Original Article

Gene methylation status in focus of advanced prostate cancer diagnostics and improved individual outcomes

Weixun Zhang[1,2#], Song Zeng[1,2#], Lian Gong[1,2#], Di Zhang[1,2], Xiaopeng Hu[1,2]

[1]Department of Urology, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China; [2]Institute of Urology, Capital Medical University, Beijing, China

Contributions: (I) Conception and design: X Hu, W Zhang, S Zeng; (II) Administrative support: X Hu; (III) Provision of study materials or patients: W Zhang, L Gong; (IV) Collection and assembly of data: S Zeng, L Gong; (V) Data analysis and interpretation: W Zhang, D Zhang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Xiaopeng Hu, MD. Department of Urology, Beijing Chao-Yang Hospital, Capital Medical University, 8th Gongti South Rd., Beijing 100020, China; Institute of Urology, Capital Medical University, Beijing, China. Email: xiaopeng_hu@sina.com.

Background: Prostate cancer (PCa) is the most prevalent type of male genitourinary tumor, remains the second leading cause of deaths due to cancer in the United States in men. The aim of this study was to perform an integrative epigenetic analysis to explore the epigenetic abnormalities involved in the development and progression of PCa, and present advanced diagnostics and improved individual outcomes.

Methods: Genome-wide DNA methylation profiles obtained from The Cancer Genome Atlas (TCGA) were analyzed and a diagnostic model was constructed. For validation, we employed profiles from the Gene Expression Omnibus (GEO) and methylation data derived from clinical samples. Gene set enrichment analysis (GSEA) and the Tumor Immune Estimation Resource (TIMER) were employed for GSEA and to assess immune cell infiltration, respectively.

Results: An accurate diagnostic method for PCa was established based on the methylation level of Cyclin-D2 (*CCND2*) and glutathione S-transferase pi-1 (*GSTP1*), with an impressive area under the curve (AUC) value of 0.937. The model's reliability was further confirmed through validation using four GEO datasets GSE76938 (AUC =0.930), GSE26126 (AUC =0.906), GSE112047 (AUC =1.000), GSE84749 (AUC =0.938) and clinical samples (AUC =0.980). Notably, the TIMER analysis indicated that hypermethylation of *CCND2* and *GSTP1* was associated with reduced immune cell infiltration, higher tumor purity, and an increased risk of tumor progression.

Conclusions: In conclusion, our study provides a robust and reliable methylation-based diagnostic model for PCa. This model holds promise as an improved approach for screening and diagnosing PCa, potentially enhancing early detection and patient outcomes, as well as for an advanced clinical management for PCa in the framework of predictive, preventive and personalised medicine.

Keywords: Prostate cancer (PCa); DNA methylation; advanced diagnostics; patient stratification; improved individual outcomes

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Introduction

Prostate cancer (PCa) is the most prevalent type of male genitourinary tumor. The incidence and mortality of PCa in developing countries are steadily increasing, likely due to lifestyle changes and the widespread adoption of screening methods. Currently, PCa ranks as the second leading cause of cancer-related deaths worldwide (1,2).

Besides, the incidence of PCa showed younger trend

with an increasing rate of metastatic PCa brought heavier social and economic burden (3). In this context, predictive, prevention and personalisation (3P) medicine is required for clinical management of PCa, which brings developing methods for early sensitive and economical diagnosis of PCa into focus (4,5).

The utilization of prostate-specific antigen (PSA) testing has significantly improved early detection, offering opportunities for timely intervention and better prognosis. However, PSA has its limitations. Conditions like benign prostatic hyperplasia (BPH) and digital rectal examination (DRE), as well as certain procedures, can elevate PSA levels, leading to unnecessary prostate biopsies or invasive tests. Moreover, a negative PSA result cannot entirely exclude the presence of cancer, particularly in patients with PSA levels ranging from 4 to 10 ng/mL, where distinguishing between benign and malignant cases based solely on screening results is challenging (6). Therefore, a more accurate screening method for PCa is necessary to enhance diagnostic precision and reduce false-positive results.

Tumor epigenetic changes, such as DNA methylation and histone modifications, have been extensively studied and are known to be closely associated with tumor initiation and progression (7). Detection of DNA methylation can help in the diagnosis of precancerous lesions and cancer (8). Furthermore, advancements in gene sequencing technology have lowered the cost of genetic testing, making epigenetic tumor testing more widely accessible. In this study, we established a reliable PCa DNA methylation diagnostic model by leveraging a DNA methylation database and validated it with clinical samples. This innovative approach conforms with principles of 3P medicine, and provides new insights and potential solutions for PCa screening and diagnosis. We present this article in accordance with the TRIPOD reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-23-405/rc).

Highlight box

Key findings

- This study introduces a dual-gene methylation diagnostic model for prostate cancer (PCa), featuring *CCND2* and *GSTP1*, which exhibited excellent diagnostic accuracy.
- The model offering high sensitivity and specificity in diverse datasets and clinical samples.
- Pathway analysis revealed associations with cell cycle regulation and immune infiltration, indicating potential prognostic value.

What is known and what is new?

- DNA methylation plays a crucial role in cancer development, with specific genes displaying increased methylation in tumor tissues.
- This study introduces a novel dual-gene model, *CCND2* and *GSTP1*, to enhance PCa diagnosis.

What is the implication, and what should change now?

- The dual-gene diagnostic model offers a promising approach for non-invasive PCa screening, potentially replacing traditional PSA-based methods. Further validation with liquid biopsy data is essential for clinical applications, enabling personalized and precise management.

Methods

Patients and samples

A total of 541 Genome-Wide DNA methylation profiles were collected from The Cancer Genome Atlas (TCGA) database, consisting of 491 PCa samples and 50 adjacent non-cancerous samples (with 50 paired cancerous samples included in the 491 tumor samples). These data were used to establish the diagnostic model. Additionally, we obtained 388 DNA methylation profiles from the Gene Expression Omnibus (GEO) database (GSE76938, GSE26126, GSE112047, and GSE84749), which included 219 PCa samples and 169 nontumor samples, for the purpose of validating the model. The development data and validation data shared the same criteria in eligibility outcomes, and predictors.

