Overexpression of MUC1 and Genomic Alterations in Its Network Associate with Prostate Cancer Progression

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Abstract

We investigate the association of MUC1 with castration-resistant prostate cancer (CRPC), bone metastasis, and PC recurrence. MUC1 expression was studied in patient-derived bone metastasis and CRPCs produced by prostate-specific PTEN−/− mice and LNCaP xenografts. Elevations in MUC1 expression occur in CRPC. Among nine patients with hormone-naïve bone metastasis, eight express MUC1 in 61% to 100% of PC cells. Utilizing cBioPortal PC genomic data, we organized a training (n = 300), testing (n = 185), and validation (n = 194) cohort. Using the Cox model, a nine-gene signature was derived, including eight genes from a MUC1-related network (APC, CTNNB1/β-catenin, GALNT10, GRB2, LYN, SIGLEC1, SOS1, and ZAP70) and FAM84B. Genomic alterations in these genes reduce disease-free survival (DFS) in the training (P = .00161), testing (P = .00699), entire (training + testing, P = 5.557e-5), and a validation cohort (P = 3.326e-5). The signature independently predicts PC recurrence [hazard ratio (HR) = 1.731; 95% confidence interval (CI): 1.104-2.712; P = .0167] after adjusting for known clinical factors and stratifies patients with high risk of PC recurrence using the median (HR 2.072; 95% CI: 1.245-3.450, P = .0051) and quartile 3 (HR 3.707, 95% CI: 1.949-7.052, P = 6.51e-5) scores. Several novel β-catenin mutants are identified in PCs leading to a rapid onset of death and recurrence. Genomic alterations in APC and CTNNB1/β-catenin reduce DFS in two independent PC cohorts (n = 485, P = .0369; n = 84, P = .0437). The nine-gene signature also associates with reductions in overall survival (P = .0458) and DFS (P = .0163) in melanoma patients (n = 367). MUC1 upregulation is associated with CRPC and bone metastasis. A nine-gene signature derived from a MUC1 network predicts PC recurrence.

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Abbreviations: ADT, androgen deprivation therapy; BR, biochemical recurrence; RP, radical prostatectomy; WHO, the World Health Organization

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Introduction
Prostate cancer (PC) is the most common male-specific malignancy in the developed world [1]. The disease progresses with a heterogeneous path. A large proportion of the low-grade [Gleason score 6/WHO grade (group) I] tumors are indolent. Approximately 30% of patients after radical prostatectomy (RP) will experience a rise in serum prostate-specific antigen (PSA) [2]; this biochemical recurrence (BR) significantly increases risk of PC metastasis and the development of castration-resistant prostate cancer (CRPC) [3]. PC predominantly metastasizes to the bone [4]; the standard treatment for this condition is androgen deprivation therapy (ADT). The treatment is palliative, and patients eventually develop CRPC. Although the recent developments have resulted in three sets of mRNA-based signatures, Oncotype DX (Genomic Prostate Score/GPS), Prolaris, and Decipher (Genomic Classifier), that evaluate the risk of PC progression [5–7], our ability to stratify PCs with high risk of progression remains poor. It is therefore critical to understand our understanding of factors contributing to PC recurrence, metastasis, and CRPC.

Mucin 1 (MUC1) is the most thoroughly studied tumor-associated antigen [8–10]. This is a cell membrane glycoprotein expressed on the apical surface of most epithelial tissues, including the pancreas, breast, lung, and gastrointestinal tract [11,12]. The expression plays a protective role for the mucosal epithelial surface [13]. This polarity of apical presence in epithelial cells is lost in cancer cells; MUC1 is upregulated and altered in its pattern of glycosylation in over 70% of cancers [9,11]. In PC, elevations in the MUC1 protein and aberrant MUC1 glycosylation have been observed [14–16]. These changes are associated with increases in angiogenesis [17], adverse clinical features, and higher Gleason scores [18]. MUC1 upregulation is weakly related with reductions in disease-free survival (DFS) and overall survival (OS) [18] and associates with adverse histopathology after RP [19]. A panel of three proteins (AZGP1, MUC1, and p53) predicts death in men with local PC [20]. Increases in MUC1 mRNA were observed in PC metastasis, genomic alterations in the MUC1 gene were detected in CRPC, and genomic changes in a 25-factor MUC1 network marginally correlated with PC recurrence [21]. Collectively, MUC1’s involvement in PC recurrence, metastasis, and CRPC development shows great potential that warrants further investigations.

