petri dishes or T-75 cell culture flasks for each cell line. After 2 weeks, the developing individual colonies were stained with crystal violet. The number of colonies formed after 2 weeks by LNCaP parent cells (250–280) and LNCaP cells overexpressing *PCGEM1* (3000–3600) were counted in three independent experiments, and the average values were analysed by the *t*-test. The full-length *PCGEM1* cDNA was obtained in a eucaryotic expression vector (pEAK8, Edge BioSystems) by screening a prostate cDNA library (Edge BioSystems). A sequence-verified *PCGEM1* cDNA gene in the pEAK8 expression vector was transfected into both LNCaP and NIH3T3 cells using lipofectamine (Invitrogen, Carlsbad, CA, USA). Transfectants were selected by 0.2 mg/l (LNCaP) or 5 mg/l (NIH3T3) puromycin (Edge BioSystems). **(c)** Detection of Rb phosphorylation in LNCaP cells with and without *PCGEM1* expression. Protein lysates from exponentially growing cells were quantitated using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA) and 30 μ g aliquots were subjected to Western blotting. Antibodies raised against phosphorylated forms of cell cycle-related proteins CDC2, CDC25, p53, Rb Ser780, Rb Ser795 and Rb Ser807/811, Chk1, as well as the Rb control antibody, were obtained from Cell Signaling Technology (Beverly, MA, USA)

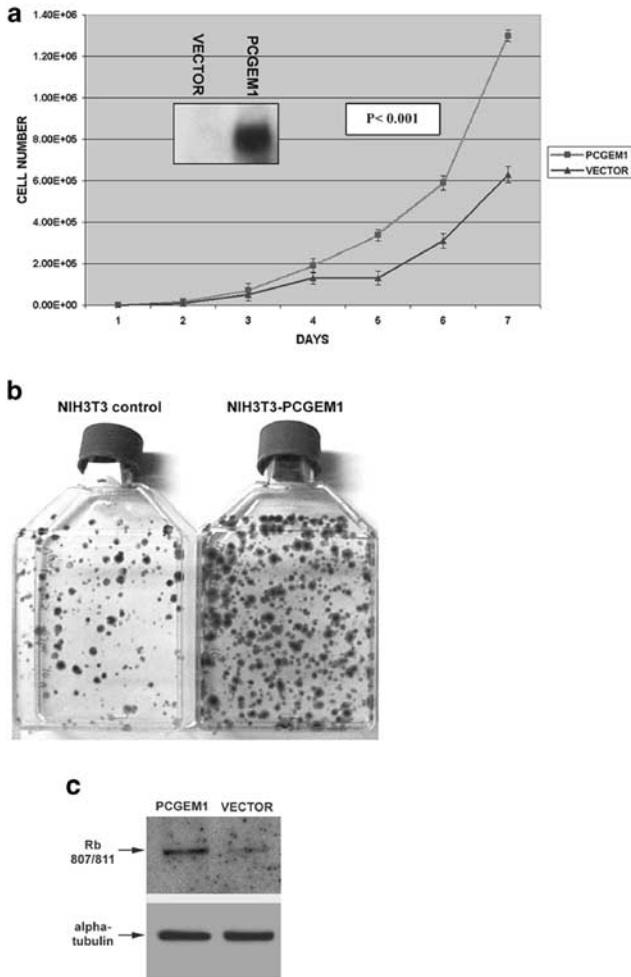


Figure 2 Cell proliferation and colony formation of NIH3T3 cells increases with *PCGEM1* overexpression. (a) Cell proliferation (plotted as daily cell number counts) and (b) colony formation of NIH3T3 cells overexpressing *PCGEM1*. Experiments were performed as described in Figure 1. Inset: Detection of *PCGEM1* RNA by Northern blot in NIH3T3 *PCGEM1* transfectants. (c) Detection of Rb phosphorylation in NIH3T3 cells with and without *PCGEM1* expression using the same procedure as described in Figure 1c. Alpha-tubulin antibody served as an internal control (Santa Cruz Biotechnology, Santa Cruz, CA, USA)

