Observational Study

Medicine

OPEN

Construction of DNA methylation-based nomogram for predicting biochemical-recurrence-free survival in prostate cancer

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Abstract

This study aimed to develop a DNA methylation-based nomogram for predicting biochemical recurrence in patients with prostate cancer. A DNA methylation signature was obtained via univariate, lasso, and stepwise multivariate Cox regression models. A 11-DNA methylation signature yielded a high evaluative performance for biochemical-recurrence-free survival. Cox regression analysis indicated that 11-DNA methylation signature and Gleason score served as independent risk factors. A nomogram was constructed based on the 11-DNA methylation signature and Gleason score, and C-index as well as the calibration plots demonstrated good performance and clinical application of the nomogram. A DNA methylation-associated nomogram serve as a prognosis stratification tool to predict the biochemical recurrence of prostate cancer patients after radical prostatectomy.

Abbreviations: BCR = biochemical recurrence, BRFS = biochemical recurrence-free survival, K–M = Kaplan–Meier, MAPK8 = mitogen-activated protein kinase 8, NA = not available, PCa = prostate cancer, PHF17 = plant homeodomain protein Jade-1, RP = radical prostatectomy.

Keywords: biochemical recurrence, methylation, nomogram, prostate cancer, radical prostatectomy

1. Introduction

Prostate cancer (PCa) is the most frequently diagnosed cancer and the fifth leading cause of cancer-related death worldwide.[1] Currently, the curative treatment for localized PCa include radical prostatectomy (RP) and radical radiation,[2,3] which significantly reduced the mortality. However, biochemical recurrence (BCR) occurs about 20 to 40% of PCa patients who undergo RP treatment.[4–6] BCR was considered as a decisive risk factor for PCa-specific mortality and overall mortality.[7] Without secondary treatment, a round 30% of patients with BCR would develop local recurrence or distant metastasis within 5 to 8 years, and 32 to 45% of these patients with clinical progression would suffer PCa-specific mortality within 15 years.[8] Hence, it has great clinical value to develop novel biomarkers for evaluating the BCR risk early and accurately.

DNA methylation is a typical epigenetic modification modulating gene transcription, and aberrant DNA methylation was reported to be closely associated with tumor progression.[9] Growing evidence demonstrated that DNA methylation is implicated in the initiation, development, and progression of human cancers and may serve as potential prognostic biomarkers. For instance, in prostate cancer, hypermethylation of RASSF1 in cancerous tissue and urine was reported to be a potential prognostic indicator for BCR after RP.[10] PCDH8 methylation in plasma predicted worse prognosis of prostate cancer patients with low Gleason score after surgery.[11] However, these studies focused on few specific genes were limited by small sample size and generally generated unstable predictive robustness. Recently, DNA methylation signatures were identified to predict recurrence risk based on the whole-genome methylation profiles from the TCGA database for a variety of cancers, including lung cancer,[12] thyroid papillary carcinoma,[13] and gastric cancer.[14]

In the current study, we aimed to identify the prognostic DNA methylation sites for PCa patients by analyzing the whole-genome DNA methylation profiles that retrieved from public database, and established a risk model for biochemical recurrence-free survival (BRFS) prediction by combining the prognostic DNA methylation signature and clinicopathological parameters of PCa patients.

2. Material and Methods

2.1. Data resource

We obtained the DNA methylation data and corresponding clinical data from the Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/) database by using R TCGAbiolinks package.[15] All DNA methylation data were generated from the Illumina Infinium Human Methylation 450 platform and the levels of DNA methylation were expressed as β values, and

The authors declare that they have no competing interests.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

This article does not contain any studies with human participants or animals performed by any of the authors.

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How to cite this article: Zhu J, Zhang L. Construction of DNA methylation-based nomogram for predicting biochemical-recurrence-free survival in prostate cancer. Medicine 2022;101:49(e32205).

Received: 3 October 2022 / Received in final form: 15 November 2022 / Accepted: 16 November 2022

http://dx.doi.org/10.1097/MD.0000000000032205

calculated as M/(M + U + 100). M and U represent the signal from methylated beads and unmethylated beads at the targets CpG sites, respectively. The methylomic data from patients whose recurrence survival information was complete were selected to evaluate the relevance between DNA methylation levels and the BRFS in PCa. The clinical information and methylation data of a total of 480 PCa samples were download and analyzed in this study, and the samples were randomly classified into training cohort (320 samples) and validation cohort (160 samples) randomly. Prognostic DNA methylation signature was identified based on the training cohort data, and the evaluation of the predictive ability was performed on the basis of the validation cohort data.