In this study, we examined MUC1 expression in CRPC produced by xenografts and prostate-specific PTEN−/− transgenic mice, and in patients with hormone-naive PC metastasized to bone. We also determined the association of a MUC1 network with PC recurrence using the PC data sets within the cBioPortal database. We report here 1) a relationship of MUC1 upregulation with CRPC and PC bone metastasis and 2) a nine-gene signature that is strongly associated with PC recurrence.

Materials and Methods
Collecting PC Bone Metastasis
Bone tissues containing metastatic PC were obtained from Hamilton Health Sciences, Hamilton, Ontario, Canada, under approval from the local Research Ethics Board (REB #11-3472).

Generation of CRPC Using Animal Models
LNCaP cells (5 × 10⁵) were used to produce subcutaneous xenograft tumors in 8-week-old male NOD/SCID mice (The Jackson Laboratory); tumor volume was monitored according to our published systems [21]. Tumor growth was measured by serum PSA levels (PSA kit, Abcam). Mice were surgically castrated when tumor reached 100 to 200 mm³. Serum PSA was determined before and following castration. Rise in serum PSA indicates CRPC growth. Animals were sacrificed once tumors reached a volume ≥1000 mm³.

Prostate-specific PTEN−/− mice were produced using PTENloxp/−; (C;129S4-Pten−/−J; the Jackson Laboratory) and PB-Cre4 mice [B6.Cg-Tg(Pbn-cre)-4Prb, the NCI Mouse Repository] following our published conditions [22]. Surgical castration was performed when mice were 23 weeks old and subsequently monitored for 13 weeks. All animal protocols were approved by the McMaster University Animal Research Ethics Board.

Quantitative Real-Time PCR Analysis of MUC1 Expression
Real-time PCR using RNA samples was performed as previously described [21,22]. All samples were run in triplicate using the following primers: MUC1 (forward): 5′-TGCCGGCCGAAGAC TACG-3′, MUC1 (reverse): 5′-TGGGTTACTGCTGTCATAG GAT-3′, β-actin (forward): 5′-ACCGAGCCGGCTACAG-3′, β-actin (reverse): 5′-CTTAATGTCAACGACGATTTCC-3′.

Immunohistochemistry (IHC)
Slide preparation, processing, and antigen retrieval were carried out according to our established protocols [21,22]. Slides were blocked in PBS, 1% BSA, and 10% normal goat serum (Vector Laboratories) for 1 hour. MUC1-N (N-terminus) (1:100, BD), MUC1-C (1:50, Fisher Scientific), and prostate acid phosphatase (PAP) (1:300, Abcam) antibodies were added at 4°C overnight. Biotinylated goat anti-mouse IgG or anti-hamster IgG secondary antibodies, and Vector ABC reagent (Vector Laboratories) were incubated according to the manufacturer’s instructions. Secondary antibody only was used as negative control. Images were acquired and analyzed using ImageScope software (Leica Microsystems Inc.). Quantification of MUC1-positive PC cells was performed by counting up to 10,000 cells in several regions for MUC1-positive and PAP-positive cells. Percentage of MUC1-positive cells was estimated as MU1 positivity/ PAP positivity × 100.