Regarding the datasets involved in this study, the DNA methylation data were generated using the Illumina Infinium Human Methylation 450 platform. Quality control and standardization for methylation followed the methods of the original research. In summary, the methylation beta score was calculated as: β = IntensityMethylated/(IntensityMethylated + IntensityUnmethylated). Beta scores with an associated detection P value of >0.01 were converted to "missing values", and data were normalized using the ComBat R package. To further validate the model, we recruited 20 patients who underwent radical prostatectomy at Beijing Chao-Yang Hospital between June and October 2019. These patients had a pathological diagnosis of primary PCa and no history of radiotherapy, chemotherapy, or endocrine therapy before surgery. For each patient, we obtained both cancerous and adjacent non-cancerous tissue samples for DNA methylation analysis. The pathological results, as well as the judgment of tumors and adjacent samples, were carried out by pathological professionals.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study

was approved by the ethics committee of Beijing Chao-Yang Hospital (No. 2021-54), and written informed consents for participants and publication were provided by all participants. The downloaded data in this study fully complied with the data access policies of GEO and TCGA.

Screening for differentially expressed genes and analysis of gene expression and DNA methylation

In previous studies and systematic reviews, six genes were identified as potential methylation markers in PCa (9-12). To validate the DNA methylation status of these genes, we employed the "minfi" R package, which allowed us to analyze the DNA methylation data from both TCGA and GEO databases. Additionally, we used the "MethylMix" R package to predict the impact of DNA methylation on gene expression, thereby identifying genes that were likely driven by DNA methylation. To assess the diagnostic performance of the identified genes, we employed receiver operating characteristic (ROC) curves.

Generation and validation of the diagnostic model

The diagnostic model was constructed using the Cox proportional hazards model. To evaluate the discriminatory ability of different categories within the Cox proportional hazards regression model, we calculated the Bayesian information criterion (BIC) values and Mallows' Cp values. A lower BIC value or Mallows' Cp value indicated a more effective diagnostic model.

To validate the model's performance, we applied it to independent datasets, including GSE76938, GSE26126, GSE112047, GSE84749, and our own dataset. Risk score of each sample was calculated based on regression coefficients. We categorized samples into high- and low-risk groups based on the median risk score derived from the model. A higher model score was associated with a higher probability of cancer. Based on the scores obtained, we categorized all data into specific groups for further analysis and comparison.

DNA extraction and analysis of DNA methylation

Cancer and para-cancerous tissues for validation were obtained immediately after surgery, and isolated from paraffin section samples. DNA extraction was carried out using the GeneRead DNA FFPE kit (QIAGEN, 180134, Duesseldorf, Germany). Primers for the candidate genes were designed using the "Methylation FastTarget V4.1" and "primer3" (http://primer3.ut.ee) software. The optimized primers were combined into a multiplex polymerase chain reaction (PCR) primer panel. Subsequently, the EZ DNA Methylation-Gold Kit (Zymo Research, D5005, Orange County, California, USA) was utilized for DNA sample processing.

The target region of the transformed samples was amplified using the multiplex PCR primer panel, and the resulting PCR products from the same sample were mixed in equal proportions. Index sequences and specific tag sequences compatible with the Illumina platform were introduced via PCR. After the sample recovery process, the Agilent 2100 Bioanalyzer was employed to verify the length of sample DNA fragments. Subsequently, high-throughput sequencing was performed on the Illumina Hiseq platform using the 2×150 bp paired-end sequencing mode by a qualified genetic testing company.

Gene set enrichment analysis (GSEA) and immune infiltration

GSEA was utilized to explore potential biological processes and pathways with annotations from the msigdb.v7.0.entrez.gmt database. Pathway screening was performed by considering absolute values of negative normalized enrichment score (NES) greater than 2.0 and q-values less than or equal to 0.05.

For immune infiltration analysis, we utilized the Tumor Immune Estimation Resource (TIMER) database. Data from TCGA PCa samples were obtained, and the relationship between the expression of the target genes and the levels of tumor purity, B cells, $CD4^+$ T cells, $CD8^+$ T cells, macrophages, neutrophils, and dendritic cells was analyzed.

Statistical analysis

Statistical analysis was conducted through R (version 4.2.0). Differences in methylation levels were assessed by Wilcoxon rank sum test, $P<0.05$ was considered statistically significant.

Results

Identification and verification of the differentially expressed genes in PCa

In previous studies and systematic reviews (9-12), we

identified *APC*, Cyclin-D2 (*CCND2*), glutathione S-transferase pi-1 (*GSTP1*), *PRKY*, *RARB*, and *RASSF1* as potential methylation markers in PCa. To validate the differential methylation patterns of these six genes in PCa, we analyzed 491 PCa samples and 50 adjacent normal samples from the TCGA database (*Figure 1A*), characteristics of the datasets we used are shown in Table S1.

Comparing the methylation levels in PCa samples to adjacent normal samples, we found that each of the selected genes exhibited hypermethylation in PCa cases (*Figure 1B*). Additionally, we conducted ROC analysis, which demonstrated that each gene had a favorable diagnostic ability and could effectively distinguish tumor tissues from adjacent normal tissues. Among the six single-gene diagnostic models, *GSTP1* showed the highest diagnostic accuracy, with an area under the curve (AUC) of 0.939 (*Figure 1C*).

Dual-gene diagnostic model for PCa

To enhance the diagnostic efficacy of our model, we sought to identify a more effective diagnostic combination from the six candidate genes. By evaluating the BIC and Mallows' Cp value, we determined that the model comprising two genes exhibited the most favorable BIC and Mallows' Cp values, indicating superior diagnostic performance (*Figure 2A,2B*).

We further assessed the adjusted R^2 values (adjR2) of each gene and found that *CCND2* and *GSTP1* had the two highest adjR2 values. Thus, we selected *CCND2* and *GSTP1* as the optimal combination for the PCa diagnostic model. Additionally, to ensure that there was no correlation or interaction between the two genes, we calculated the Variance Inflation Factor (VIF), which confirmed that the VIF for these two genes was 1.314, signifying no significant interaction between them (*Figure 2C*).

Remarkably, the dual-gene model (regression coefficients of *CCND2*, 0.5469989; *GSTP1*, 2.6733119) demonstrated excellent diagnostic ability, achieving an AUC of 0.937, outperforming the diagnostic ability of either the *GSTP1* or *CCND2* single-gene models (*Figure 2D-2F*).

Evaluation of diagnostic efficacy

To validate the robustness of the *CCND2* and *GSTP1* dual-gene diagnostic model, we tested its diagnostic performance on four external datasets (GSE76938, GSE26126, GSE112047, GSE84749). In all four external datasets, the methylation levels of the *CCND2* and *GSTP1* genes were significantly elevated in tumor tissues compared to normal tissues (*Figure 3*).

After calculating risk scores of each sample by our model based on *CCND2* and *GSTP1* methylation, the ROC demonstrated outstanding diagnostic performance. Specifically, the AUC values for the GSE76938, GSE26126, GSE112047, and GSE84749 datasets were 0.930, 0.906, 1.000, and 0.938, respectively. Importantly, the diagnostic performance of the dual-gene model surpassed that of any single-gene diagnostic models based on *CCND2* or *GSTP1* alone (*Figure 3*). Detailed data can be found in the online table (available at https://cdn.amegroups.cn/static/public/tau-23-405-1.pdf).