2.2. Preprocess of DNA methylation data

Preprocess of the DNA methylation data was essential before the statistical analyses and predictive model establishment. First of all, we count the number of the methylation sites with not available (NA) beta value, and removed the sites (>10% of the total samples). The remaining NA data was assumed with "impute.knn" function from impute package.[16] Then, the methylation β values were normalized using "betaqn" function from wateRmelon package,[17] and transformed to M value according to the formulation: M = log(β/(1-β)) All the samples were divided into recurrence and no recurrence group, and the methylation sites with significant different level between the recurrence and no recurrence group were identified based on M value by using "dmpFinder" function from minfi package.[18]

2.3. Statistical analyses

BRFS was defined as the time from the beginning of treatment to the BCR. The univariate Cox proportional hazard analysis was implemented in the training cohort to screen methylation sites that are significantly ($P < .05$) related to PCa patients' BRFS. Then, the LASSO Cox regression analysis was performed using "glmnet" R package to screen the key methylation sites affecting the BRFS of PCa(17). Subsequently, key methylation sites from LASSO analysis were further included in the multivariate Cox regression analysis. Finally, a 11-DNA methylation signature was identified to construct risk prediction model. The risk score (RS) for every patients was calculated as follows: RS=∑(βi*coefi) ("i"=the number of prognostic methylation site, "βi" represents the beta value of each methylation site, "coefi" represents the coefficient of each methyaltion site. Then, PCa patients were divided into the high-risk and low-risk groups according to the median score. The differences in BRFS between the high-risk and low-risk group were analyzed using Kaplan–Meier (K-M) method, and K-M survival curves were generated using the a public R package "survival."[19] A receiver operating characteristic (ROC) curve was used to evaluate the RS model performance with the "survivalROC" package.

2.4. Construction and validation of the nomogram

To elucidate a quantitative method to predict a patient's probability of BRFS, we constructed a nomogram based on the "rms" R package.[20] The univariate Cox proportional hazard analysis and multivariate Cox proportional hazard analysis were performed to identify the independent prognostic factors of PCa patients' BRFS, and the factors with $P \leq .05$ from multivariate Cox proportional hazard analysis were applied to develop nomogram. C-index and calibration plots were executed to weigh the predictive performance of the established nomogram.

3. Results

3.1. Clinical characteristics of the study populations

In total, 480 PCa patients with complete methylation and clinical data were included in this study. The median age at diagnosis was 61 years (range, 41–78) and the median occurrence time of BCR were 652.5 days. The 5-year BCR rate of all patients was 14.10%. The T stage of PCa patients ranged from II to IV, and 184 (38.33%) patients were in stage I, 286 (59.58%) patients were in stage III, and 10 (2.08%) patients were in stage IV. Race of the study patients included white 398 (82.92%), Asian 12 (2.50%), Black 56 (11.67%) and uncertain race 14 (2.92%), respectively. Patients were divided into 3 groups according to Gleason score, that is < 7 43 (8.96%), =7 241 (50.21%), >7 196 (40.83%). In addition, laterality of PCa patients included left/right (unilateral) group and both (bilateral) group. The both group was the most common type 418 (87.08%) (Table 1). All patients were randomly divided into the training cohort and validation cohort at a 2:1 ratio. Figure 1 showed the overall design and flowchart of the present study.

Table 1

Clinical characteristics of included patients.

	Total (n = 480)		Training dataset (n = 320)		Testing dataset (n = 160)	
Characteristics	n	%	n	%	n	%
Age						
≤55	108	22.50	75	23.44	33	20.63
>55	372	77.50	245	76.56	127	79.38
Race						
White	398	82.92	272	85.00	126	78.75
Asian	12	2.50	9	2.81	3	1.88
Black	56	11.67	31	9.69	25	15.63
Not reported	14	2.92	8	2.50	6	3.75
Laterality						
Left/right	62	12.92	41	12.81	21	13.13
Both	418	87.08	279	87.19	139	86.88
Gleason score						
<7	43	8.96	23	7.19	20	12.50
=7	241	50.21	167	52.19	74	46.25
>7	196	40.83	130	40.63	66	41.25
T stage						
T2	184	38.33	123	38.44	61	38.13
T3	286	59.58	190	59.38	96	60.00
T4	10	2.08	7	2.19	3	1.88

3.2. Identification of methylation signature associated with BRFS

A total of 262 differentially expressed methylation sites were identified between the recurrence and no recurrence group, and 241 of these DNA methylation sites identified to be related to BRFS of PCa patients ($P < .05$) according to the univariate Cox regression analysis. Subsequently, LASSO Cox regression analysis further screened 35 key methylation sites as prognostic factors for BRFS (Fig. 2A and 2B). Finally, multivariate Cox regression model was performed based on the 35 DNA methylation sites and a risk score formula of 11 methylation sites was constructed