Establishing of a Nine-Gene Genomic Signature from the MUC1 Network
The largest TCGA data set (n = 499), which includes 485 patients with follow-up data, within the cBioPortal database [23,24] (http://www.cbioportal.org/index.do) was extracted and randomly divided into 10 sets of training (n = 300) and testing (n = 185) cohorts using the RandomizationR package in R. The 25 genes of the MUC1 network [21] plus FAM84B [22] (Supplementary Table 1) were inputted into the Cox model to select for their contributions to hazard ratio (HR) by either forward addition or backward elimination of covariates using SPSS Statistics version 23. The resultant 9 genes were then examined on the 10 testing cohorts and validated on an independent cohort (MSKCC, eBioPortal, n = 194) for effects on DFS, OS, and HR using the Survival package in R.

Statistical analysis
Statistical analysis was performed using Student’s t test. Kaplan-Meier survival curves, log-rank test, receiver-operating characteristic (ROC) curve, and univariate and multivariate Cox proportional hazards regression analyses (Survival package in R and SPSS Statistics version 23). A value of P < .05 is considered statistically significant.
Results

Upregulation of MUC1 in CRPC

CRPC is the leading cause of PC fatalities; specific genomic alteration in the MUC1 gene was observed in CRPC [21]. To examine MUC1 expression in CRPC, we implanted LNCaP cells, a well-established androgen-dependent PC cell line, into NOD/SCID mice. Surgical castration initially reduced tumor growth, followed by a subsequent tumor regrowth (Figure 1A). The regrown tumors (CRPCs) exhibited a significant increase in MUC1 mRNA compared to the xenografts developed in intact mice (Figure 1B). Elevations in MUC1 protein in CRPC were also demonstrated; cell surface expression and a special clustering pattern for MUC1 were observed (Figure 1C), which is consistent with MUC1 being a cell surface protein [11,12] and its detection in prostate cancer stem-like cells [21].

Furthermore, we have generated prostate-specific $PTEN^{-/-}$ mice (Supplementary Figure 1A), castrated the animals at 23 weeks, and euthanized the mice 13 weeks later. CRPCs were clearly developed (Supplementary Figure 1B). Compared to tumors in intact mice, tumors in castrated mice express an increased level of MUC1 (Figure 2). Intriguingly, the MUC1 protein shows a preference of expression in the luminal layer epithelial cells in mouse PINs.

Figure 1. MUC1 upregulation in animal models of CRPC. (A) PSA levels in NOD/SCID mice bearing LNCaP cell-derived xenograft tumors prior to and after castration. (B) Real-time PCR analysis of MUC1 mRNA in hormone-naive ($n = 3$) and castration-resistant ($n = 3$) LNCaP xenograft tumors. Statistical analysis was performed using Student’s $t$ test (two-tailed). (C) IHC staining of MUC1 in LNCaP xenograft tumors produced in intact ($n = 3$) and castrated mice ($n = 3$). Typical images from three different regions of individual tumors are shown. The indicated regions were enlarged three-fold.
produced in castrated PTEN−/− mice (Figure 2), and this preference is lost in carcinoma (Figure 2), resembling the MUC1’s expression pattern in nontumor [13] and tumor tissues [9,11]. Collectively, we provide direct evidence for MUC1 upregulation in CRPC.

**Derivation of a Nine-Gene Signature from a MUC1 Network with a Robust Predicting Value of PC Recurrence**

BR is associated with significant increases in risk for PC metastasis and CRPC [3]. Despite extensive research effort searching for...
BR-associated biomarkers, effective biomarkers are not available. Our observed MUC1 upregulation in CRPC and bone metastasis suggests a relationship between MUC1 and BR. Indeed, a weak association of MUC1 and PC recurrence was recently reported [18]; MUC1 associates with adverse pathology after RP [19]; and MUC1 along with AZGP1 and p53 predicts death in patients with local prostate tumor [20]. We reasoned that our recently identified MUC1 network consisting of 25 genes [21] would enhance the effectiveness of BR prediction.