These results confirm that the *CCND2* and *GSTP1* dual-gene diagnostic model exhibits consistent and superior diagnostic ability across multiple datasets, making it a promising tool for PCa diagnosis.

CCND2 and GSTP1 may be methylation driver genes

In light of recent research, it has been observed that several genes do not show changes in gene expression despite significant alterations in their methylation levels. To investigate this further, we proceeded to examine the correlation between gene expression and methylation levels in *CCND2* and *GSTP1*. For this purpose, we utilized the TCGA PCa dataset and the GSE84749 dataset.

The scatter plot analysis demonstrated a significant negative correlation between methylation levels and gene expression for both *CCND2* and *GSTP1* in both datasets (TCGA and GEO). This finding indicates that increased methylation levels of *CCND2* and *GSTP1* are associated with down-regulation of gene expression, suggesting that these two genes may be influenced by DNA methylation (*Figure 4A,4B*).

Subsequently, we performed correlation analysis of gene methylation and expression using the Cancer Cell Line Encyclopedia (CCLE) dataset. In pan-cancer samples (n=1,457), we observed significant negative correlations between *CCND2* and *GSTP1* methylation and expression, with correlation coefficients of –0.41 and –0.83, respectively. When specifically focusing on PCa samples (n=6), the small sample size limited statistical significance, but a negative correlation trend between methylation and expression still existed (*Figure 4C,4D*).

These results provide compelling evidence that the methylation levels of *CCND2* and *GSTP1* inversely influence their gene expression, supporting the notion that

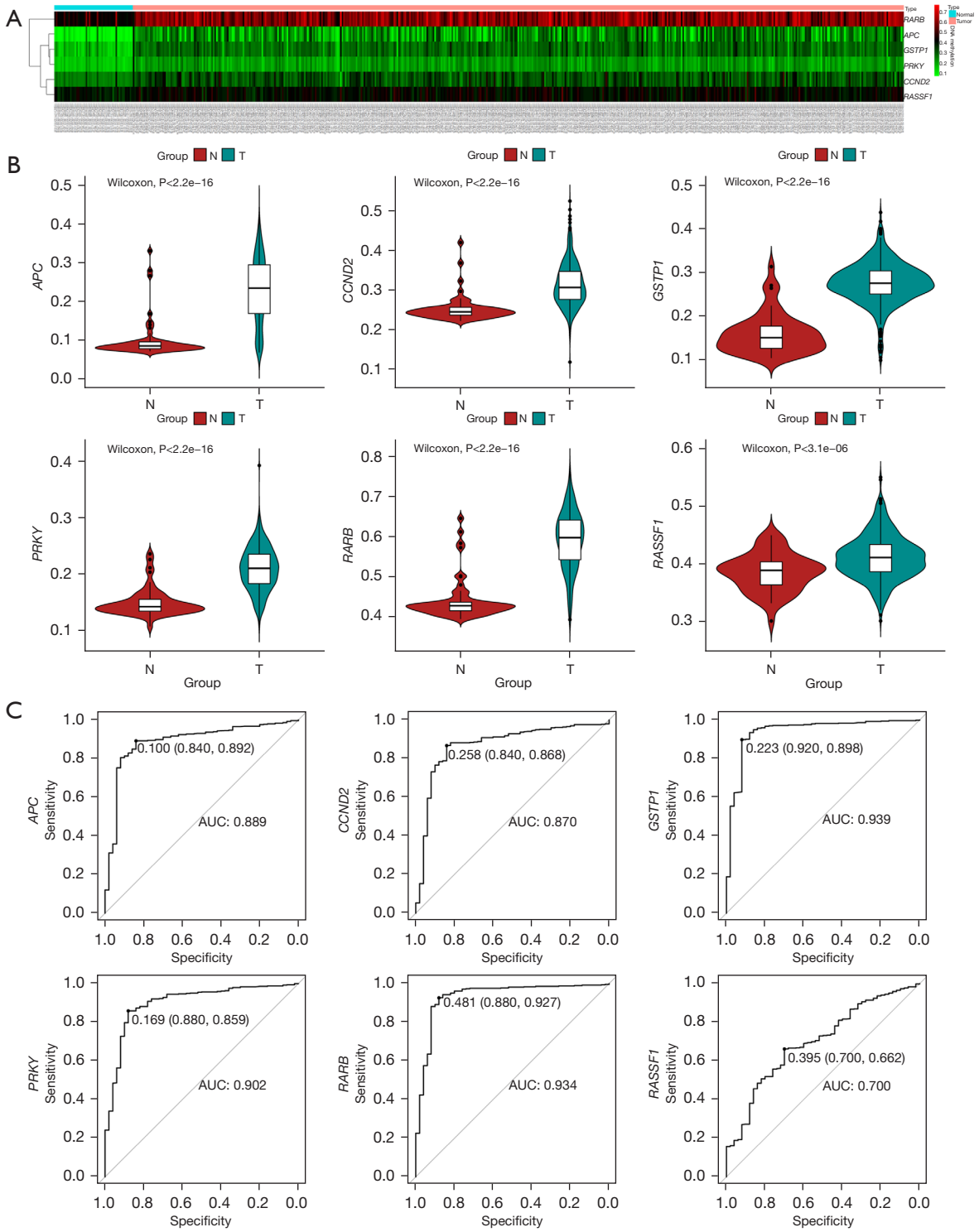


Figure 1 Identification and verification of the differentially expressed genes in prostate cancer. (A) Heat map of *RARB*, *APC*, *GSTP1*, *PRKY*, *CCND2*, and *RASSF1* genes DNA methylation in prostate cancer tissues (tumor) and paracancerous tissues (normal) in TCGA database. (B) Violin plot of methylation levels of *APC*, *CCND2*, *GSTP1*, *PRKY*, *RARB*, and *RASSF1* genes in prostate cancer tissues (T) and paracancerous tissues (N). (C) ROC of single-gene diagnostic performance. *CCND2*, Cyclin-D2; *GSTP1*, glutathione S-transferase pi-1; AUC, area under the curve; TCGA, The Cancer Genome Atlas; ROC, receiver operating characteristic.