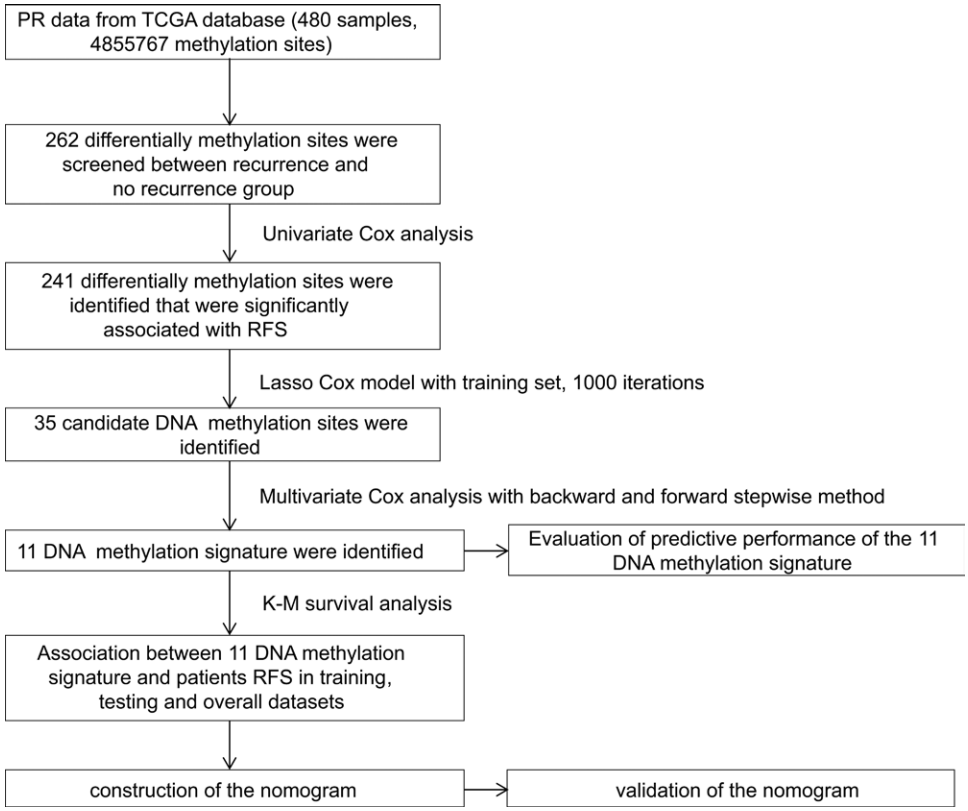

Figure 1. Flowchart of the present study.

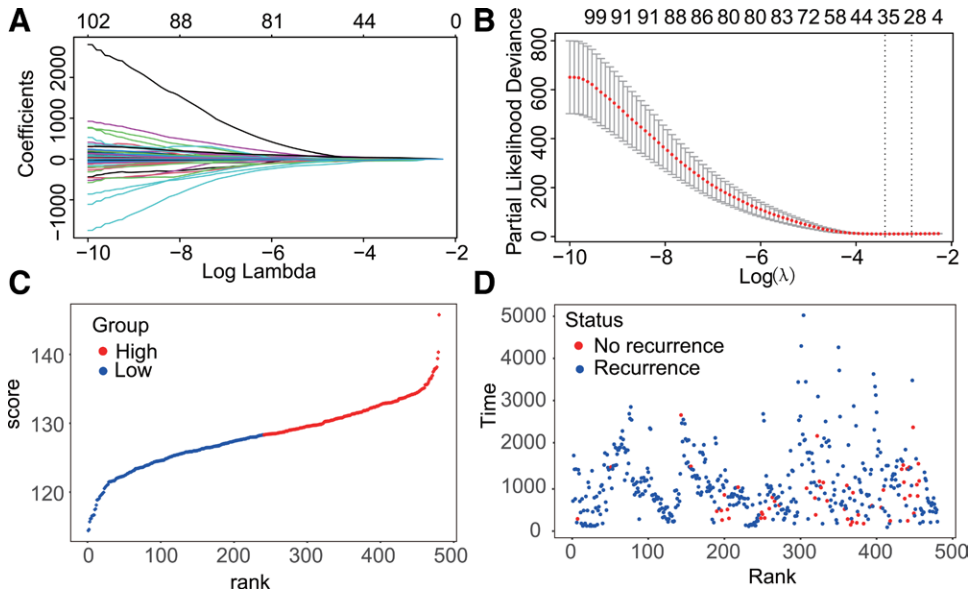

Figure 2. Candidate methylation site selection using the LASSO Cox regression model and construction of the methylation-related signature. (A) 10-fold cross-validation for tuning parameter selection in the LASSO model via minimum criteria (the 1-SE criteria). (B) LASSO coefficient profiles of the 242 methylation sites. A coefficient profile plot was produced against log(lambda) sequence. Vertical line was drawn at the value selected using 10-fold cross-validation, where optimal lambda resulted in 35 non-zero coefficients. (C) Methylation risk score distribution against the rank of risk score. Median risk score is the cutoff point. (D) Recurrence status of PCa patients against the rank of risk score.

