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Research Progress of Abnormal DNA Methylation in the Development, Diagnosis, and Treatment of Prostate Cancer

Zhen Ren[1], Ming Yu[1], Yi Xia[2]

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Abstract

Prostate cancer (PCa) is one of the most common malignancy in men around the world. In recent years, studies have shown that the development of PCa is driven by epigenetic modifications to a great extent, mediated through abnormal DNA methylation. Aberrant methylation of DNA promoters leads to abnormal expression of genes that regulate the development and progression of PCa. In addition, the current diagnosis of PCa still relies on tissue invasiveness. Prostate biopsy is an invasive process whereas non-invasive liquid biopsy based measurement of abnormal DNA methylation is expected to become the future diagnostic method and may be established as a therapeutic target. This article reviews the progress on DNA methylation in the development, early diagnosis, prognosis, and treatment of PCa.

Key words DNA methylation, epigenetics, prostate cancer, diagnosis, treatment

1. Department of Ultrasound, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China.

2. Department of Neurosurgery, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, China.

Correspondence: Yi Xia (Department of Neurosurgery, Tangdu Hospital, Fourth Military Medical University, Xinshi Road, Xi'an, Shaanxi Province, P.R. China, 710038; Email: shylock_xy@163.com) and Ming Yu (Department of Ultrasound, Xijing Hospital, Fourth Military Medical University, Changle West Road, Xi'an, Shaanxi Province, P.R. China, 710000; Email: yumingfmmu@126.com).

Introduction

Prostate cancer (PCa) is one of the most common urological malignancies. According to recent data, approximately 268,490 new PCa cases and 34,500 PCa death occured in 2022 alone in the United States [1]. Although the 5-year survival rate of PCa is more than 90%, a considerable number of patients with advanced disease progress to castration-resistant Pca after treatment, or even develop distant metastases, resulting in poor prognosis [2, 3]. Therefore, prognosis of PCa is dependent on early diagnosis of the disease. However, the current means of early diagnosis of Pca are very limited, mainly relying on prostate specific antigen (PSA) test and imaging examination, both have a high false positive and false negative rates. Therefore, it is very important to develop biological markers of PCa with high sensitivity and specificity for the early diagnosis and prognosis assessment. Numerous studies have shown that epigenetic modification(s) caused by abnormal DNA methylation plays a crucial role in the development and progression of PCa [4-6]. More importantly, DNA methylation is a reversible process and can be stably detected in tissues or body fluids with high specificity. It is projected that DNA methylation may be a developing trend of PCa diagnosis and therapy in the future. In this review, the molecular mechanism(s) of abnormal DNA methylation in the development of PCa, as well as the research progress in early diagnosis, prognosis, and treatment are reviewed.

DNA methylation and Pca

DNA methylation is one of the most well-studied epigenetic regulatory mechanisms, which can change the genomic traits of a species without altering the genetic sequence of DNA. In terms of molecular mechanism, DNA methylation is the addition of a methyl group to the 5' carbon of the cytosine nucleotide (CpG dinucleotide) adjacent to guanine in the genome under the action of DNA methyltransferase, generating 5-methylcytosine [7]. DNA methylation is a key regulator of gene expression, in which hypermethylation of tumor suppressor gene promoter region and hypomethylation of oncogene promoter region can lead to the silencing of tumor suppressor genes and abnormal activation of oncogenes, thus regulating tumor [8]. In recent years, more and more studies have confirmed that the phenomenon of abnormal DNA methylation is widespread in PCa. Although the proportion of driver genes of DNA methylation is small, they are mainly enriched in important cancer signaling pathways and therefore play an important role in PCa development [9]. Xu et al. [10] used PCa methylation and gene transcription data from TCGA and GEO databases to conduct GO enrichment analysis and KEGG pathway analysis. It was found that driver genes of DNA methylation were significantly enriched in REDOX processes, extracellular exosomes, electron carrier activity, reactive oxygen species, and aldehyde dehydrogenase [NAD (P) +] activity, with drug metabolism (cytochrome p450), phenylalanine metabolism, histidine metabolism, glutathione metabolism and tyrosine metabolism, suggesting that abnormal DNA methylation may mediate the tumor microenvironment and regulate related metabolic processes to achieve tumor development. In addition, Zhao et al. [11] found that 22% of epigenomic subtypes in metastatic castration-resistant prostate cancer (mCRPC) are related to *TET2*, *DNMT3B* and *IDH1*. Hypermethylation of *BRAF* gene is related to somatic mutation, and the methylation modification in the interregion of PCa driver genes such as *AR*, *MYC* and *ERG* is significantly related to gene expression levels, which indicates that abnormal DNA methylation plays an important regulatory role in the process of PCa metastasis and castration resistance. These aberrant DNA methylation sites are potential therapeutic targets for mCRPC. Another study analyzed DNA methylation in PCa patients in China [12] and found that the PCa genome was hypomethylated relative to normal prostate tissue, with 5' non-coding regions and CpG islands being relatively hypermethylated, while exons, introns and repeat units were hypomethylated. At the same time, the authors found a large number of differentially methylated region (DMRs) in PCa cells that are significantly enriched in promoters and enhancers,which is closely related to PCa gene regulation and cell growth and development. This study provides molecular basis for personalized diagnosis and treatment based on abnormal DNA methylation in the Chinese population with PCa. In summary, we can find that the DNA abnormal methylation patterns play an important regulatory role in the formation and development of PCa, suggesting that abnormal DNA methylation sites might be effective therapeutic targets.