Although the number of noncoding RNA genes identified has sharply increased in recent years (<http://biobases.ibch.poznan.pl/ncRNA/>), functional data are available only on a small subset of these genes (Szymanski and Barciszewski, 2002). H19, one of the most well-characterized noncoding RNAs, exhibited tumor suppressor activity when overexpressed in embryonal carcinoma cells (Hao *et al.*, 1993). Recent results, however, indicated that in breast epithelial cells, H19 overexpression promotes tumor progression without affecting cell proliferation (Lottin *et al.*, 2002). His-1 (Askew *et al.*, 1994) and Bic (Tam *et al.*, 1997) are noncoding RNA genes whose transcription is activated by retroviral insertion, and they are implicated in the pathogenesis of hematological malignancies. Avian bic

cooperates with *c-myc* in oncogenesis, and in enhancing the growth of chicken embryo fibroblasts (Tam *et al.*, 2002). No functional data are reported on the prostate-specific noncoding gene DD3, which has a highly CaP-specific expression (Bussemakers *et al.*, 1999).

In an attempt to assess potential clinical utility of *PCGEM1*, its expression levels in both cancer and normal prostate epithelial cells derived from radical prostatectomy specimens of CaP patients were analysed. Using OCT-embedded frozen sections of prostate tissues, prostate epithelial cells with normal and cancer phenotypes, as defined by hematoxylin–eosin (H&E) staining, were laser capture microdissected (LCM). A total of 180 RNA specimens representing microdissected paired normal and tumor cells of 90 CaP patients were quantified and used for expression analyses.

As a quality control, each LCM RNA sample was assayed by RT–PCR for the expression of NKX 3.1, a prostate epithelial cell marker (Xu *et al.*, 2000). In a subset of 40 patients, the expression of *DD3*, a sensitive and specific marker of prostate tumor cells (DeKok *et al.*, 2002), was also determined. Over 80% of the patients exhibited higher *DD3* expression in their prostate tumor cells than in the normal prostate epithelium (data not shown). This bank of paired normal and tumor cell-derived RNAs were screened by real-time multiplex quantitative RT–PCR (TaqMan) for the expression of *PCGEM1*. The expression data were normalized to *GAPDH* expression levels, which was measured in parallel in the same tubes (multiplex PCR).

The association of *PCGEM1* expression data with 35 different clinicopathological parameters linked to the CaP patients were analysed (full list in legends to Table 1). A summary of *PCGEM1* association with selected clinicopathological features is presented in Table 1. The statistical analyses revealed a striking association between *PCGEM1* expression levels and the ethnic origin of CaP patients. Tumor cells of African-American CaP patients ($n=22$) harbored significantly higher *PCGEM1* expression ($P=0.0002$) compared to those of Caucasian-American CaP patients ($n=66$) (Figure 3a). In contrast, *PCGEM1* expression in normal prostate epithelial cells of these two patient groups was not significantly different ($P=0.6001$) (Figure 3b). In the African-American patient population, *PCGEM1* expression was increased in their prostate tumor cells compared to matched normal prostate epithelial cells in 68.2% of the cases (15/22). On the other hand, 41% (27/66) of Caucasian-American CaP patients showed tumor cell-associated *PCGEM1* overexpression in comparison to matched normal epithelial cells. To our knowledge, this is the first observation of a prostate-specific gene with a cell growth-promoting function that shows elevated expression in African-American CaP patients, the population with the highest CaP incidence in the world. At this point, however, we cannot conclude that *PCGEM1* overexpression is cancer specific, because *PCGEM1* expression has not been evaluated in other pathological conditions of the prostate (BPH, prostatitis).

Table 1 Relationship of *PCGEM1* expression in prostate epithelial cells to clinicopathological features of CaP patients undergoing radical prostatectomy