according to their methylation level and coefficients: Risk score = 173.923632*cg01223512 + 21.340183*cg05241265 - 10.025613*cg08005809 + 21.447533*cg09129050 + 58.818317*cg16046505 - 8.338201*cg17183215 - 17.669399*cg18770149 - 32.874199*cg19527159 + 93.486761*cg24250070 + 79.609362*cg26108999 - 105.900271*ch.1.159725313. As a result, the hypermethylation levels of cg01223512, cg05241265, cg09129050, cg16046505, cg24250070, and cg26108999 were correlated with a higher risk of BCR. Nevertheless, the hypomethylation levels of cg08005809, cg17183215, cg18770149, cg19527159, and ch.1.159725313 were associated with a higher risk of BCR (Fig. 3). Furthermore, the risk scores were ranked and the patients were divided into the high-risk and low-risk groups (Fig. 2C), and the patients' survival status were also drew in Fig. 2D.

K–M analysis was performed in the training and validation cohort as well as the entire cohort to determine the BRFS of patients in the low- versus high-risk group. Intuitively, the patients in the high-risk group had worse BRFS in training cohort (P = 4.983e–10) (Fig. 4A), similar results were discovered in the validation cohort (P = 2.757e–02) (Fig. 4C) and entire cohort (P = 1.597e–10) (Fig. 4E). To evaluate the predictive ability of the risk model, a time-dependent ROC curves were drew based on risk score and the AUC values were calculated. The results showed that the risk model yielded a high predictive ability in the training, validation and entire cohort. In the training cohort, the AUCs for 1, 3, and 5 years BRFS rates in were 0.94, 0.911, and 0.877, respectively (Fig. 4B). In the validation and entire cohort, the AUCs for 1, 3, and 5 years BRFS rates were (0.828, 0.717, 0.706) (Fig. 4D) and (0.912, 0.851, 0.836) (Fig. 4F), respectively. These data indicated that the risk model had good predictive accuracy and may serve as a biomarkers to predict the BRFS of patients with PCa.

3.3. Nomogram development and assessment

Univariate Cox survival analysis demonstrated that Gleason score, t stage and 11-DNA methylation signature were prognostic factors (P < .05), and the results of multi-Cox regression analysis demonstrated that the 11-DNA methylation