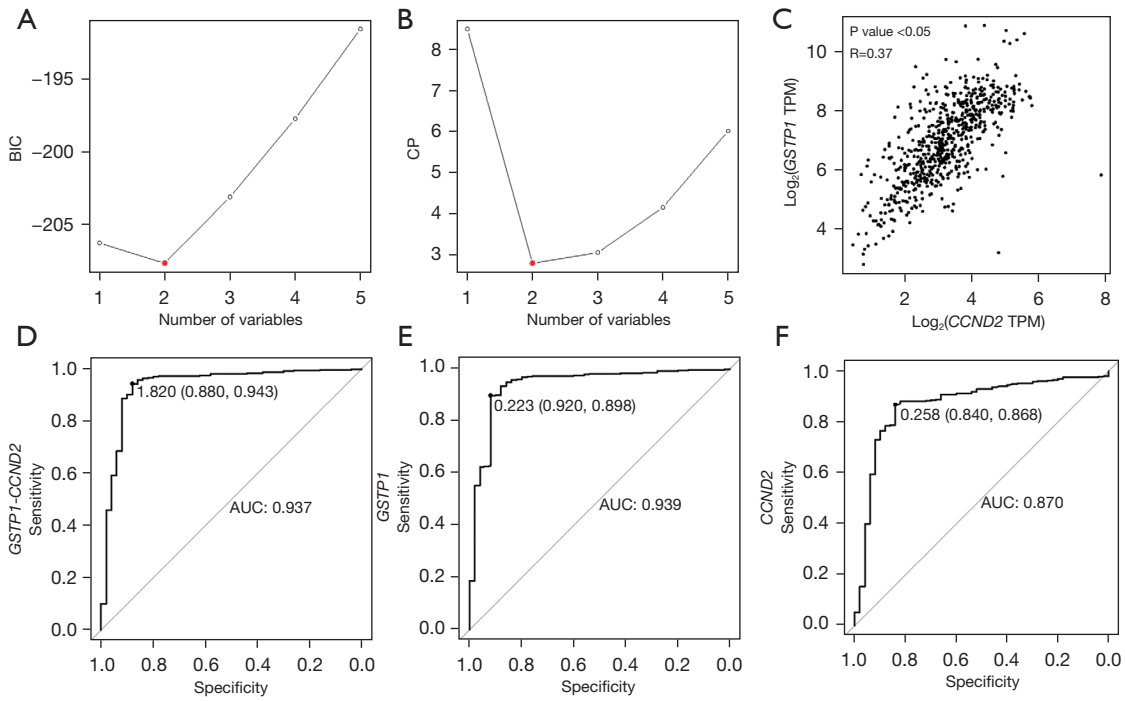


Figure 2 Dual-gene diagnostic model for prostate cancer. (A,B) BIC and Mallows' Cp value for the best diagnostic model. (C) Correlation between the expression of *CCND2* and *GSTP1* gene, VIF =1.314. (D) ROC of *GSTP1-CCND2* dual-gene diagnostic model in TCGA database. (E) ROC of *GSTP1* single-gene diagnostic model in TCGA database. (F) ROC of *CCND2* single-gene diagnostic model in TCGA database. BIC, Bayesian information criterion; TPM, transcripts per million; *CCND2*, Cyclin-D2; *GSTP1*, glutathione S-transferase pi-1; AUC, area under the curve; VIF, Variance Inflation Factor; TCGA, The Cancer Genome Atlas; ROC, receiver operating characteristic.

these genes are likely regulated by DNA methylation.

Pathway enrichment and immunoassay

The GSEA revealed that after screening, 2,977 signaling pathways or biological processes exhibited varying degrees of enrichment in high-score samples. Among these, ten pathways were related to PCa, cell cycle regulation, and immune infiltration, displaying high NES values. The cell cycle regulation pathways were positively correlated with the model score, while immune cell migration, chemotaxis, and immune regulation pathways showed a negative correlation with the model score. This suggests that higher model scores are associated with inhibited migration and chemotaxis of immune cells.

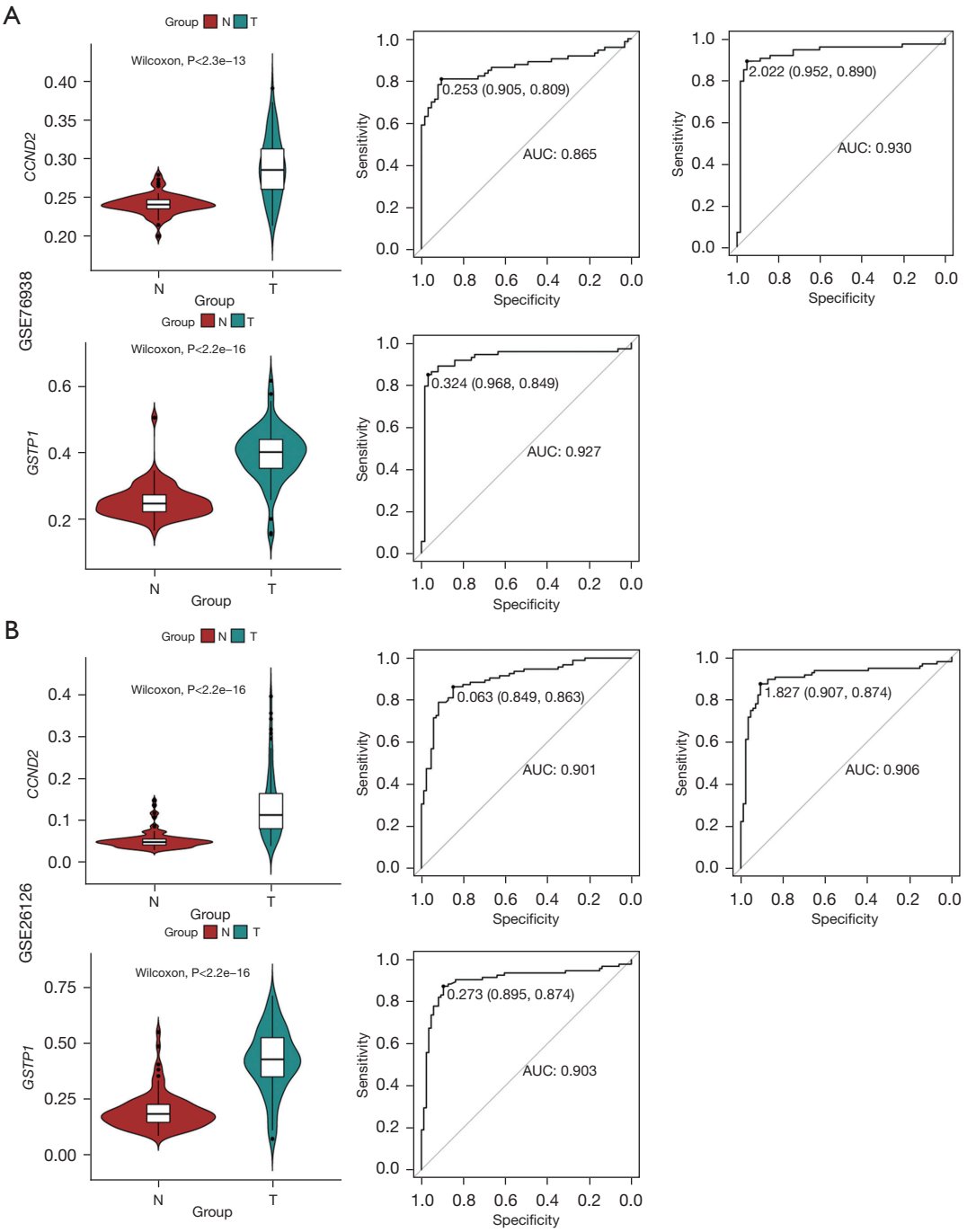
Moreover, the pathways classified by the model score displayed the same enrichment trend as PCa-related pathways in multiple gene sets, indicating the accuracy of the two-gene model for PCa diagnosis (*Figure 5*).