Molecular mechanism of abnormal DNA methylation and the development of PCa

There are numerous DMRs in PCa cells, and hypermethylated DMRs can regulate the downstream expression of tumor suppressor genes, DNA repair genes, and hormone receptor regulation genes. While hypomethylated DMRs can lead to genomic instability and abnormal gene expression, and these abnormal DNA methylation states often determine the progression of tumor development [13]. At present, many studies have confirmed that abnormal DNA methylation plays an important role in the occurrence and development of PCa (**Figure 1**). Coding region of abnormal methylation of DNA is closely related to the occurrence and development of PCa. Yang et al. [14] found that demethylation of RUNt-related transcription factor 3 (*RUNX3*) gene promoted the expression of *RUNX3*, and *RUNX3* inhibited the proliferation of PCa cells by regulating cell cycle, promoting cell apoptosis, thus playing an anti-tumor role. Chen et al. [15] found that hypermethylation in metastasis suppressor 1(*MTSS1*) gene is associated with poor prognosis in PCa. Demethylation of *MTSS1* gene inhibits proliferation, migration, and invasion of PCa cells, and blocks cell cycle in G0/ G1 phase. In addition, *MTSS1* is involved in the regulation of epithelial cell adhesion molecule (EMT) by up-regulating E-cadherin and inhibiting the expression of zinc finger protein. Li et al. [16] found that high methylation of olfactomedin 4 (*OLFM4*) promoter region will downregulate the expression of *OLFM4*, and further lead to the increase of SHH expression, thus activating the hedgehog signaling pathway, inhibiting tumor cell proliferation and EMT, and achieving tumor inhibition. Gao et al. [17] found that the expression of type 2 17β-hydroxysteroidal solid ol dehydrogenase (*17βHSD2*) is regulated by multiple mechanisms such as DNA methylation, androgen stimulation and mRNA selective splicing, and demethylation can lead to the upregulation of *17βHSD2* expression. The conversion of testosterone and DHT into upstream precursor small molecules leads to androgen inactivation in vivo, which inhibits androgen-induced tumor cell proliferation and xenograft tumor growth. Leng et al. [18] found that the gene testis-specific protein Y-encoded 1 (*TSPY1*) on the Y chromosome was an oncogene targeted and regulated by the androgen to androgen receptor (*AR*) axis, and the *TSPY1* promoter CpG. The hypomethylation of the island can enhance the *TSPY1* transcription level and promote proliferation of PCa cells. In conclusion, abnormal methylation of CpG islands in many gene promoter regions is closely related to the proliferation, apoptosis, invasion, and migration of PCa cells. Methylation of non-coding regions of DNA is also involved in the formation and development of PCa. Mazzu et al. [19] found that the expression of miR-193b was affected by CpG island methylation, and miR-193b could induce apoptosis and inhibit tumor cell invasion by targeting the expression of *FOXM1* and *RRM2* genes. Shi et al. [20] found that