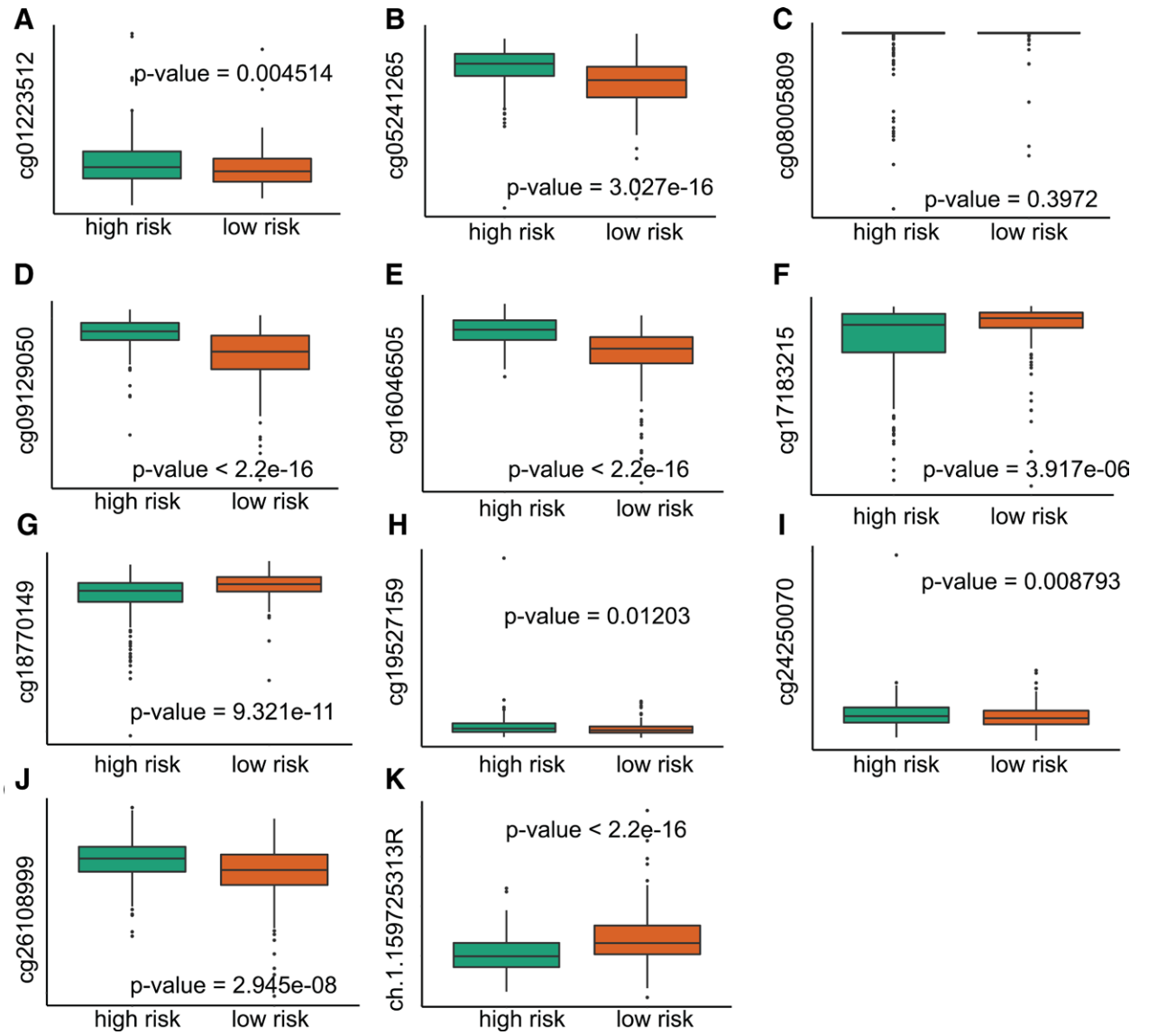

Figure 3. Boxplots of methylation β values against risk group in the entire TCGA dataset. "high risk" and "low risk" represent the high-risk and low-risk group, respectively. The median risk score was taken as a cutoff. Y-axis represents the β-value of 11-DNA methylation sites, respectively. The differences between the 2 groups were estimated by Mann–Whitney *U* test.