The TIMER analysis further revealed that in PCa samples, the expression of *CCND2* and *GSTP1* genes was negatively correlated with tumor purity, with R-values of –0.448 and –0.577, respectively. This indicates that increased expression of these two genes is linked to a significant reduction in tumor purity. Conversely, the infiltration of B cells, $CD4^+$ T cells, $CD8^+$ T cells, macrophages, neutrophils, and dendritic cells is enhanced with upregulated gene expression.

In summary, the findings suggest that the increased expression of *CCND2* and *GSTP1* leads to elevated immune cell infiltration, reduced tumor purity, and decreased tumor risk, which aligns with the outcomes predicted by our model.

Validation in clinical samples

To further validate the diagnostic performance of the dual-gene model in clinical samples, we obtained DNA from PCa and adjacent tissues of 20 PCa patients who underwent radical prostatectomy at our hospital. DNA methylation



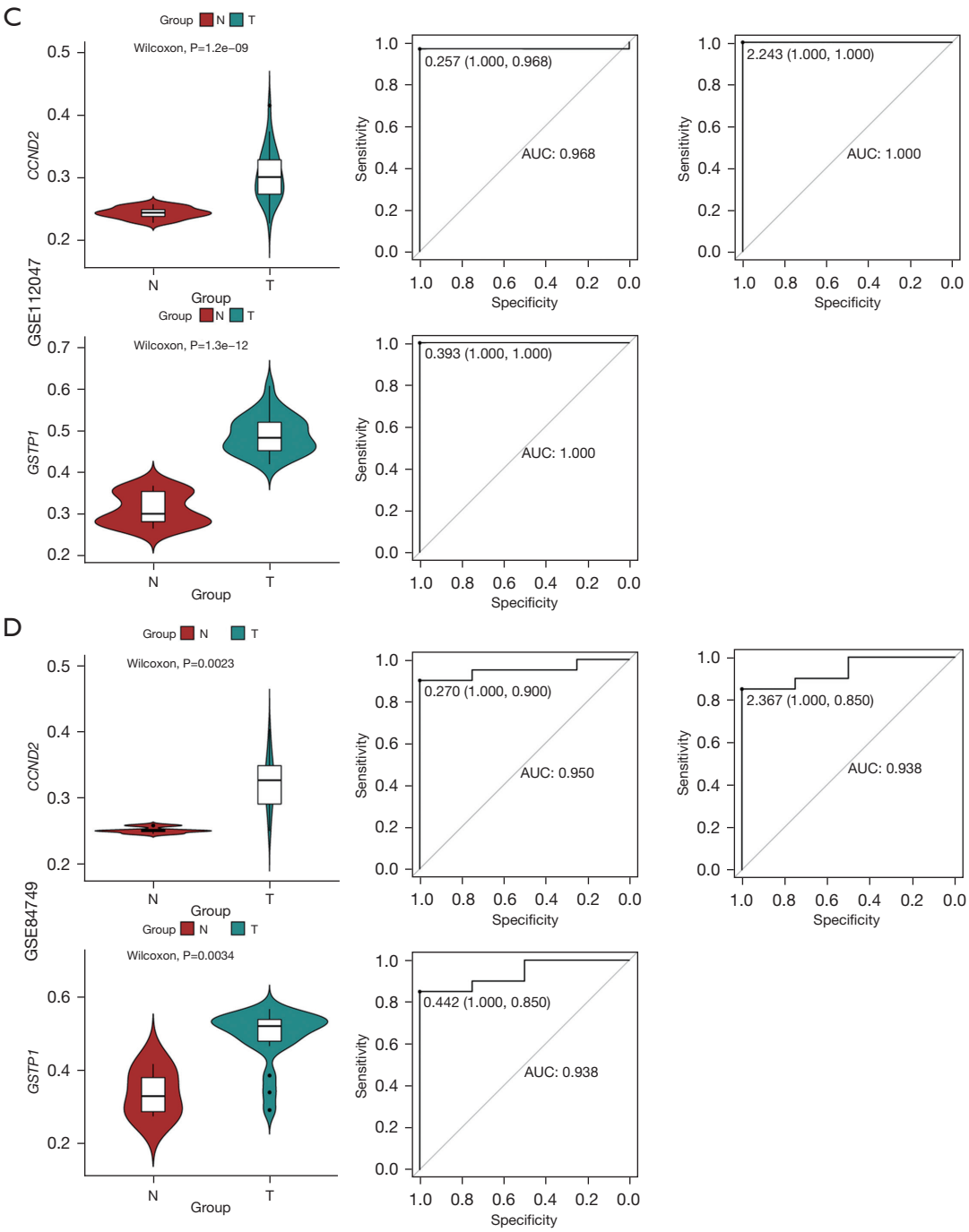


Figure 3 Evaluation of diagnostic efficacy. Methylation level and single-gene diagnostic performance of *CCND2* and *GSTP1* gene in GSE76938 (A), GSE26126 (B), GSE112047 (C), and GSE84749 (D) datasets. ROC shows the dual-gene diagnostic model in each dataset. T = prostate cancer tissues; N = paracancerous tissues. *CCND2*, Cyclin-D2; *GSTP1*, glutathione S-transferase pi-1; AUC, area under the curve; ROC, receiver operating characteristic.

