

Prognostic Significance of *EZH2*-Related Gene Variants in Patients With Prostate Cancer Undergoing Androgen Deprivation Therapy

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Abstract

Background/Aim: Prostate cancer remains a major global health burden, with treatment resistance posing a significant challenge. Enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*), a histone methyltransferase, is frequently overexpressed in prostate cancer, contributing to tumor progression and castration resistance. Clinical trials of *EZH2* inhibitors may have therapeutic benefits. This study aimed to evaluate the impact of genetic variants in *EZH2*-related genes on survival outcomes in prostate cancer.

Patients and Methods: We conducted a genetic association study evaluating 76 single nucleotide polymorphisms (SNPs) across 10 *EZH2*-related genes in 630 patients with prostate cancer undergoing androgen deprivation therapy (ADT). Functional analyses, including gene ontology and pathway enrichment assessments, were performed to elucidate the biological significance of key genes across multiple datasets.

continued

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Results: *DNMT3A* rs77993651 was significantly associated with both cancer-specific survival [hazard ratio (HR)=0.82, $p=0.042$] and overall survival (HR=0.80, $p=0.011$). Functional annotation indicated that rs77993651 resides within enhancer histone marks, potentially regulating *DNMT3A* expression. Elevated *DNMT3A* expression was observed in prostate tumor tissues and correlated with more aggressive features and shorter progression-free survival. Gene set enrichment analysis revealed that *DNMT3A* expression was strongly associated with cell cycle G₂/M checkpoint regulation, implicating a role in prostate cancer progression.

Conclusion: The prognostic significance of *DNMT3A* and its genetic variant rs77993651 in prostate cancer is herein highlighted. Targeting *DNMT3A*-mediated pathways may offer novel therapeutic strategies for prostate cancer management.

Keywords: Prostate cancer, EZH2, epigenetic regulation, gene set enrichment analysis, survival.

Introduction

Prostate cancer is a significant health issue, particularly among older men, with an estimated 313,780 new cases and 35,770 deaths in the United States by 2025 (1). The disease disproportionately impacts African-American men and individuals of Caribbean descent. Treatment options vary and include watchful waiting, surgery, radiotherapy, androgen deprivation therapy (ADT), chemotherapy, radiopharmaceuticals, and proton beam radiation. The choice of treatment depends on the cancer stage, patient's overall health, and personal preferences. Predicting the progression of prostate cancer involves several clinical factors, such as age, prostate-specific antigen (PSA) level, family history, smoking status, alcohol consumption, and cholesterol level (2). Elevated PSA levels are significant, and combining these with other risk factors can improve prediction accuracy. However, the management of prostate cancer presents challenges, including difficulties in early detection, complex treatment decisions, treatment resistance, and the impact of individual patient factors. Genetic factors are important, with approximately 60% of prostate cancer risk attributed to inherited factors (3). Germline mutations in genes, such as *BRCA1* and *BRCA2*, are associated with a higher risk and more aggressive forms of cancer (4). Genetic testing can help identify individuals at high risk and guide personalized treatment strategies (5, 6). Despite advancements, the slow progression of prostate

cancer and potential for treatment resistance highlight the need for further research and novel therapeutic approaches.

Enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*), a histone methyltransferase, aids in prostate cancer by catalyzing the trimethylation of histone H3 at lysine 27, leading to gene silencing (7). Overexpression of *EZH2* is frequently observed in prostate cancer and associated with aggressive tumor behavior, metastasis, and poor prognosis (8). Mechanistically, *EZH2* drives cancer progression by silencing tumor suppressor genes, interacting with androgen receptors (AR) to promote castration-resistant prostate cancer, and methylating nonhistone proteins involved in DNA damage repair and apoptosis (9, 10). DNA methyltransferases (DNMTs), which add methyl groups to DNA, contribute to tumor progression by cooperating with *EZH2* to silence tumor-suppressor genes (11, 12). Clinical trials of *EZH2* inhibitors, such as mevinmetostat, have demonstrated promising results, particularly combined with enzalutamide, leading to improved median radiographic progression-free survival and a manageable safety profile (13). Single nucleotide polymorphisms (SNPs) in *EZH2* and *DNMT* may significantly influence cancer risk and prognosis (14). For example, *EZH2* rs2302427 is associated with susceptibility to cancer, including prostate cancer (15), whereas *DNMT3B* rs1569686 has been implicated in the risk of gastric cancer (16).

Given the emerging role of *EZH2*-related genes in cancer progression, we hypothesized that genetic variants of these genes may influence survival outcomes in patients with prostate cancer undergoing ADT. Therefore, this comprehensive genetic association study aimed to evaluate 76 SNPs across 10 *EZH2*-related genes in a cohort of 630 patients with prostate cancer. To further elucidate the biological mechanisms underlying these associations, we performed functional analyses, including gene ontology and pathway enrichment assessments, to explore the role of *EZH2*-responsive genes in prostate cancer progression.

Patients and Methods

Patient and response assessment. This study included 630 patients with prostate cancer who underwent ADT at the National Taiwan University Hospital, Kaohsiung Medical University Hospital, and Kaohsiung Veterans General Hospital (17, 18). The study was approved by the Institutional Review Board of the Kaohsiung Medical University Hospital (KMU-HIRB-2013132) and conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all participants. Clinicopathological data were extracted from hospital medical records, and the endpoints of the study were cancer-specific survival (CSS) and overall survival (OS), defined as the time from the start of ADT to death from prostate cancer or any other cause. Over a median follow-up period of 165.8 months, 414 patients died, with 314 succumbing to prostate cancer (19). Clinical factors, such as age, PSA level at ADT initiation, clinical stage, Gleason score at diagnosis, PSA nadir, and time to PSA nadir, were significantly associated with OS and CSS ($p < 0.05$).