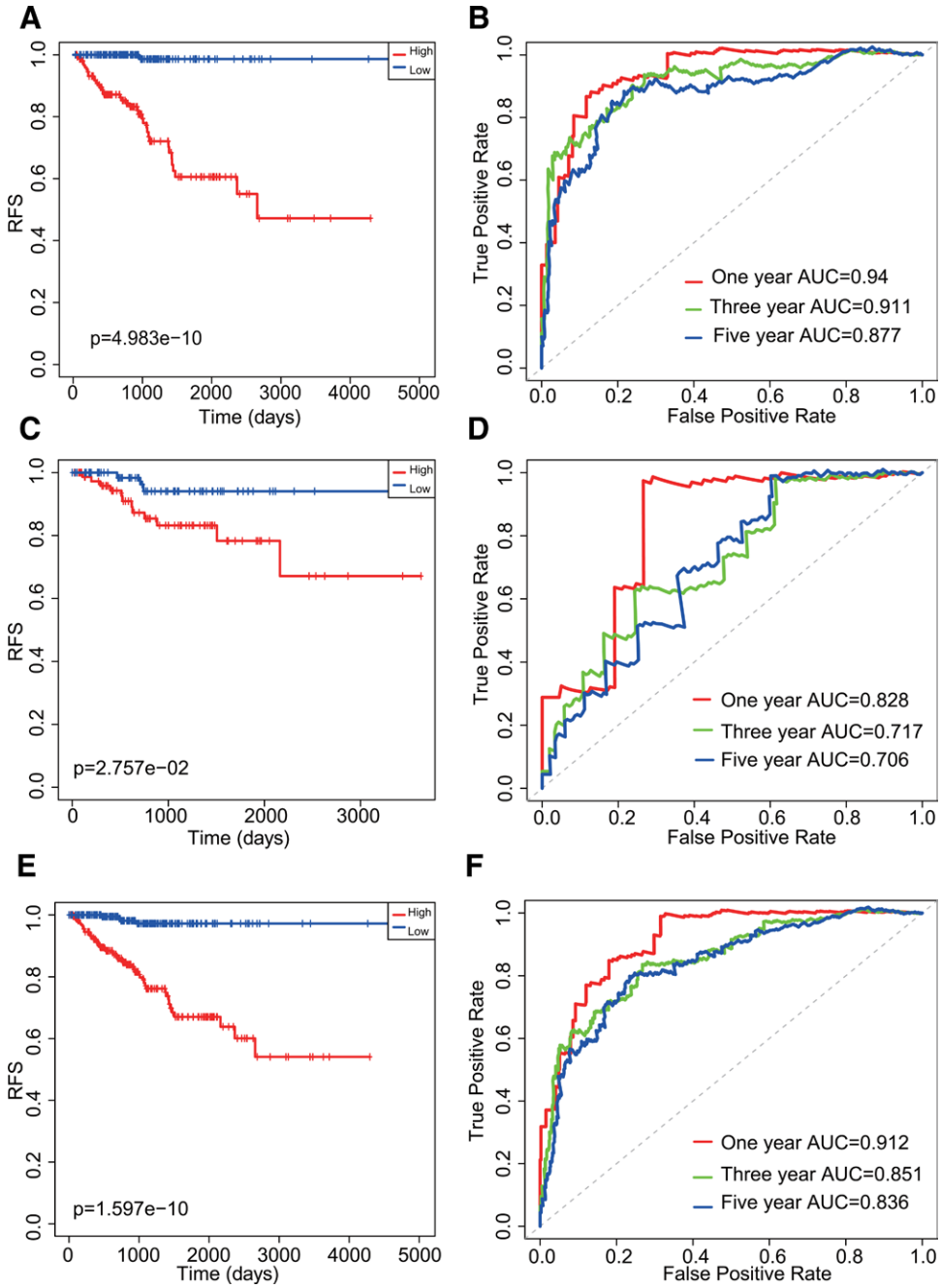

Figure 4. Kaplan–Meier and ROC analysis of patients with PCa in the training and validation cohort as well as entire cohort. (A, C and E) Kaplan–Meier analysis with 2-sided log-rank test was performed to estimate the differences in BRFS between the low-risk and high-risk group patients. (B, D and F) 1-, 3- and 5-year ROC curves of the 11-DNA methylation signature were used to demonstrate the sensitivity and specificity in predicting the BRFS of PCa patients. "High" and "Low" represent the high-risk score group and low-risk score group, respectively. The median risk score was taken as a cutoff. BRFS = biochemical recurrence-free survival, PCa = prostate cancer.

signature (P < 2e–16, HR 1.461, 95%CI: 1.342–1.591) and Gleason score (P = .0176, HR 2.068, 95%CI: 1.135–3.7680) were still significantly associated with PCa patients' BRFS (Table 2). A nomogram (Fig. 5A) was developed by integrating the risk score model and Gleason score to predict BRFS. The concordance index (C-index) of the nomogram was 0.868 (95%CI: 0.822–0.914) in the entire cohort. Simultaneously, the calibration curves also exhibited a good predictive accuracy simultaneously (Fig. 5B–D). These results revealed that the established nomogram provided a high reliability to serve as a tool for predicting the BRFS of PCa patients.

4. Discussion

BCR has been considered as a decisive risk factor for clinical recurrence and the metastasis of PCa, about 20–40% of patients with PCa yield a BCR after RP, which brings a huge challenge for public health worldwide. The accurate prediction of BCR risk will help to access the prognosis and tailor patient-specific follow-up and management. However, the accuracy of the traditional clinicopathological parameters, such as TNM staging and Gleason scores required further to be improved for predicting the prognosis of PCa. A variety of molecular markers have been developed to forecast the prognosis in various tumors, and the application of DNA methylation as a prognostic biomarker

Table 2

Univariate Cox regression analysis and multivariate Cox regression analysis outcome based on methylation risk score and other clinical factors.

	Univariate Cox analysis				Multivariate Cox analysis			
Characteristics	HR	HR.95L	HR.95H	*P* value	HR	HR.95L	HR.95H	*P* value
Age	0.612	0.299	1.255	.18				
Race	1.035	0.752	1.424	.834				
Laterality	0.864	0.369	2.02	.736				
Gleason score	3.406	1.985	5.842	**8.57e−06**	2.068	1.135	3.7680	**.0176**
T stage	3.085	1.801	5.283	**4.08e−05**	1.366	0.700	2.664	.3608
Risk score	1.484	1.368	1.609	**<2e−16**	1.461	1.342	1.591	**<2e−16**