| Clinicopathological features (n) | Tumor cells | | Normal cells | | P-values | |
|----------------------------------|------------------------------------|-------------------------------------|------------------------------------|-------------------------------------|----------|--------|
| | <i>PCGEM1</i> expression | | <i>PCGEM1</i> expression | | Tumor | Normal |
| | 0–3 'low' Patient number (n=90) | 4–5 'high' Patient number (n=90) | 0–3 'low' Patient number (n=90) | 4–5 'high' Patient number (n=90) | | |
| Cell differentiation | 58 | 31 | 61 | 28 | 0.1138 | 0.6158 |
| Well (46) | 32 | 14 | 31 | 15 | | |
| Moderate (28) | 14 | 14 | 18 | 10 | | |
| Poor (15) | 12 | 3 | 12 | 3 | | |
| Race | 59 | 31 | 62 | 28 | 0.0002 | 0.6001 |
| African-American (22) | 7 | 15 | 14 | 8 | | |
| Caucasian-American (66) | 51 | 15 | 46 | 20 | | |
| Age (years) | 59 | 31 | 62 | 28 | 0.4100 | 0.5260 |
| 40–54 (29) | 12 | 8 | 16 | 4 | | |
| 55–59 (23) | 18 | 5 | 16 | 7 | | |
| 60–64 (27) | 18 | 9 | 16 | 11 | | |
| 65–75 (20) | 11 | 9 | 14 | 6 | | |
| Prebiopsy PSA | 58 | 31 | 61 | 28 | 0.2031 | 0.6705 |
| 0–4 (11) | 9 | 2 | 7 | 4 | | |
| 4.1–7 (42) | 25 | 12 | 26 | 11 | | |
| 7.1–10 (18) | 13 | 5 | 14 | 4 | | |
| 10.1+ (23) | 11 | 12 | 14 | 9 | | |
| Family history of CaP | 55 | 28 | 56 | 27 | 0.7984 | 0.0400 |
| Yes (24) | 15 | 9 | 12 | 12 | | |
| No (59) | 40 | 19 | 44 | 15 | | |
| T stage | 57 | 30 | 60 | 27 | 0.6016 | 0.6476 |
| T2 (33) | 24 | 9 | 22 | 11 | | |
| T3a, b (39) | 24 | 15 | 26 | 13 | | |
| T3c (15) | 9 | 6 | 12 | 3 | | |
| Margins | 58 | 31 | 61 | 28 | 0.8174 | 0.1468 |
| Neg (59) | 39 | 20 | 37 | 22 | | |
| Pos (30) | 19 | 11 | 24 | 6 | | |
| Seminal vesicle | 58 | 31 | 61 | 28 | 0.3745 | 0.3723 |
| Neg (74) | 50 | 24 | 49 | 25 | | |
| Pos (15) | 8 | 7 | 12 | 3 | | |

RNA isolated (MicroRNA kit, Stratagene, La Jolla, CA, USA) from LCM samples of tumor and normal epithelial cells of radical prostatectomy patients were quantitated (RiboGreen dye, Molecular Probes, Eugene, OR and VersaFluor fluorimeter, BioRad, Hercules, CA, USA) and assayed by real-time quantitative RT-PCR (TaqMan). In all, 1 ng LCM RNA was used to produce cDNA, sufficient for 10 PCR reactions. Real-time quantitative PCR analysis was performed using TaqMan detection chemistry on the ABI Prism 7700 Sequence Detection System according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). PCR primers were: forward primer, 5'-TTCAATTAGGCAG CAACCTTT-3'; reverse primer, 5'-CATTGAGCTCTATGAATCTGCTTAA-3'; and Taqman probe, FAM-CCGTAACCTGTGTCTG CAACTTCTCTAATT-TAMRA. Each PCR was multiplexed using the GAPDH detection control mix (Applied Biosystems, Foster City, CA, USA) as an internal control in the same tube with the *PCGEM1* mix. GAPDH control cycle threshold (C_T) values obtained from the real-time PCR assays were subtracted from the *PCGEM1* C_T values. The resulting values represent *PCGEM1* expression levels normalized to GAPDH (dC_T values). Normalized *PCGEM1* expression levels were categorized as 1 ($dC_T > 10$), 2 ($6 < dC_T < 10$), 3 ($3 < dC_T < 6$), 4 ($1 < dC_T < 3$), 5 ($dC_T < 1$), and 0 (no detectable *PCGEM1* expression). Statistical analysis was performed with the SAS software package (SAS Institute Inc., Cary, NC, USA), comparing *PCGEM1* expression categories 0–3 ('low' and no *PCGEM1* expression) to categories 4–5 ('high' *PCGEM1* expression). The association between *PCGEM1* expression and clinicopathological features was analysed using Fisher's exact test. The number of patients is in brackets. $P < 0.05$ was considered statistically significant. The prostate tissue specimens were obtained under an IRB-approved protocol from patients treated at Walter Reed Army Medical Center (WRAMC) and Uniformed Services University of the Health Sciences (USUHS). The patient database included the following clinicopathological parameters: race, prebiopsy PSA, diagnosis date, surgery date, age at surgery, left Gleason sum, right Gleason sum, worst Gleason sum, prostatitis at diagnosis, PIN, HGPIN, pretreatment testosterone, family history of CaP, T stage, neoadjuvant date, margins, tumor number; PSA recurrence: months after surgery, date, prostatitis pathology; bone metastasis: months after surgery, date; follow-up: months after surgery, latest contact date, capsule, seminal vesicle, nodes, worst grade, bladder neck, multifocal, diagnosis PSA, worst nuclear grade, prostate weight, hormone refractory date, differentiation at LCM microenvironment