SNP selection and genotyping. Haplotype-tagging SNPs (htSNPs) were selected to capture the majority of genetic variability across 10 *EZH2*-related genes, including *BMI1* polycomb ring finger proto-oncogene (*BMI1*), *DNA methyltransferase 1* (*DNMT1*), *3A* (*DNMT3A*), *3B* (*DNMT3B*),

embryonic ectoderm development (*EED*), *enhancer of zeste 2 polycomb repressive complex 2 subunit* (*EZH2*), *histone deacetylase 1* (*HDAC1*), *jumonji and AT-rich interaction domain containing 2* (*JARID2*), *RB binding protein 4*, *chromatin remodeling factor* (*RBBP4*), *RB binding protein 7* (*RBBP7*), and *SUZ12 polycomb repressive complex 2 subunit* (*SUZ12*). Haploview 4.2 and the tagger algorithm (20) were used for htSNP selection, based on genotype data from the 1000 Genomes Project for Han Chinese in Beijing and Southern Han Chinese populations. htSNPs were identified with a minor allele frequency (MAF) > 0.05 and a pairwise linkage disequilibrium threshold of $r^2 > 0.8$, ensuring efficient coverage of common variants with a minimal set of SNPs. Genomic DNA was extracted from whole blood using the QIAamp DNA blood kit (Qiagen, Germantown, MD, USA) and genotyped at the National Center for Genome Medicine using the Affymetrix Axiom Genotyping Array system (Thermo Fisher Scientific, Waltham, MA, USA) (21). SNPs with MAF < 0.03 , genotyping call rates < 0.94 , and significant deviations from Hardy-Weinberg equilibrium ($p < 0.0001$) were excluded. The final analysis included 76 htSNPs.

Bioinformatic analyses. To functionally annotate rs77993651 and investigate its potential regulatory roles, we integrated data from HaploReg v4.2 and FIVEx databases (22, 23). HaploReg was used to assess the impact of rs77993651 on regulatory elements, transcription factor binding, and evolutionary conservation. Expression quantitative trait loci (eQTL) analysis was performed via FIVEx, using linear regression models to associate the rs77993651 genotype with *DNMT3A* expression. To explore *DNMT3A* expression levels and their correlation with prostate cancer outcomes, publicly available datasets, including PCaDB (24), the Gene Expression Database of Normal and Tumor Tissues 2 (25), and The Cancer Genome Atlas Prostate Adenocarcinoma (TCGA PRAD), were used to examine the clinical significance of *DNMT3A* in prostate cancer. The molecular mechanisms linked to *DNMT3A* were explored using LinkedOmics via gene ontology and

hallmark pathway enrichment analyses employing gene set enrichment analysis (GSEA) (26). GSEA, using Pearson correlation coefficients to rank genes based on their correlation with *DNMT3A* expression, identified enriched gene sets by a weighted Kolmogorov–Smirnov-like running sum statistic (27). Statistical significance was determined using 1000 gene set label permutations and Benjamini–Hochberg false discovery rate (FDR) correction. The prognostic significance of *DNMT3A* and G₂/M checkpoint genes, including *aurora kinase B* (*AURKB*), *cyclin A2* (*CCNA2*), and *cyclin-dependent kinase 1* (*CDK1*), was assessed using TCGA PRAD data.

Statistical analyses. Statistical analyses were conducted using Statistical Product and Service Solutions version 19.0.0 (IBM, Armonk, NY, USA), with statistical significance set at two-sided $p < 0.05$. Kaplan–Meier analysis and log-rank tests were used to compare survival curves, whereas univariate and multivariate Cox regression analyses were performed to assess the associations between clinico-pathological features and patient prognosis, with hazard ratios (HRs) and 95% confidence intervals (CIs). The correlation between *DNMT3A* expression and tumor characteristics was evaluated using Spearman’s and Pearson’s correlations. A pooled analysis of *DNMT3A* expression in prostate cancer and normal tissues was conducted using Review Manager version 5.4.1 (Cochrane, London, UK) by applying a random-effects model to account for study heterogeneity, with standardized mean differences (SMDs) and corresponding 95% CIs.

Results

To explore the relationship between *EZH2*-related genes and prostate cancer progression, we examined the association of 76 htSNPs within 10 functional partner genes of *EZH2* with CSS and OS in patients with prostate cancer. Among these SNPs, two (rs77993651 in *DNMT3A* and rs794782 in *JARID2*) were significantly associated with CSS, whereas four (rs77993651 in *DNMT3A*, rs17576436 and rs17576233 in *JARID2*, and

rs2288937 in *DNMT1*) were significantly associated with OS ($p < 0.05$, Figure 1). Notably, rs77993651 in *DNMT3A* was the only SNP that showed a significant correlation with both CSS and OS. Specifically, each additional minor A allele of rs77993651 corresponded to a 18% reduction in cancer-specific mortality risk (HR=0.82, 95% CI=0.68-0.99, $p=0.042$, Table I) and 20% decrease in all-cause mortality risk (HR=0.80, 95% CI=0.68-0.95, $p=0.011$). Multivariate analysis further confirmed that rs77993651 in *DNMT3A* was an independent prognostic factor for OS after adjusting for clinical covariates ($p=0.025$, Table I).

To assess the potential functional role of rs77993651, we used HaploReg, which suggested that this SNP is located within enhancer histone marks across various tissues, indicating a possible regulatory function (Figure 2A). The FIVEx database provided additional support, revealing that the protective A allele of rs77993651 was linked to decreased *DNMT3A* expression in memory and naïve regulatory T cells ($p \leq 0.039$, Figure 2B). However, direct eQTL analysis of the prostate tissue remains unavailable.