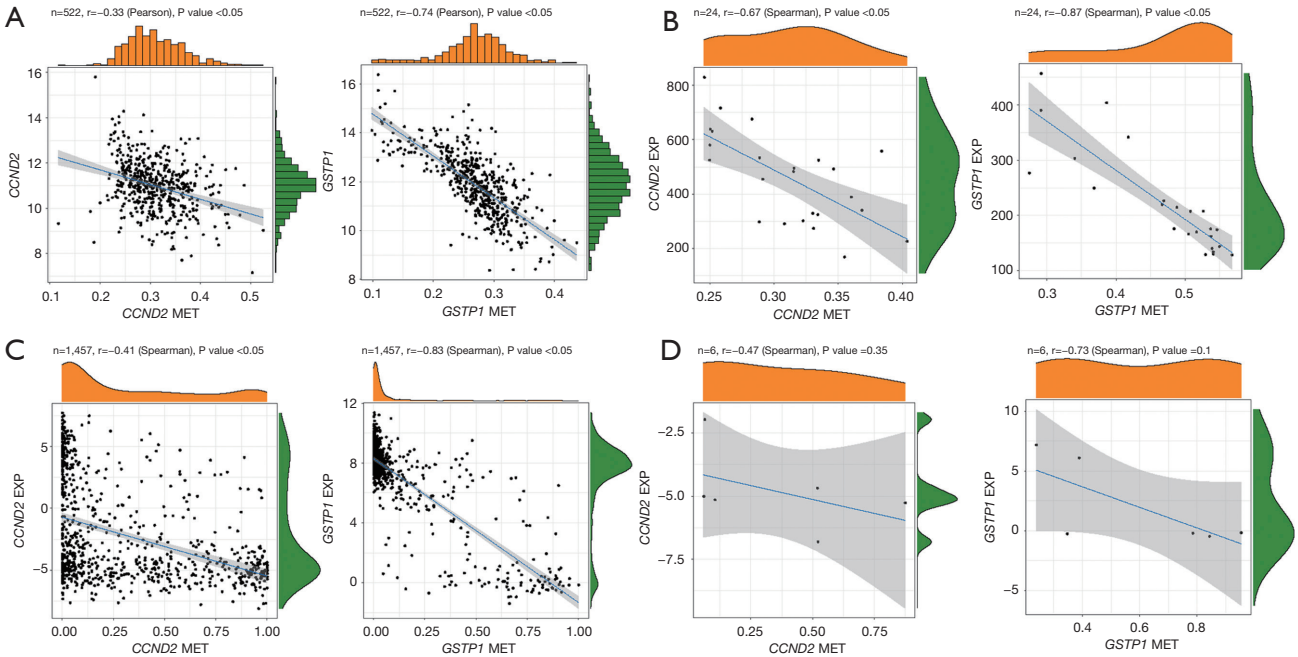


Figure 4 *CCND2* and *GSTP1* may be methylation driver genes. (A) Correlation of DNA methylation and *CCND2* and *GSTP1* gene expression in TCGA prostate cancer dataset. (B) Correlation of DNA methylation and *CCND2* and *GSTP1* gene expression in the GSE84749 dataset. (C) Correlation of DNA methylation and *CCND2* and *GSTP1* gene expression in the CCLE pan-cancer dataset. (D) Correlation of DNA methylation and *CCND2* and *GSTP1* gene expression in the CCLE prostate cancer dataset. *CCND2*, Cyclin-D2; *GSTP1*, glutathione S-transferase pi-1; EXP, expression; MET, methylation; TCGA, The Cancer Genome Atlas; CCLE, Cancer Cell Line Encyclopedia.

sequencing targeting the six candidate genes was conducted on these samples.

Among the 20 paired samples, it was found that six genes exhibited significantly elevated methylation levels in PCa tissues compared to adjacent tissues. Both *CCND2* and *GSTP1* demonstrated excellent diagnostic performance in the clinical samples. Remarkably, when these two targets were combined, the AUC reached 0.980 in the ROC analysis. This result provides compelling evidence that our model exhibits high-level diagnostic accuracy in clinical samples (*Figure 6*).

The findings from our analysis of clinical samples further support the robustness and reliability of the *CCND2* and *GSTP1* dual-gene diagnostic model for PCa diagnosis in a real-world clinical setting.

Discussion

In recent years, identifying the molecular characteristics of tumor tissue to determine tumor occurrence and prognosis has become a novel and essential research area (13). DNA methylation, an important epigenetic modification pathway, is frequently associated with the development and progression of tumors through changes in the methylation levels of proto-oncogenes and tumor suppressor genes (14). For instance, the *APC* gene exhibits high methylation levels in liver cancer, colorectal cancer, PCa, and other tumor tissues (15-17). Similarly, the *GSTP1* gene shows high methylation levels in PCa, breast cancer, lung cancer, hepatocellular carcinoma, and other tumor tissues (18). Thus, the detection of methylation levels in specific genes can play a crucial role in tumor diagnosis. Moreover, epigenetic changes such as DNA methylation are more stable in tumors, making them promising biomarkers for clinical use (19).

In our study, we screened six genes with elevated methylation levels in various tumor tissues through a systematic review of research related to DNA methylation. These six genes exhibited acceptable diagnostic performance in PCa based on methylation data. However, the diagnostic performance of individual single-gene models has not yet reached the desired level of accuracy. To enhance the