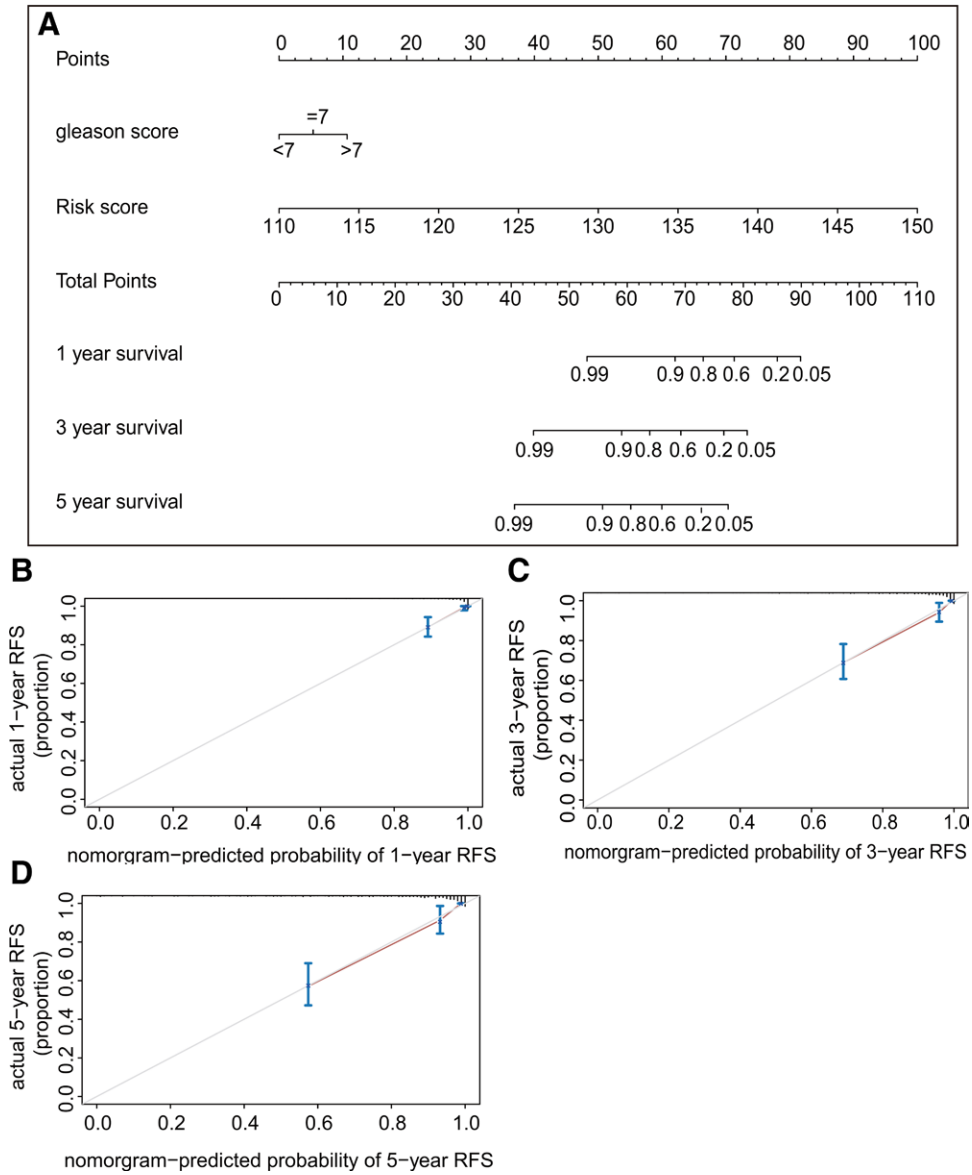

Figure 5. Methylation nomogram for the prediction of PCa patients' BRFS and validation of methylation nomogram in entire cohort. (A) The nomogram was developed in the entire TCGA cohort, with the methylation risk score and Gleason score. B, C, and D, represent the 1-, 3-, and 5-year nomogram calibration curves, respectively. The closer the dotted line fit to the ideal line, the better the predictive accuracy of the nomogram is. BRFS = biochemical recurrence-free survival, PCa = prostate cancer.

has a few merits over other molecular biomarkers, including higher stability,[21] smaller sample size requirement,[22] and relative higher accuracy.[23] Accumulating evidence has reported that DNA methylation signatures achieved satisfactory results in the prognostic prediction of multiple types of cancer. For example, a 13-DNA methylation signature was discovered to yield a high evaluative performance in the RFS prediction in stage I lung cancer.[12] Another recent study revealed that a 6-DNA methylation signature display a better value for predicting recurrence-free survival of thyroid papillary cancer.[13] In gastric cancer, Ma et al

found that DNA methylation signature performed well in prognostic prediction and established a nomogram model based on a 11-DNA methylation sites and clinicopathological indicators.[14] However, a quantitative method to predict a PCa patient's probability of BRFS based on DNA methylation signature was not developed yet.