To address this possibility, we extracted a population (n = 485) with genomic alterations and pathological data from the TCGA data set (n = 499) within the cBioPortal database. Ten random sets of training (n = 300) and testing (n = 185) cohorts were generated (Table 2). FAM84B is a novel factor associated with PC progression [22]. A systematic variable selection from the 25 genes of the MUC1 network (Supplementary Figure 3) and FAM84B for their specific genomic alterations (Supplementary Table 1) using the Cox model yielded 16 candidate genes (Table 3). The top eight genes appeared in at least two training cohorts (Table 3); LYN and GALNT15 have P values approaching the significant level (P < .05) and HR > 1 (Table 3); the top eight genes (GALNT10, SOS1, ZAP70, FAM84B, GRB2, SIGLEC1, CTNNB1, and APC) plus LYN and GALNT15 (Table 3) were thus further considered. The 95% confidence interval (CI) for GALNT15 was large (Table 3); its removal from this 10-gene list improved HR and P values in 6 training cohorts (Supplementary Table 2). We thus defined the nine-gene signature for their specific genomic alterations (Table 3).

Reanalysis of the 9-gene signature revealed a robust association with reductions of DFS in all 10 training cohorts (smallest P = 6.11e-7) and 8 of 10 testing cohorts (smallest P = 5.75e-5) (Figure 4, data not shown). Of note, the 9-gene signature was a risk factor for BR in all training and 8 of 10 testing populations based on HR (Supplementary Table 3). The signature was not associated with reductions in DFS (data not shown), and neither was it a risk factor in the same two testing cohorts (Supplementary Table 3), which likely resulted from patient randomization. It is thus important to use multiple sets of randomized training and testing cohorts. We further examined this nine-gene signature in the entire TCGA cohort (n =

![Figure 3. Extensive expression of MUC1 in PC-derived bone metastases from hormone-naïve patients. Bone tissues with hormone-naïve PC were obtained from patients #3 and #4 and were IHC stained for PAP and MUC1 using an antibody to a MUC1 N-terminal region. Matched images of a low magnification for PAP and MUC1 staining are shown (top panels). Regions marked with the same number in the PAP and MUC1 image of individual patients are matched. Please note that the #2 region in the PAP image of patient 4 was dislocated during IHC staining. Matched images of PAP and MUC1 staining for patient #3 in a higher magnification are also included (bottom panel).](image-url)

| Table 2. Demographics of Patient Populations |
|---------------------------------------------|
| Characteristics                      | Training Set* (n = 300) | Testing Set* (n = 185) |
|-------------------------------------------|-------------------------|------------------------|
| Age (years)                               | Mean ± median ± SD      | Mean ± median ± SD     |
| Q1(SD)-Q3(SD)                             | 61.2 ± 0.4               | 61.5 ± 0.67            |
| Follow-up (months)                        | 56.1 (0.32)-65.9 (0.3)   | 56.3 (0.64)-66 (0.63)  |
| Mean median ± SD                          | 27 ± 1.33                | 27.2 ± 2.07            |
| Q1(SD)-Q3(SD)                             | 14.7 (0.71)-45.1 (1.2)   | 14 (1.5)-44.5 (2.1)    |
| Recurred                                  | No (mean ± SD, %)        | 245.5 ± 4.8, 82%       |
| Yes (mean ± SD, %)                        | 53.5 ± 4.8, 18%          | 149.5 ± 4.8, 81%       |
| Tumor stages                              |                         |                       |
| T2a (mean ± SD, %)                        | 7.7 ± 2.2, 26%           | 5.3 ± 2.2, 29%         |
| T2b (mean ± SD, %)                        | 5.2 ± 1.2, 17%           | 1.8 ± 1.2, 1%          |
| T2c (mean ± SD, %)                        | 10.2 ± 4.2, 34%          | 61.9 ± 4.2, 35.5%      |
| T3a (mean ± SD, %)                        | 94.7 ± 2.8, 31.6%        | 60.4 ± 2.8, 32.6%      |
| T3b (mean ± SD, %)                        | 81.6 ± 5.4, 27.2%        | 48.4 ± 5.4, 26.2%      |
| T4 (mean ± SD, %)                         | 5.7 ± 1.3, 19%           | 4.3 ± 1.3, 23%         |
| Surgical margin                           |                         |                       |
| R0 (mean ± SD, %)                         | 188 ± 4.5, 62.7%         | 121 ± 4.5, 65.4%       |
| R1 (mean ± SD, %)                         | 90.8 ± 4.2, 30.3%        | 51.2 ± 4.2, 27.7%      |
| R2 (mean ± SD, %)                         | 2.5 ± 0.7, 0.8%          | 2.2 ± 0.8, 1.2%        |
| Rx (mean ± SD, %)                         | 9.8 ± 2.6, 3.3%          | 5.2 ± 2.6, 2.8%        |