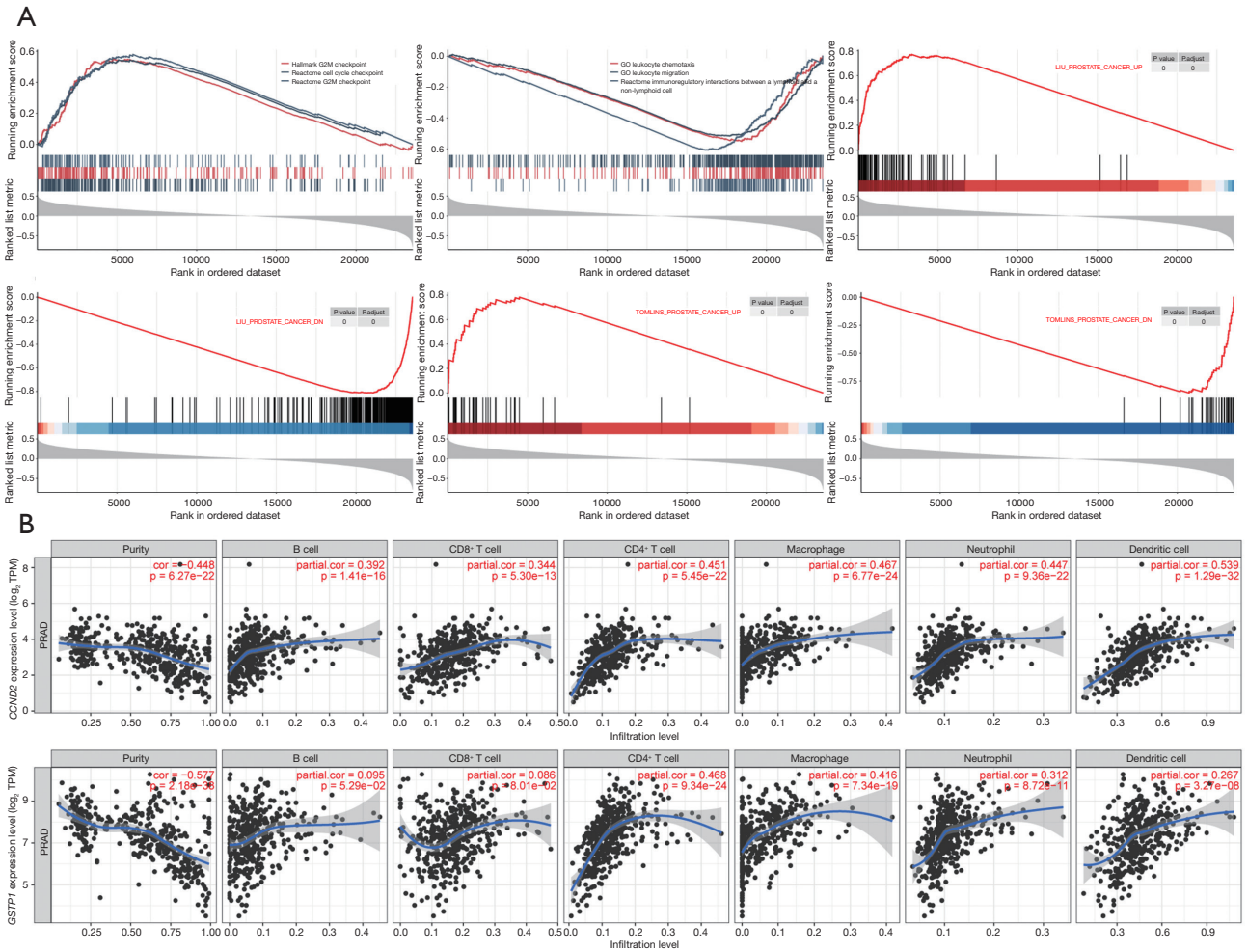


Figure 5 Pathway enrichment and immunoassay. (A) Differentially expressed pathways in Gene Set Enrichment Analysis of multiple gene sets. (B) Correlation of gene expression and immune infiltration in *CCND2* and *GSTP1* gene. *CCND2*, Cyclin-D2; *GSTP1*, glutathione S-transferase pi-1; TPM, transcripts per million; PRAD, prostate adenocarcinoma; cor, correlation.

diagnostic ability of the model, we developed a dual-gene PCa diagnostic model consisting of the *CCND2* and *GSTP1* genes from the pool of six candidate genes. This dual-gene model demonstrated excellent diagnostic performance in the TCGA dataset with an AUC of 0.937, specificity of 88%, and sensitivity of 94.3%.

GSTP1 is a 3.2 kb gene comprising nine exons. It plays a crucial role in safeguarding cells from carcinogens and cytotoxins, exhibiting diverse physiological functions, such as neutralizing potentially genotoxic complexes, metabolizing various carcinogens, and minimizing cellular DNA damage (18). In lung cancer research, *GSTP1* is responsible for eliminating carcinogens present in tobacco and is involved in the development of smoking-related lung cancer (20). In the context of epigenetic modifications, the promoter region of *GSTP1* is frequently influenced by methylation. Hypermethylation in this region can lead to the silencing of *GSTP1* expression, resulting in a compromised detoxification and antioxidant capacity. Numerous studies have established a significant association between *GSTP1* methylation and the occurrence, recurrence, and prognosis of PCa, suggesting its potential as an informative epigenetic marker (21-23).

CCND2 is a crucial cell cycle regulator gene that has been identified as a proto-oncogene. Its overexpression has been linked to the progression and unfavorable prognosis of gastric cancer, colon cancer, ovarian cancer, and other tumors (24-26). Interestingly, elevated methylation in the