To determine the clinical significance of *DNMT3A* expression in prostate cancer, we analyzed 2,461 tumor samples and 986 normal prostate tissues across 35 public datasets. *DNMT3A* expression was significantly elevated in tumor tissues compared to normal tissues (SMD=0.21, 95% CI=0.01-0.40, $p=0.04$, Figure 3). Furthermore, analysis of TCGA PRAD data revealed a strong correlation between increased *DNMT3A* expression and higher Gleason scores and advanced tumor stage ($p < 0.001$, Figure 4, left and middle). Moreover, patients with higher *DNMT3A* expression exhibited significantly shorter progression-free survival in both the GSE21032 and TCGA PRAD datasets ($p \leq 0.045$, Figure 4, right), suggesting that *DNMT3A* may contribute to tumor progression.

To elucidate the biological role of *DNMT3A* in prostate cancer, we identified genes correlated with *DNMT3A* expression in TCGA PRAD data. In total, 5,136 genes were positively correlated, whereas 4,459 genes were negatively correlated with *DNMT3A* (Pearson’s correlation

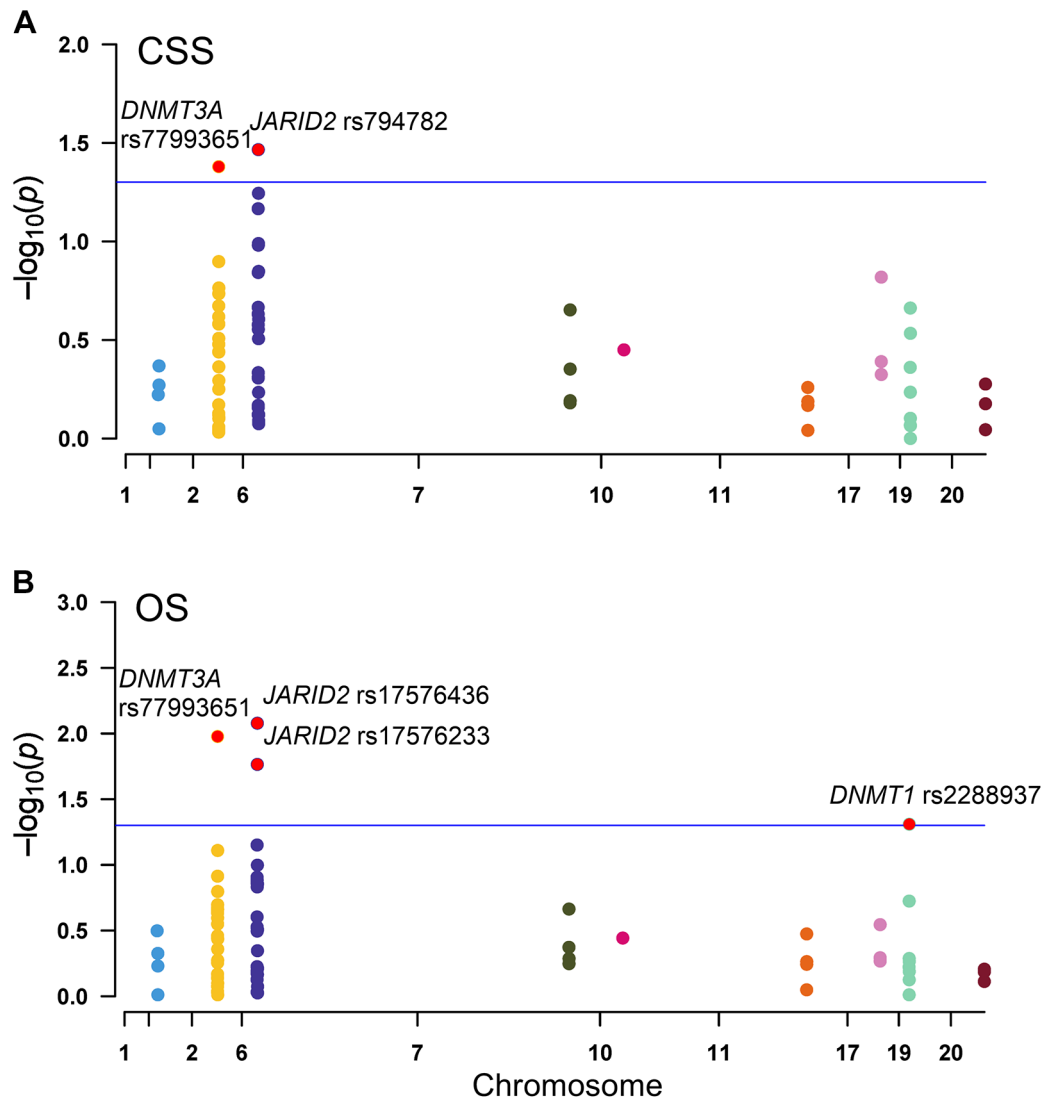


Figure 1. Manhattan plots depicting the association between 76 single-nucleotide polymorphisms (SNPs) across 10 EZH2-related genes and (A) cancer-specific survival and (B) overall survival in patients with prostate cancer undergoing androgen deprivation therapy. The Y-axis represents $-\log_{10}(p)$ values, whereas the X-axis indicates chromosomal positions of the SNPs. The nominal significance threshold ($p=0.05$) (blue horizontal line marks). SNPs meeting the significance criteria (red circles).