* Ten random pairs of training and testing sets were generated; all numbers here are the respective means ± SD.
Figure 4. The nine-gene signature associates with reductions in DFS in training and testing cohorts. See Table 3 for the identities of nine genes and the types of genomic alterations being tested in the signature. The signature was evaluated in 10 individual sets of training and testing populations; typical results in 2 cohort sets are shown. Kaplan-Meier and log-rank tests were performed using the R Survival Package. Recurr, recurrence; T, total. For training cohort, n = 300; for testing cohort, n = 185.
S33F, and T41A in PC [28,30]. Nonetheless, we describe for the first time a set of missense mutations at D32, S33, S37, T41, and S45 in PC (Table 4) and novel missense mutations detected outside of the destruction box [28], N387K, W383G, and R225H. These novel mutations are observed in tumors causing either PC death or recurrence (Table 4). While whether all mutations described here promote PC progression remains unknown, their mutual exclusivity with genomic alterations of the APC tumor suppressor (Figure 5), a well-demonstrated theme in colon cancer [28], suggests their contributions together with APC in PC recurrence. Indeed, we provide the first evidence that genomic alterations in APC and β-catenin significantly shorten DFS in the TCGA (n = 492, P = .0369) and MSKCC (n = 103, P = .0437) cohorts (cBioPortal) (Supplementary Figure 6).

**Figure 5.** The nine-gene signature robustly correlates with decreases in DFS and OS in PC. (A) The indicated types of genomic alterations for the nine genes in the TCGA data set (n = 492) within the cBioPortal database [23,24] are shown; only the proportion of cohorts containing the nine-gene signature are included. Each column is for individual tumor. Note: This cohort was used to generate the training and testing subcohorts. DF, disease free; NA, not available. (B) Analysis of DFS using the TCGA cohort. Total#, total number of cases; relap#, number of relapsed cases; MMDFS, median months disease-free survival. (C, D) Genomic alterations for the nine genes in a subcohort (n = 194) within a MSKCC data set (cBioPortal) [25] (C) and the effects of the nine-gene signature on DFS in this cohort (D). (E) Analysis of patients with the nine-gene signature–positive or –negative tumors for their overall survival using the TCGA data set. Dec#, number of deceased cases; MMS, median months survival.

The Nine-Gene Signature Stratifies Patients with High Risk of PC Recurrence and Is an Independent Risk Factor for PC Recurrence

To examine whether the nine-gene signature stratify patients with elevated risk in PC recurrence, we scored each patients based on their nine-gene signature using $\sum (f_{i})_{n} (f_{i}: \text{Cox coefficient of gene}_{i}, n = 9)$ (Supplementary Table 4). Scores derived from the signature have an AUC value of 0.64 (P = .002) in predicting PC recurrence (Supplementary Figure 7A). The median (±0.657) and Q3
Scores ≥ 1.089 classify patients into high- and low-risk group of PC recurrence (Supplementary Figure 7, B and C). While the 9-gene signature has DFS = 73.4 months (95% CI: 37.1-109.5, \( P = 5.557 \times 10^{-5} \)), the median and Q3 scores (Supplementary Table 4) show respective DFS = 63.2 months (95% CI: 23.2-103.2, \( P = 8.91 \times 10^{-5} \)) and 29.4 months (95% CI: 3.5-55.2, \( P = 1.23 \times 10^{-5} \)).