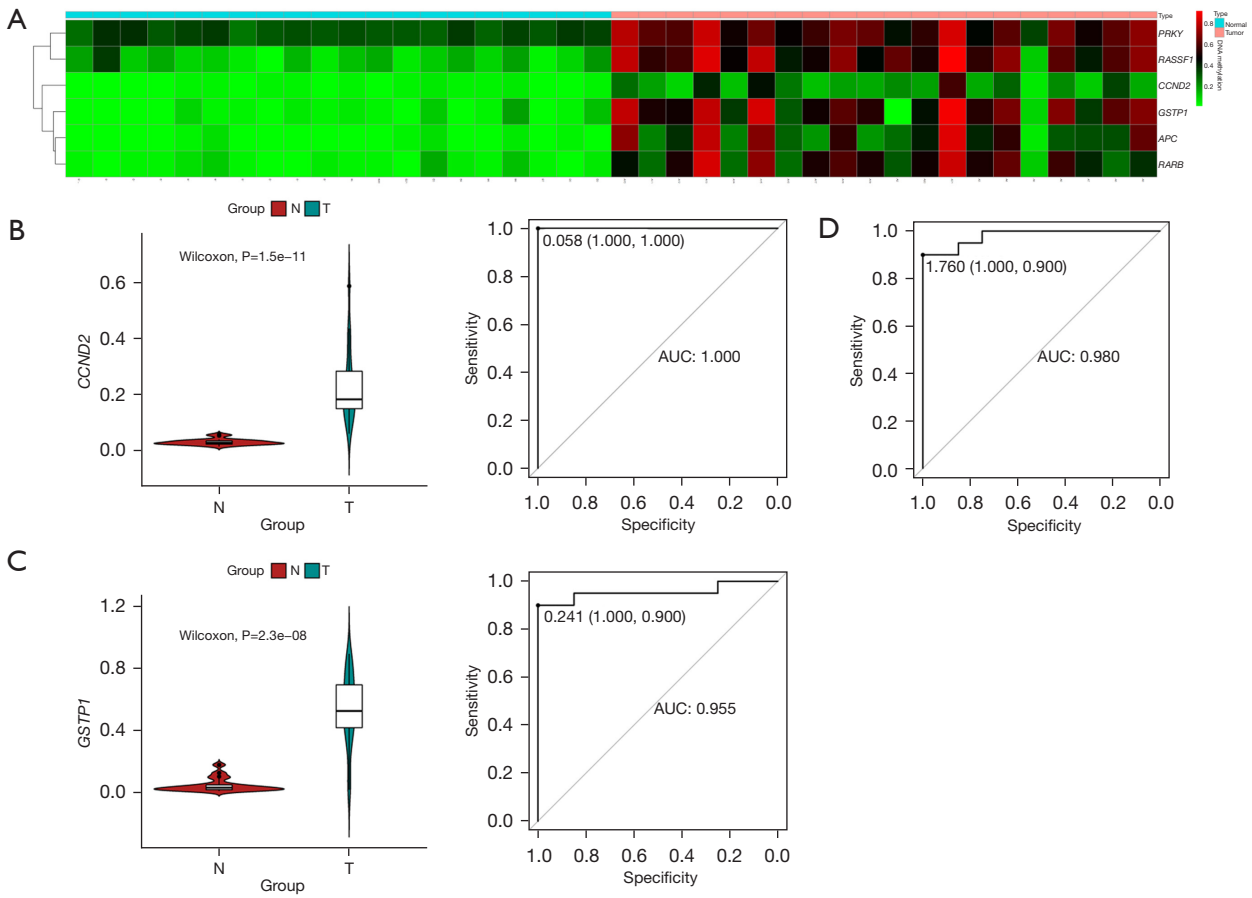


Figure 6 Validation in clinical samples. (A) Heat map of *PRKY*, *RASSF1*, *CCND2*, *GSTP1*, *APC*, and *RARB* genes DNA methylation in prostate cancer tissues (tumor) and paracancerous tissues (normal) of clinical samples. (B) Violin plot of methylation levels and ROC of single-gene diagnostic performance in *CCND2*. (C) Violin plot of methylation levels and ROC of single-gene diagnostic performance in *GSTP1*. (D) ROC of the dual-gene diagnostic model in of clinical samples. T = prostate cancer tissues; N = paracancerous tissues. *CCND2*, Cyclin-D2; *GSTP1*, glutathione S-transferase pi-1; AUC, area under the curve; ROC, receiver operating characteristic.

promoter region of the *CCND2* gene hinders its expression. Considering its role as a proto-oncogene, it might be expected that hypermethylation would inhibit tumor occurrence. However, it has been observed that reduced *CCND2* expression resulting from promoter methylation is also associated with the advancement of breast cancer, lung cancer, and pancreatic cancer, suggesting that *CCND2* silencing may contribute to tumor promotion (27,28).

Similarly, in PCa, increased *CCND2* promoter methylation is linked to reduced *CCND2* mRNA expression and is correlated with higher Gleason scores and tumor aggressiveness (29). Nevertheless, the potential of *CCND2* as a biomarker for the early diagnosis of PCa has not been fully substantiated. Further research is required to elucidate its precise role and diagnostic significance in PCa detection.

In our study, we introduced a novel dual-gene model comprising *CCND2* and *GSTP1* as a combined diagnostic tool for PCa. This approach significantly enhanced the sensitivity and specificity of PCa diagnosis. The diagnostic performance of the model was further confirmed in multiple datasets, with specificity exceeding 85% and sensitivity exceeding 90.7%.

To bolster the reliability of our model, we included an additional 20 PCa samples in our study. These samples were analyzed to further validate and strengthen the diagnostic accuracy of the dual-gene model in real-world clinical settings.

The methylation sequencing analysis demonstrated hypermethylation of *CCND2* and *GSTP1* genes in clinical samples. The dual-gene diagnostic model exhibited

excellent diagnostic performance in these samples, achieving an AUC of 0.980, sensitivity of 90%, and specificity of 100%. Compared to the PSA test, our model demonstrated evident diagnostic advantages, with significantly improved sensitivity and specificity. Although our model demonstrated excellent accuracy in diagnosis, 20 paired clinical samples are still relatively small, a larger number of samples are needed to further prove the accuracy of the model.

In the context of tumor development, only a few genes tend to play dominant or driving roles, while most gene expression changes are often associated with tumor progression. DNA methylation exhibits a similar effect, and it is important to identify whether changes in gene DNA methylation drive alterations in gene expression. Genes whose expression levels are influenced by methylation changes are often referred to as methylation-driven genes (30). To ascertain whether the genes involved in our diagnostic model are methylation-driven, we analyzed the TCGA PCa dataset and GSE84749 dataset. The expression of *CCND2* and *GSTP1* genes decreased significantly with an increase in their respective methylation levels, indicating that these two genes might be methylation-driven. This finding was further supported by the analysis of the CCLE database, where a significant negative correlation trend between methylation levels and gene expression was observed in pan-cancer tissue samples.

We conducted GSEA analysis to compare the gene expression profiles of different model scores and the pathways from multiple gene sets. We found that immune cell migration, chemotaxis, and immune regulation pathways were down-regulated in samples with high model scores, suggesting that our model has good performance in diagnosing PCa and may also have prognostic value.

Furthermore, our study analyzed the immune infiltration and tumor purity of *CCND2* and *GSTP1* genes in PCa tissue. It was confirmed that in PCa samples, higher expression levels of these two genes were associated with significantly decreased tumor purity and increased infiltration of B cells, T cells, macrophages, and other immune cells. This suggests that the overexpression of *CCND2* and *GSTP1* is linked to higher immune cell infiltration and lower tumor purity, and the methylation process of these two genes may reduce their expression, thereby increasing tumor risk.

Conclusions

In conclusion, the dual-gene methylation diagnosis model can serve as an independent and effective method for PCa diagnosis. When combined with liquid biopsy technology, it has the potential to enhance the accuracy of non-invasive diagnosis. Liquid biopsy methods are currently widely used for detecting various components like tumor cells, tumor DNA, RNA, and exosomes (31). A significant portion of cellular components present in urine originates from the prostate, making it an attractive choice for non-invasive PCa screening (32,33).

The diagnostic model developed in our study will undergo further validation using urine samples' sequencing data. This new approach holds promise as a non-invasive and reliable PCa screening method, potentially replacing the traditional PSA-based screening method. By integrating the dual-gene diagnostic model with liquid biopsy-based urine testing, clinicians may have a more accurate and less invasive tool for early detection and diagnosis of PCa.

Urine-based liquid biopsies also facilitated stratification for PCa patients, as well as prognostic prediction (34-36), all of which contribute to personalized and precise management of individual patient to achieve the purpose of predictive preventive personalised medicine.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-23-405/rc

Data Sharing Statement: Available at https://tau.amegroups.com/article/view/10.21037/tau-23-405/dss

Peer Review File: Available at https://tau.amegroups.com/article/view/10.21037/tau-23-405/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-23-405/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was

conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics committee of Beijing Chao-Yang Hospital (No. 2021-54), and written informed consents for participants and publication were provided by all participants.

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