Table I. The association of DNMT3A rs77993651 with cancer-specific and overall survival in patients with prostate cancer receiving androgen deprivation therapy.

Genotype	Frequency	CSS	HR (95% CI)	<i>p</i> -Value	HR (95% CI) ^a	<i>p</i> -Value ^a	OS	HR (95% CI)	<i>p</i> -Value	HR (95% CI) ^a	<i>p</i> -Value ^a
GG/GA/AA	372/224/ 34	188/115/ 11	0.82 (0.68-0.99)	0.042	0.87 (0.71-1.06)	0.152	254/142/ 18	0.80 (0.68-0.95)	0.011	0.82 (0.69-0.98)	0.025

CSS, Cancer-specific survival; OS, overall survival; HR, hazard ratio; CI, confidence interval. ^aHRs were adjusted for age, stage, Gleason score at diagnosis, PSA level at ADT initiation, PSA nadir, and time to PSA nadir.

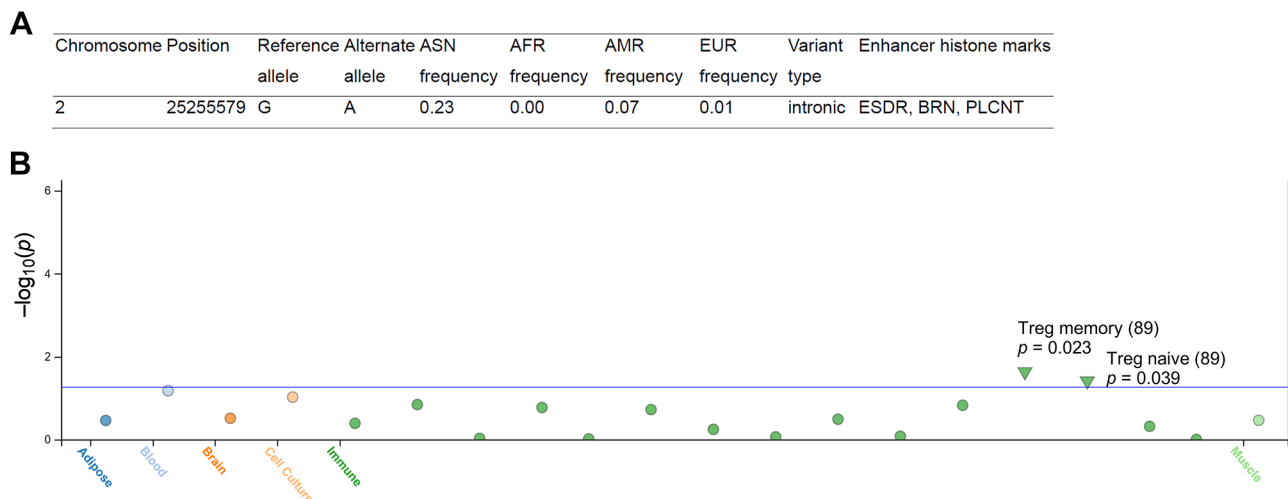


Figure 2. Functional impact of *DNMT3A* rs77993651. (A) Regulatory annotations for rs77993651 derived from HaploReg. (B) Expression quantitative trait loci analysis demonstrates the relationship between rs77993651 and *DNMT3A* expression levels across various human tissues. The nominal significance threshold ($p=0.05$) (blue horizontal line marks). The negative (inverted triangle) and non-significant (circles) effects of rs77993651 on *DNMT3A* expression. Sample sizes for each subgroup (numbers in brackets).

FDR<0.01). GSEA using the ranked gene list indicated the enrichment of positively correlated genes in cellular components, such as the chromosomal region, nuclear chromosome, and condensed chromosome (Figure 5A). Additionally, these genes are involved in biological processes, such as heterochromatin organization, chromosome segregation, and epigenetic regulation of gene expression (Figure 5B), and molecular functions, including helicase activity, histone binding, and catalytic activity acting on DNA (Figure 5C). Hallmark pathway analysis revealed enrichment of pathways associated with cell cycle progression, particularly at the G₂/M checkpoint, E2F targets, and mitotic spindle assembly (Figure 5D).

GSEA identified G₂/M checkpoint regulation as the most significantly enriched pathway (normalized enrichment score=2.404, FDR<2.2×10⁻¹⁶), implying a potential role for *DNMT3A* in this checkpoint. Therefore, we examined the correlation between *DNMT3A* expression and key hub genes within the protein-protein interaction network of the G₂/M checkpoint, including *AURKB*, *CCNA2*, and *CDK1*. Given the central role of *DNMT3A* in DNA methylation, we investigated its potential influence on the G₂/M checkpoint gene expression by

modulating DNA methylation. Our findings showed a negative correlation between *DNMT3A* expression and the DNA methylation status of *AURKB*, *CCNA2*, and *CDK1* in TCGA PRAD dataset (Figure 6, left). This pattern was consistent with the positive correlation observed between *DNMT3A* expression and the expression levels of these hub genes (Figure 6, middle left). Additionally, these genes were significantly upregulated during prostate cancer progression (Figure 6, middle right). Moreover, the elevated expression of *AURKB*, *CCNA2*, and *CDK1* was associated with worse survival outcomes, reinforcing the hypothesis that *DNMT3A* exerts an oncogenic effect by activating G₂/M checkpoint genes during prostate cancer progression.