By analyzing whole-genomic methylation profiles in 480 samples, we found that 11 DNA methylation sites was related to BRFS in patients with PCa. Higher levels of 6-DNA methylation sites (cg01223512, cg05241265, cg09129050, cg16046505, cg24250070, cg26108999) were associated with better survival, while, higher level of 5 DNA methylation sites (cg08005809, cg17183215, cg18770149, cg19527159, ch.1.159725313R) were associated with worse survival. Previous similar study reported a 10-DNA methylation signature which showed better accuracy in predicting disease-free survival of PCa, including cg02801786, cg00516513, cg21938261, cg06945936, cg08814105, cg20081453, cg22583065, cg01139508, cg25741646 and cg23258881.[24] These methylation sites were projected into 11 genes (KIF20A, SPAG5, FOXM1, CDCA5, TPX2, PLK1, PRC1, KIF4A, CDKN3, UBE2C, and MYBL2), which were demonstrated to promote the progress of PCa, except for CDCA5 and MYBL2. Obviously, there was no overlapped sites between our findings and the 10-DNA methylation sites. Although the methods used in the 2 study were basically the same, we failed to draw a accurate conclusion in term of the reason for the absence of overlapping sites due to certain differences in the data sets used. This may be due to the differences in the molecular mechanisms behind the different outcomes (disease-free survival vs BRFS). This is consistent with the high specificity of the methylation signature as prognostic hallmark. In this study, the 11-DNA methylation signature was capable to distinguish patients with low- or high-risk BCR, and it was also an independent factors for PCa patients' BRFS after adjusting by race, age, laterality, Gleason score, and T stage. Moreover, we constructed a risk model on the basis of the 11 DNA methylation sites, which yielded good accuracy in predicting the PCa patients' BRFS.

DNA methylation regulates the transcription of target genes. In the present study, the 11 DNA methylation sites were associated with ten genes, including plant homeodomain protein Jade-1 (PHF17), MAPK8, NRXN2, SMYD3, C8orf59, COQ10B, SCRIB, SKI, MTMR11, and SIL1. Some of these genes have been reported to involved in cancer progression. For instance, PHF17 is a candidate suppressor which was stabilized by pVHL in renal tumor.[25] Mitogen-activated protein kinase 8 (MAPK8), known as c-JUN N-terminal kinase (JNK), is a member of the MAPK family.[26] Numerous studies reported the various roles of MAPK8 in cancer progression, such as chemoresistance[27] and recurrence.[28] SMYD3 is an oncogenic driver and independent prognostic factors of PCa, and was found to stimulate androgen receptor transcription[29] or targets Cyclin D2 through H4K20me3[30] to provide a more aggressive phenotype of PCa. SCRIB is a lysine methylase which plays a important role in cancer progression and invasion. In PCa, the deregulation of SCRIB was revealed to be associated with poor survival. Mechanistically, Scrib can negatively regulate MAPK to suppress tumorigenesis.[31] SKI is a corepressor of Smad2/3, in Nodal and TGF-β signaling in prostate cancer cells, regulating the proliferation and migration of PCa cells.[32] SIL1 is a cochaperone of BiP, it functions as an oncogene accelerate the progression of breast cancer[33] and glioma.[34] Except for these reported genes, the remained genes may also play crucial roles in the PCa progression which required further investigation.

Apart from the inspiring results, there are also several limitations in our study. Firstly, the 11-DNA methylation signature were identified from the TCGA database, lacking of external validation cohort. This may generate hazard of selection bias. Secondly, high cost of methylation test limits its clinical application, but this is being resolved with the advancement of technology. Despite the above-mentioned limitations, our study still provided some valuable implications. Firstly, employing LASSO method to identify BRFS-related methylation sites in the study solved the multicollinearity problem and generated more reliable results. Secondly, the 11-DNA methylation signature of PCa was capable to separate PCa patients into high- and low-risk groups and predicted BRFS with robust accuracy. Thirdly, Due to the insufficient sample size, we cannot perform subgroup analysis of different races. Taking into account the natural environment, diet, medical conditions, and other reasons, different DNA methylation profiles are anticipated in different racial groups. Therefore, with the continuous update and expansion of the database, it is very necessary to conduct subgroup analysis among different races in the future. Moreover, the established nomogram by integrating clinical indicators and methylation signature provided a quantitative method for accurate BRFS prediction of PCa patients, which will contribute to development of the field of personalized medicine for PCa.

5. Conclusion

In this study, we identified a 11-DNA methylation signature that may serve as a independent prognostic biomarker for predicting the BRFS of PCa patients and constructed a risk model based on the 11-DNA methylation sites to discriminate high- and low-risk of BCR patients. A nomogram that integrated the 11-DNA methylation signature and Gleason score were also established with satisfactory performance to predict BRFS of PCa. Our results shed light on methylation biology of PCa and promote the development of effective prognostic biomarkers for PCa.

Author contributions

Jiayu Zhu designed, extracted, analyzed, and interpreted the data from TCGA databases. Jiayu Zhu, and Le Zhang wrote and made substantial contributions to the conception of the work and substantively revised it. All authors have read and approved the final manuscript.
Conceptualization: Jiayu Zhu.
Data curation: Jiayu Zhu.
Formal analysis: Jiayu Zhu.
Methodology: Jiayu Zhu.
Software: Jiayu Zhu.
Writing—original draft: Le Zhang, Jiayu Zhu.
Writing—review and editing: Le Zhang, Jiayu Zhu.

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