Genetic, hormonal, and environmental factors may contribute to a higher risk of CaP in African-American men (Brawley *et al.*, 1998; Powell, 1998; Cussenot and

Valeri, 2001). Polymorphism of genes, for example, CYP3A4 (Paris *et al.*, 1999), androgen receptor (Platz *et al.*, 2000), and vitamin D receptor (Taylor *et al.*,

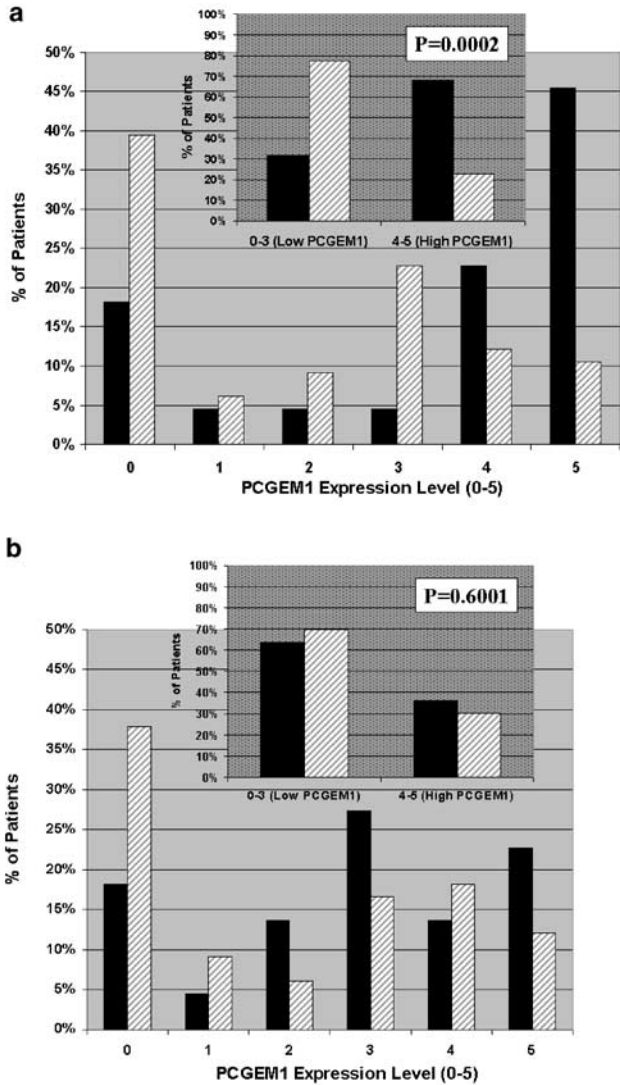


Figure 3 *PCGEM1* expression in microdissected tumor (a) and normal (b) prostate epithelial cells of African-American and Caucasian-American CaP patients. *PCGEM1* expression data were obtained and analysed as described in Table 1. The percentage of African-American (black columns) and Caucasian-American (striped columns) CaP patients expressing *PCGEM1* at different levels (0–5) in their prostate epithelial cells are represented by the columns. The *P*-value (in box) was calculated by Fisher’s exact test

1996), may predispose African-American men to higher CaP risk in comparison to Caucasian-Americans. Bcl-2, an antiapoptotic protein (Guo *et al.*, 2000) and caveolin-1, a membrane protein with tumor suppressor activity (Yang *et al.*, 2000), show differential expression in CaP cells of African-American men compared to Caucasian-Americans. *PCGEM1* belongs to this latter category, with several interesting features: it is a prostate-specific gene, it promotes cell proliferation/colony formation, and it shows highly significant prostate tumor cell-specific overexpression in African-Americans versus Caucasian-Americans.