Discussion

This study identified *DNMT3A* and its genetic variant, rs77993651, as critical factors influencing prostate cancer survival. The rs77993651 A allele is associated with improved survival, likely because of its role in reducing *DNMT3A* expression. Functional analyses revealed that *DNMT3A* exerts oncogenic effects as its elevated

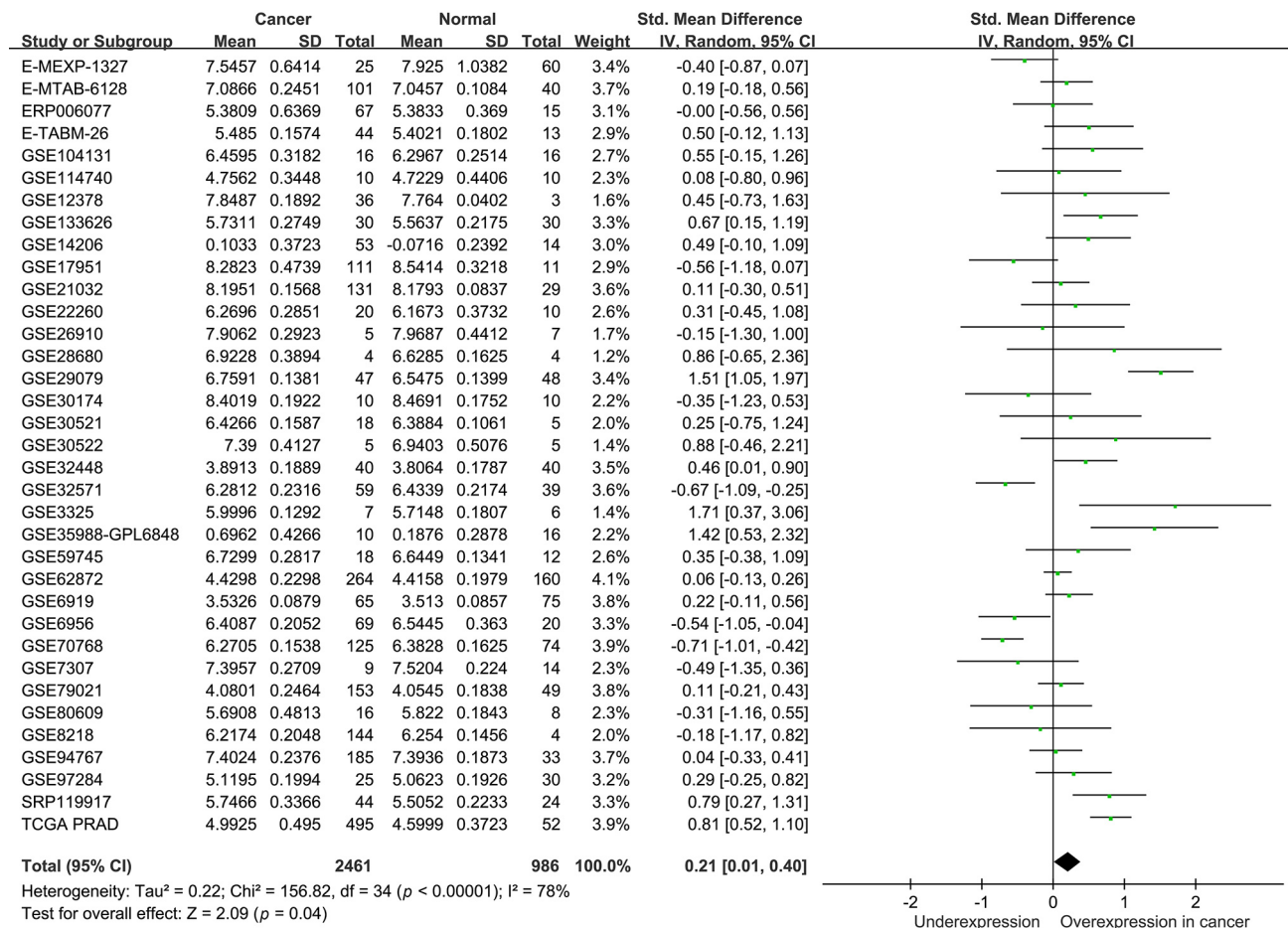


Figure 3. Pooled analysis compares *DNMT3A* expression in normal versus prostate cancer tissues across 35 independent studies. *DNMT3A* levels are significantly elevated in prostate cancer samples. SD, standard deviation. IV, inverse variance. CI, confidence interval. Std, standardized. TCGA PRAD, The Cancer Genome Atlas Prostate Adenocarcinoma. df, Degrees of freedom.

expression is significantly associated with prostate cancer progression and poor survival outcomes. Notably, *DNMT3A* expression positively correlated with key regulators of the G₂/M cell cycle checkpoint, highlighting its role in cell cycle dysregulation. These findings highlight *DNMT3A* as a promising prognostic biomarker and potential therapeutic target for prostate cancer.

SNP rs77993651 is located within an intronic region of *DNMT3A* that exhibits enhancer-like chromatin modification patterns, suggesting a potential regulatory role in *DNMT3A* expression. Our eQTL analysis revealed that the protective allele A was associated with decreased *DNMT3A*

expression in regulatory T cells. However, the potential effect of rs77993651 on *DNMT3A* expression in prostate tissues remains unexplored. *DNMT3A*, a key DNA methyltransferase responsible for *de novo* DNA methylation, aids in gene regulation and has been implicated in various cancers, including prostate cancer (28, 29). By establishing new DNA methylation patterns, *DNMT3A* often silences genes involved in cell growth and differentiation (30). It cooperates with *EZH2*, a component of polycomb repressive complex 2, to achieve robust gene silencing (11, 12). Elevated *DNMT3A* expression is frequently observed in cancers, leading to oncogene