We further demonstrated the 9-gene signature being an independent risk factor for PC recurrence (HR = 1.731, 95% CI: 1.104-2.712, \( P = .0167 \)) after adjusting for TMN tumor stage, age at diagnosis, radical prostatectomy (total) GS, and surgical margin (Table 5). Instead of total GS, we also analyzed the World Health Organization (WHO) PC grading system [WHO grade (group) I-V, see Supplementary Table 4 for details]; the signature continues to predict PC recurrence (HR = 1.769, 95% CI: 1.130-2.768, \( P = .0126 \)) after adjusting for the WHO grades, TMN tumor stage, age, and surgical margin. Of note, the high-risk group of patients stratified by the median and Q3 scores is associated with an elevated and independent HR = 2.07 (95% CI: 1.245-3.450, \( P = .0051 \))/total GS or HR = 2.054 (95% CI: 1.234-3.417, \( P = .0056 \))/WHO grades and HR = 3.707 (95% CI: 1.949-7.052, \( P = 6.51 \times 10^{-5} \))/total GS or HR = 3.832 (95% CI: 2.015-7.286, \( P = 4.18 \times 10^{-5} \))/WHO grades, respectively. Additionally, by performing similar analysis on the nine genes individually, we observed a general worsening in individual HR and \( P \) values in multivariate Cox analysis compared to univariate analysis only in the eight MUC1 network genes (Supplementary Table 5), which validates the eight genes belonging to the MUC1 network.

In view of the recent demonstration with respect to increased prostate cancer risk in men with germline mutations in BRCA1 and BRCA2 [31], we reasoned whether there is a relationship between the nine-gene signature and these mutations. Somatic mutations in BRCA1 (1 in 492) and BRCA2 (7 in 492) are not a frequent event in the TCGA cohort (Supplementary Figure 8). Of these mutations, the BRCA1 and three BRCA2 mutations co-occur with the nine-gene signature (Supplementary Figure 8). Importantly, addition of BRCA1 and BRCA2 mutations (positive cases 102/recurred cases 32, median DFS months 73.36; negative cases 383/recurred cases 57, median DFS months not reached; \( P = 1.099 \times 10^{-4} \)) does not enhance the 9-gene's predictive potency (positive cases 96/recurred cases 31, median DFS months 73.36; negative cases 389/recurred cases 58, median DFS months not reached; \( P = 5.575 \times 10^{-5} \)). Collectively, these observations provide indirect support for the importance of the intercomponent connections in the association of the nine-gene signature with reductions in DFS.

Discussion

MUC1 expression is commonly altered in multiple tumor types [9,11,12] and promotes tumor progression through activation of the EGFR, β-catenin, NF-kB, PKM2, and other pathways [9,13,32]. MUC1 thus has applications in diagnosis and therapy. However, both applications need further investigations.

We provide the first evidence for elevations of the MUC1 proteins in CRPC (Figures 1 and 2). Of note, a recent phase II clinical trial reported that a MUC1-based dendritic cells (DC) vaccination delayed...
Table 4. β-Catenin Mutations in PC and Their Impact on PC Death and Recurrence.