It is worth noting that increased PSA levels in the serum of African-American men compared to that of

Caucasian-Americans have been described (Moul *et al.*, 1995). Although *PCGEM1* is an androgen-regulated gene, we found no significant association between pretreatment PSA levels and *PCGEM1* expression in either tumor ($P=0.2031$) or normal ($P=0.6705$) prostate epithelial cells (Table 1), making it unlikely that a general induction of the androgen pathway would be responsible for the elevated *PCGEM1* RNA levels.

Intriguingly, our analysis of the relative levels of *PCGEM1* in histologically normal cells of CaP patients revealed a significant increase in *PCGEM1* expression in patients with a family history of CaP (12/24), as

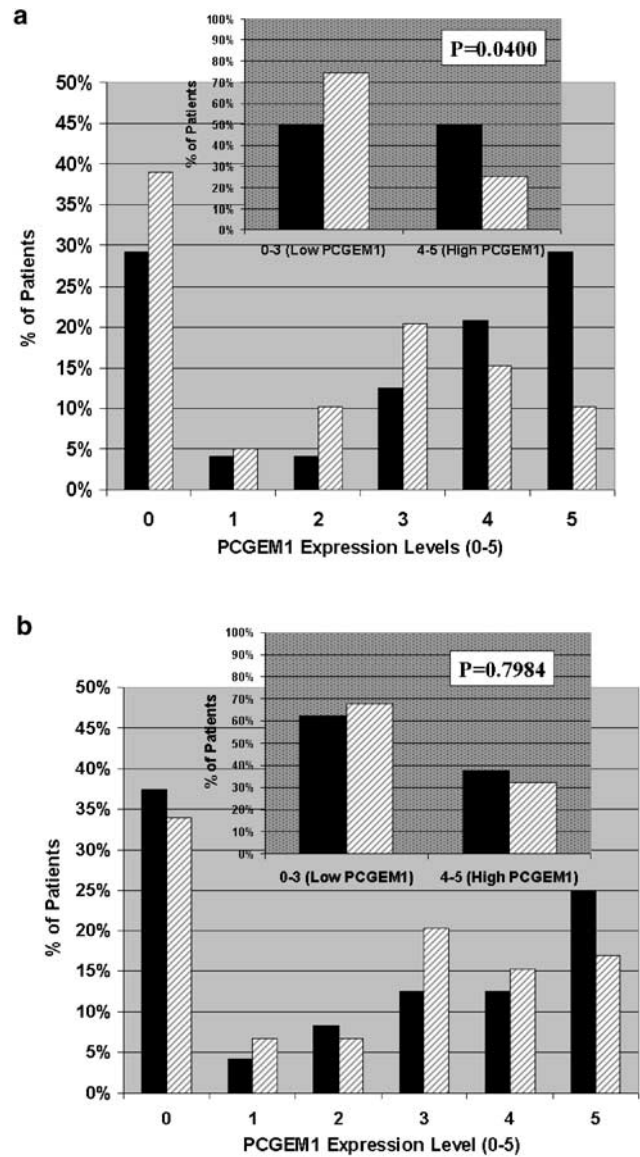


Figure 4 *PCGEM1* expression in microdissected tumor (a) and normal (b) prostate epithelial cells of CaP patients with and without family history of CaP. *PCGEM1* expression data were obtained and analysed as described in Table 1. The percentage of patients with (black columns) and without (striped columns) a family history of CaP expressing *PCGEM1* at different levels (0–5) in their prostate epithelial cells are represented by the columns. The *P*-value (in box) was calculated by Fisher’s exact test

opposed to patients with no family history (15/59) ($P=0.0400$) (Figure 4).