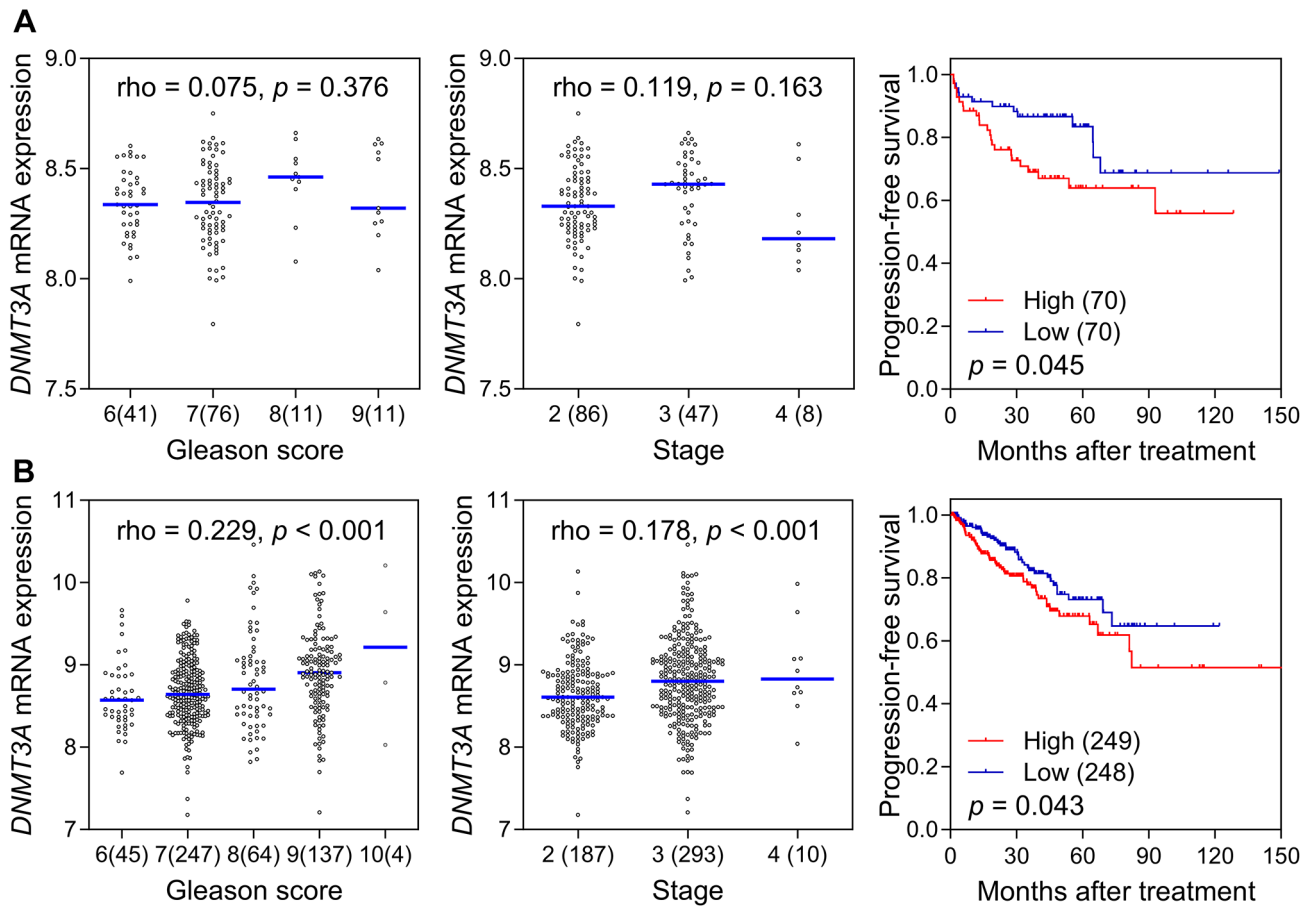


Figure 4. Clinical relevance of *DNMT3A* expression in prostate cancer. Elevated *DNMT3A* expression is linked to poorer progression-free survival in datasets (A) GSE21032 and (B) The Cancer Genome Atlas prostate adenocarcinoma (TCGA PRAD). Additionally, *DNMT3A* levels are significantly higher in tumors with increased Gleason scores and advanced staging within the TCGA PRAD cohort. Sample sizes for each subgroup (numbers in brackets).

activation, such as *CDK1* in acute myeloid leukemia (31), and silencing of tumor suppressor genes, such as the DAB2 interactive protein in colorectal cancer (32). These alterations contribute to uncontrolled cell growth and tumor progression. Additionally, *DNMT3A* regulates oncogene activation and the epithelial-to-mesenchymal transition by modulating the microRNA-200 family, thereby promoting cancer progression and metastasis (33).

Our GSEA of *DNMT3A*-associated expression networks revealed a positive relationship between *DNMT3A* expression and cell cycle G_2/M checkpoint genes, including the key hub genes *AURKB*, *CCNA2*, and *CDK1*. *AURKB* is essential for chromosome condensation, spindle assembly

checkpoints, and chromosome segregation during cell division. In prostate cancer, *AURKB* is overexpressed, which promotes tumor progression by enhancing proliferation and resistance to apoptosis. Its inhibition has shown promise in reducing tumor growth (34). *CCNA2* is pivotal in regulating the cell cycle, particularly by facilitating the G_1/S and G_2/M transitions as a cyclin-dependent kinase regulator. In prostate cancer, *CCNA2* exhibits oncogenic properties by promoting cancer cell proliferation, invasion, and metastasis, whereas its downregulation leads to cell cycle arrest (35). *CDK1* is critical for the G_2/M transition and mitotic progression, and its overexpression is linked to increased tumor proliferation and survival. Notably,