| Patient Cohort          | n   | Mutation (n); Patient Survival or Recurrence              |
|-------------------------|-----|------------------------------------------------------------|
| Michigan, Nature 2012  | 59  | T41A (1); Deceased (OS 15 months)                           |
|                         |     | D32Y (1); Deceased (OS 69 months)                          |
| Robinson et al., Cell 2015 | 150 | T41A (2); NA                                                |
|                         |     | S37C (1); NA                                               |
|                         |     | S45C (1); NA                                               |
|                         |     | S45P (1); NA                                               |
| FH, Nat Med 2016        | 56  | T41A (1); NA                                               |
|                         |     | D32Y (1); NA                                               |
|                         |     | M14 V537Y (1); NA                                         |
|                         |     | S45P (1); NA                                               |
|                         |     | M76G (1); NA                                               |
| Trento/Cornell/Broad 2016 | 81  | T41A (1); NA                                               |
|                         |     | D32N (1); NA                                               |
|                         |     | S33A (1); NA                                               |
|                         |     | A522T, S45P; Recurred                                    |
|                         |     | W383G (1); NA                                             |
| TCGA provisional        | 489 | T41A (1); HC-7738; Recurred, DF: 13.8 months               |
|                         |     | T41A (1); EJ-A65D; DF, censored: 12.9 months              |
|                         |     | D32Y (1), HC-8262; DF, censored: 22.3 months              |
|                         |     | D32V (1), EJ-5494; DF, censored: 48.5 months             |
|                         |     | D32H (1), EJ-5525; Recurred, DF: 17.9 months             |
|                         |     | S33A (1); DF, censored: 41 months                         |
|                         |     | S33V (1); DF, censored: 21.8 months                       |
|                         |     | S33C (1); Recurred, DF: 18.4 months                       |
|                         |     | S45P (1); DF                                               |
|                         |     | R225H (1); Recurred                                       |
|                         |     | R342K (1); DF, censored: 48.5 months                      |
|                         |     | T75I (1); DF                                              |

All cohorts were extracted from cBioPortal. Frequently mutated residues are bolded.

n, number of patients; Mutation (n), number of cases with the indicated mutations.

Patient ID is included only for patients with PC containing either T41A or missense mutations in D32 within the TCGA dataset.

Table 5. Univariate and Multivariate Cox Analysis of the Nine-Gene Signature for PC Recurrence

| Clinical Variables | Univariate | Multivariate |
|--------------------|------------|--------------|
|                    | HR         | 95% CI       | P Value    | HR         | CI (95%)     | P Value    |
| The 9-gene sig b   | 390        | 2.381        | 1.547-3.684 | 3.47e-5*   | 1.731       | 1.104-2.712 | .0167*     |
| 0                  | 95         |              |             |            |             |            |            |
| 1                  | 2.162      | 1.418-2.396  | .00034*     | 1.271      | 0.798-1.993 | .3203      |
| Tumor stage c      | 184        | 3.93         | 2.178-7.102 | 5.57e-6*   | 1.919       | 0.994-3.683 | .0502      |
| ≤ T2               | 295        | 1.025        | 0.993-1.058 | .128       | 1.002       | 0.970-1.036 | .8879      |
| T3 and T4          | 485        | 2.209        | 1.773-2.752 | 1.57e-12*  | 1.874       | 1.430-2.360 | 1.93e-6*   |
| Age at diagnosis   | 485        | 2.162        | 1.418-2.396 | .00034*     | 1.271      | 0.798-1.993 | .3203      |
| RP GS d            | 208        | 2.381        | 1.547-3.684 | 3.47e-5*   | 1.731       | 1.104-2.712 | .0167*     |
| 0                  | 147        |              |             |            |             |            |            |
| 1                  | 2.162      | 1.418-2.396  | .00034*     | 1.271      | 0.798-1.993 | .3203      |

* Number of cases.

b The nine-gene signature: 0: signature-negative; 1: signature-positive.

c Six cases without stage information.

d Radical prostatectomy Gleason score.

e Surgical margin status: 0, R0; 1, R1 and R2; NA, case without surgical margin information and Rx (n = 30).
present to compensate for these mutations. Additionally, three new β-catenin mutations were detected on residues outside of the destruction box, N387K, W383G, and R225H, and they occurred together with PC recurrence and fatality. Although the impact of all β-catenin mutations detected here (Table 4) on PC oncogenesis needs additional studies, the observed mutual exclusiveness between APC mutations and β-catenin mutations strongly suggests their roles in PC promotion (Table 4).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neo.2017.06.006.

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Conflict of Interest Statement
All authors declare no conflict of interest.

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