A multivariable logistic regression analysis with backward elimination of insignificant variables was performed for the association of *PCGEM1* expression with patient characteristics (race, family history of CaP, margin, prebiopsy PSA, prostate weight, worst Gleason sum, differentiation). The analysis revealed that the African-American race is significantly related to *PCGEM1* expression in tumor cells ($P=0.0007$), and a family history of CaP is significantly related to *PCGEM1* expression in normal prostate epithelial cells ($P=0.0374$). All other variables analysed were insignificant.

Potential CaP biomarkers that are consistently overexpressed in CaP cells, for example, *HEPSIN*, *AMACR*, *DD3*, and *PSMA*, do not show any reported association with ethnicity or family history of CaP (Isaacs *et al.*, 2002). Loss of *GSTP1* expression is also among the most common alterations in CaP cells (Nelson *et al.*, 2001). *PCGEM1* appears to be a distinct prostate-specific gene associated with these high-risk CaP patients. Only a few studies on cancer-associated expression of other noncoding RNA genes have been reported. H19 appears to be lost in certain embryonal tumors (Hao *et al.*, 1993), but overexpressed in a small subset of breast cancers (Lottin *et al.*, 2002). Retroviral insertion events appear to activate the expression of Bic (Tam *et al.*, 1997) and of His-1 (Askew *et al.*, 1994), but cancer-associated

expression of these genes in humans has not been reported. The expanding universe of noncoding RNAs and their functional evaluations may define additional genes with potential functions in the process of tumorigenesis.

Taken together, the cell proliferation/colony formation-promoting function of a novel prostate-specific noncoding gene, *PCGEM1*, and the association of its increased expression level with high-risk CaP patients suggest the potential roles of *PCGEM1* in CaP biology. Further, *PCGEM1* expression characteristics may provide a promising biomarker and potential therapeutic target in the high-risk CaP patients noted herein.

Abbreviations

CaP, prostate cancer; PSA, prostate-specific antigen; FBS, fetal bovine serum; LCM, laser capture microdissection; RT, reverse transcription; H&E, hematoxylin-eosin.

Acknowledgements

We thank Dr. Gregory Buzard for critically reading this manuscript. This research was supported by Grant #1R01CA85596-01 from the National Institutes of Health to SS. The opinions and assertions contained herein are the private views of the authors and are not to be considered as reflecting the views of the US Army or the Department of Defense.