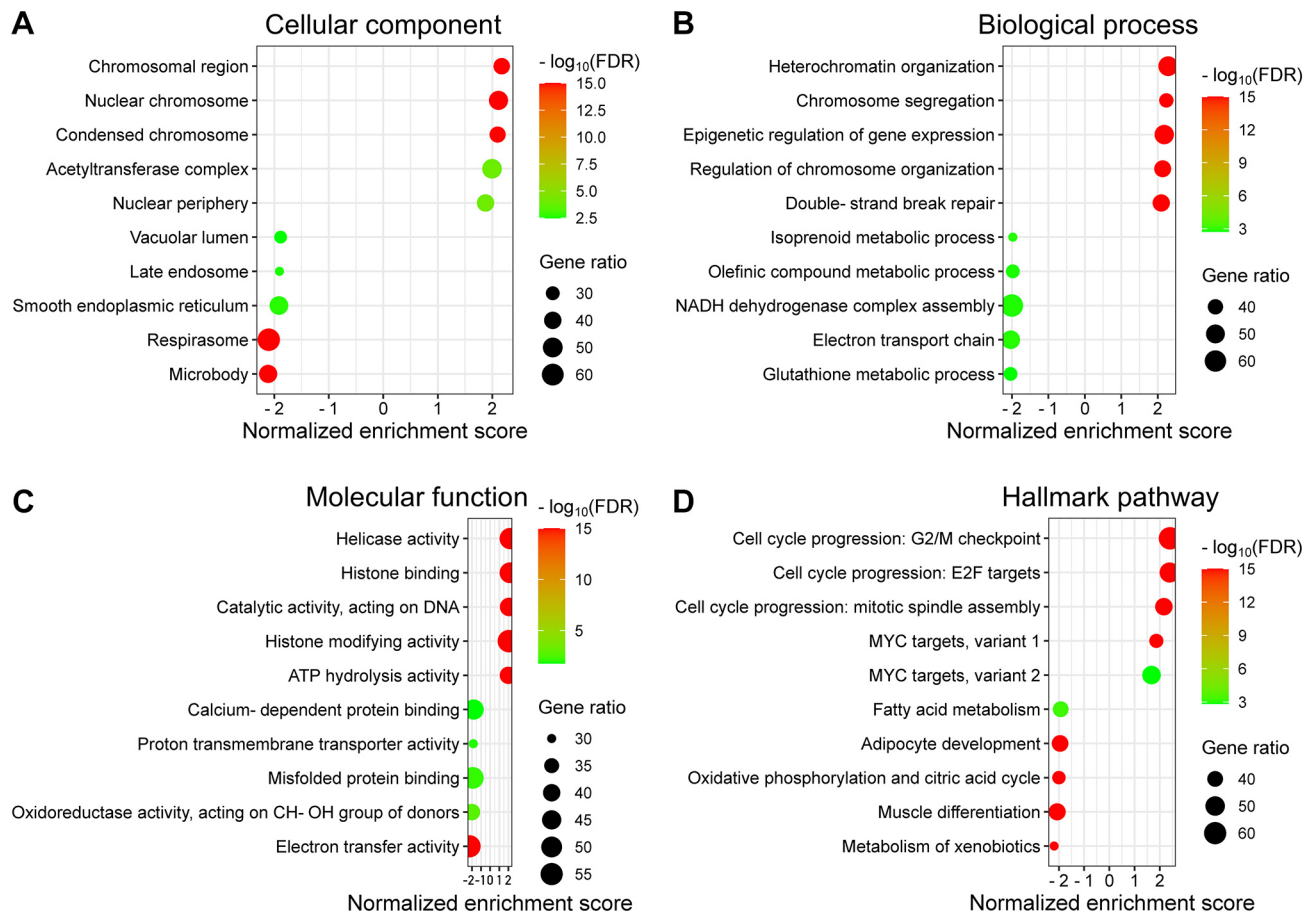


Figure 5. Gene ontology (GO) and pathway enrichment analyses of genes associated with *DNMT3A* expression. Top 10 GO terms are shown for (A) cellular components, (B) biological processes, and (C) molecular functions. (D) The most enriched Hallmark pathways. The ratio of core enrichment genes (bubble size) and its statistical significance (color scale).

CDK1 enhances AR activity by directly phosphorylating AR at Ser81, promoting prostate cancer growth, even under low-androgen conditions (36). Therapeutically, *DNMT* inhibitors, such as 5-aza-2'-deoxycytidine, have demonstrated efficacy in prostate cancer treatment by reducing tumor cell growth and enhancing sensitivity to AR inhibitors (37, 38). Furthermore, the combined inhibition of *DNMT3A* and *EZH2* enhances antitumor effects by preventing compensatory epigenetic mechanisms and activating immune pathways, as observed in colon cancer (39). These findings highlight *DNMT3A* as a key driver of prostate cancer progression and a promising therapeutic target.

Conclusion

The study provides valuable insights into the role of *EZH2*-related genetic variants in prostate cancer progression and highlights *DNMT3A* rs77993651 as a potential prognostic biomarker. A major strength of this study is the use of a well-characterized patient cohort with comprehensive clinical data, which allowed for robust association analyses. Additionally, this study's focus on epigenetic regulation offers a novel perspective on disease mechanisms. However, the limitations include the relatively small sample size, which may affect the statistical power, and lack of functional validation experiments to confirm the

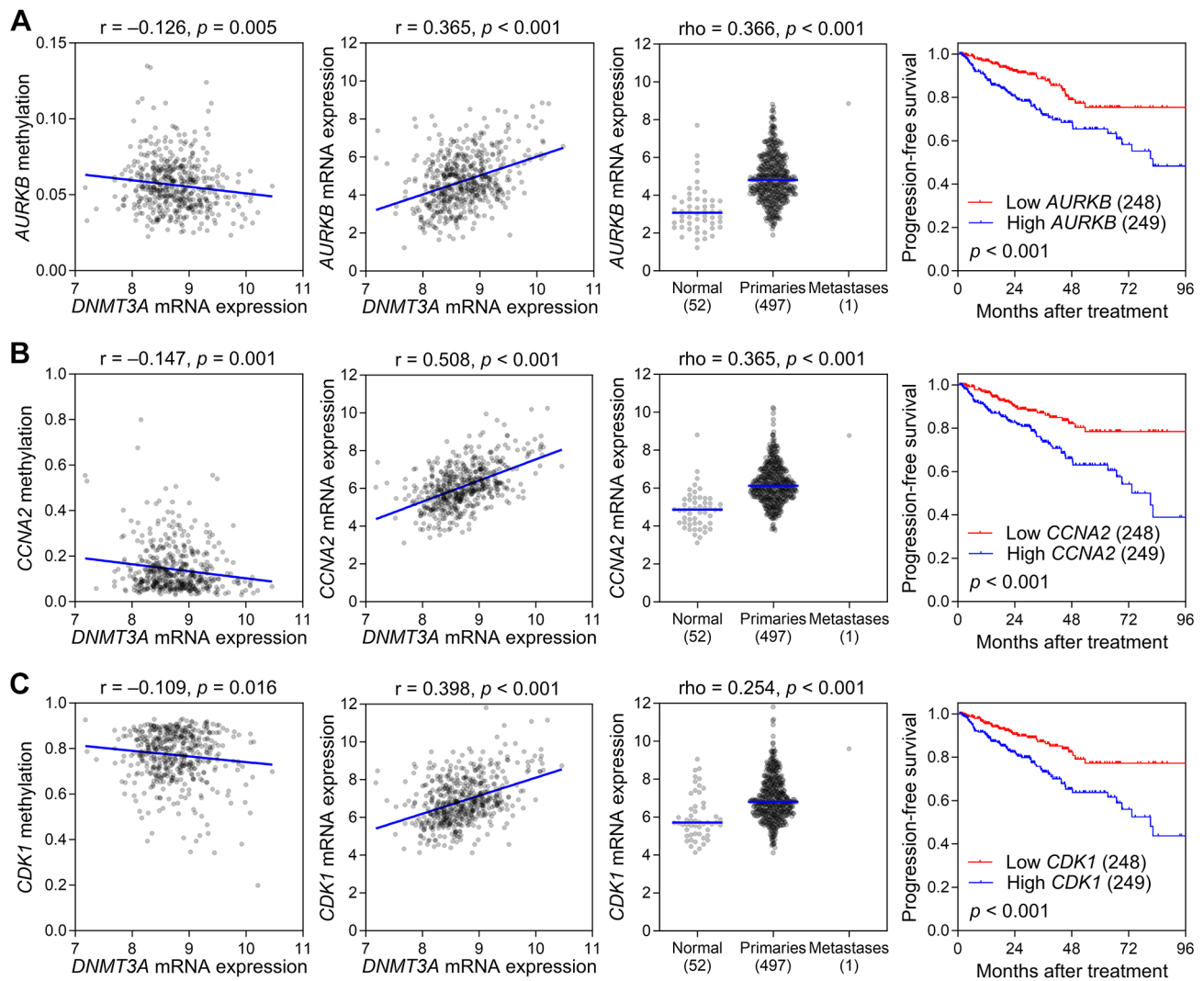


Figure 6. Correlation between *DNMT3A* expression and key regulators of the G_2/M checkpoint pathway in prostate cancer. The inverse relationship between *DNMT3A* and the methylation status of three hub genes: (A) *AURKB*, (B) *CCNA2*, and (C) *CDK1* (left panel). The positive correlation between *DNMT3A* and the expression of these genes (middle-left panel). Elevated expressions of *AURKB*, *CCNA2*, and *CDK1* in prostate cancer tissues relative to normal samples (middle-right panel). Higher expression levels of these genes are associated with worse survival outcomes in prostate cancer (right panel). Expression data were $\log_2(x+1)$ transformed RNA sequencing by expectation-maximization normalized count. Patient groups were dichotomized into low- and high-expression categories based on the median expression value. Sample sizes for each subgroup (numbers in brackets).

biological impact of the identified variants. Given the therapeutic relevance of *EZH2* inhibitors, these insights may guide personalized treatment strategies. Future research should validate these associations in larger cohorts and explore the mechanistic link between *DNMT3A* dysregulation and progression of prostate cancer. Understanding these epigenetic interactions may enhance

risk stratification and improve targeted therapies for patients with aggressive diseases.

Conflicts of Interest

The Authors declare that they have no potential conflicts of interest in regard to this study.

Authors' Contributions

SPH contributed to project development, data collection, and funding acquisition. BYB performed data collection and analysis. CYH, CCY, and VCL performed data collection. THC and TLL performed data analysis. YTC contributed to project development, data analysis, and funding acquisition. All Authors prepared and agreed to the published version of the manuscript.

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Artificial Intelligence (AI) Disclosure

During the preparation of this manuscript, a large language model (Google Gemini) was used solely for language editing and stylistic improvements in select paragraphs. No sections involving the generation, analysis, or interpretation of research data were produced by generative AI. All scientific content was created and verified by the authors.

Furthermore, no figures or visual data were generated or modified using generative AI or machine learning-based image enhancement tools.

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