References

- Askew DS, Li J and Ihle JN. (1994). *Mol. Cell. Biol.*, **14**, 1743–1751.
- Brantley MA and Harbour JW. (2000). *Cancer Res.*, **60**, 4320–4323.
- Brawley OW, Knopf K and Thompson I. (1998). *Semin. Urol. Oncol.*, **16**, 193–201.
- Bussemakers MJ, van Bokhoven A, Verhaegh GW, Smit FP, Karthaus HF, Schalken JA, Debruyne FM, Ru N and Isaacs WB. (1999). *Cancer Res.*, **59**, 5975–5979.
- Cussenot O and Valeri A. (2001). *Eur. J. Intern. Med.*, **12**, 11–16.
- DeKok JB, Verhaegh GW, Roelofs RW, Hessels D, Kiemeny LA, Aalders TW, Swinkels DW and Schalken JA. (2002). *Cancer Res.*, **62**, 2695–2698.
- Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, Pienta KJ, Rubin MA and Chinnaiyan AM. (2001). *Nature*, **412**, 822–826.
- Guo Y, Sigman DB, Borkowski A and Kyprianou N. (2000). *Prostate*, **42**, 130–136.
- Hao Y, Crenshaw T, Moulton T, Newcomb E and Tycko B. (1993). *Nature*, **365**, 764–767.
- Isaacs W, De Marzo A and Nelson WG. (2002). *Cancer Cell*, **2**, 113–116.
- Knudsen ES and Wang JY. (1996). *J. Biol. Chem.*, **271**, 8313–8320.
- Lottin S, Adrianssens E, Dupressoir T, Berteaux N, Montpeller C, Coll J, Dugimont T and Cury JJ. (2002). *Carcinogenesis*, **23**, 1885–1895.
- Luo J, Zha S, Gage WR, Dunn TA, Hicks JL, Bennett CJ, Ewing CM, Platz EA, Ferdinandusse S, Wanders RJ, Trent JM, Isaacs WB and De Marzo AM. (2002). *Cancer Res.*, **62**, 2220–2226.
- Magee JA, Araki T, Patil S, Ehrig T, True L, Humphrey PA, Catalona WJ, Watson MA and Milbrandt J. (2001). *Cancer Res.*, **61**, 5692–5696.
- Moul JW, Sesterhenn IA, Connelly RR, Douglas T, Srivastava S, Mostofi FK and McLeod DG. (1995). *JAMA*, **274**, 1277–1281.
- Nelson WG, De Marzo AM, Dewese TL, Lin X, Brooks JD, Putzi MJ, Nelson CP, Groopman JD and Kensler TW. (2001). *Ann. NY Acad. Sci.*, **952**, 135–144.
- Paris PL, Kupelian PA, Hall JM, Williams TL, Levin H, Klein EA, Casey G and Witte JS. (1999). *Cancer Epidemiol. Biomarkers Prev.*, **8**, 901–905.
- Platz EA, Rimm EB, Willett WC, Kantoff PW and Giovannucci E. (2000). *J. Natl. Cancer Inst.*, **92**, 2009–2017.
- Powell IJ. (1998). *Semin. Urol. Oncol.*, **16**, 221–226.
- Rubin MA, Zhou M, Dhanasekaran SM, Varambally S, Barrette TR, Sanda MG, Pienta KJ, Ghosh D and Chinnaiyan AM. (2002). *JAMA*, **287**, 1662–1670.
- Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C, Tamayo P, Renshaw AA, D'Amico AV, Richie JP, Lander ES, Loda M, Kantoff PW, Golub TR and Sellers WR. (2002). *Cancer Cell*, **1**, 203–209.
- Small EJ. (1998). *Curr. Opin. Oncol.*, **10**, 244–252.
- Srikantan V, Zou Z, Petrovics G, Xu LL, Augustus M, Davis L, Livezey JR, Connell T, Sesterhenn IA, Yoshino K, Buzard GS, Mostofi FK, McLeod DG, Moul JW and Srivastava S. (2000). *Proc. Natl. Acad. Sci. USA*, **97**, 12216–12221.
- Szymanski M and Barciszewski J. (2002). *Genome Biol.*, **3**, 51–58.
- Tam W, Ben-Yehuda D and Hayward WS. (1997). *Mol. Cell. Biol.*, **17**, 1490–1502.

- Tam W, Hughes SH, Hayward WS and Besmer PJ. (2002). *Virology*, **76**, 4275–4286.
- Taylor JA, Hirvonen A, Watson M, Pittman G, Mohler JL and Bell DA. (1996). *Cancer Res.*, **56**, 4108–4110.
- Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP, Rubin MA and Chinnaiyan AM. (2002). *Nature*, **419**, 624–629.
- Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, Frierson Jr HF and Hampton GM. (2001). *Cancer Res.*, **61**, 5974–5978.
- Xu J, Zheng SL, Komiya A, Mychaleckyj JC, Isaacs SD, Hu JJ, Sterling D, Lange EM, Hawkins GA, Turner A, Ewing CM, Faith DA, Johnson JR, Suzuki H, Bujnovszky P, Wiley KE, DeMarzo AM, Bova GS, Chang B, Hall MC, McCullough DL, Partin AW, Kassabian VS, Carpten D, Bailey-Wilson JE, Trent JM, Ohar J, Bleecker ER, Walsh PC, Isaacs WB and Meyers DA. (2002). *Nat. Genet.*, **32**, 321–325.
- Xu LL, Srikantan V, Sesterhenn IA, Augustus M, Dean R, Moul JW, Carter KC and Srivastava S. (2000). *J. Urol.*, **163**, 972–979.
- Yan H, Dobbie Z, Gruber SB, Markowitz S, Romans K, Giardiello FM, Kinzler KW and Vogelstein B. (2002). *Nat. Genet.*, **30**, 25–26.
- Yang G, Addai J, Ittmann M, Wheeler TM and Thompson TC. (2000). *Clin. Cancer Res.*, **6**, 3430–3433.