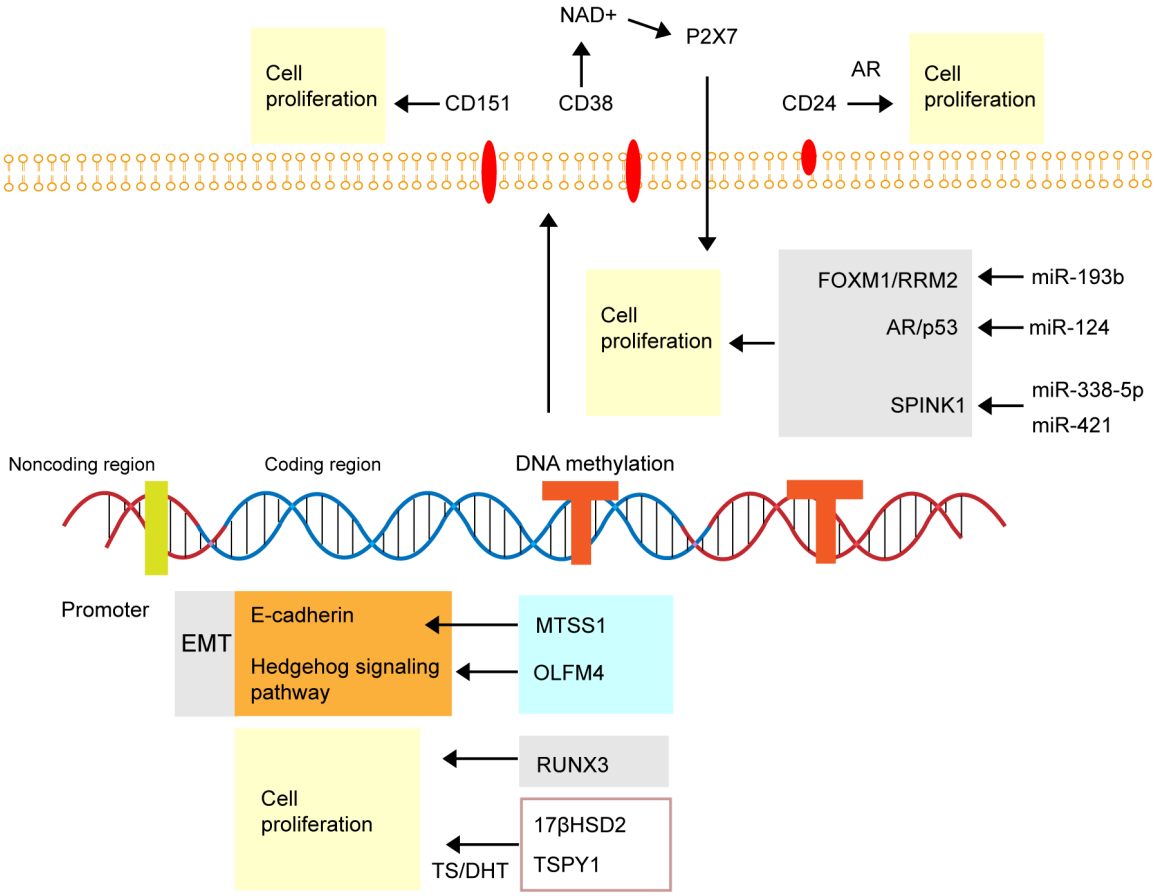

Figure 1. Graph of molecular mechanism of abnormal DNA methylation in prostate cancer. EMT, Epithelial-mesenchymal transition; MTSS1, metastasis suppressor 1; OLFM4, metastasis suppressor 1; RUNX3, RUNt-related transcription factor 3; 17βHSD2, type 2 17β-hydroxysteroidal solid ol dehydrogenase; TSPY1, testis-specific protein Y-encoded 1; AR, androgen receptor.

the methylation state of CpG island was involved in the regulation of miR-124 gene expression, and the increased miR-124 level directly targeted AR and induced the upregulation of p53, which plays a role in cancer inhibition. Bhatla et al. [21] found that *EZH2* gene, as an epigenetic switch, promoted the methylation of CpG island in the regulatory region of miR-338-5p/miR-421 through trimethylation of lysine at the 27th position of histone H3, and deactivated the target *SPINK1* gene. As a result, the expression of *SPINK1* is up-regulated, which drives the occurrence and progression of PCa. In addition, the effect of abnormal DNA methylation on transmembrane proteins is also involved in the initiation and development of PCa. Mottahedeh et al. [22] found that inhibition of CD38 expression by methylation increases the level of nicotinamide adenine dinucleotide (NAD+) outside PCa cells, and free NAD+ could activate P2X7. It affects the transport of ion channels, thus affecting the downstream signaling pathway, inducing early apoptosis of cells and changes in the immune microenvironment, thus affecting tumor progression. Han et al. [23] found that the expression of tumor-promoting factor CD151 in PCa was closely related to promoter methylation, and reduces the expression of CD151, inhibited proliferation and EMT of PCa cells, suggesting that CD151 can play a bidirectional role in the process of intercellular signal transduction, mediated by AR. There is a correlation between the transcription of AR and modulation of epigenetic inheritance. In addition, Tolkach et al. [24] found that the promoter region of *CD24* gene in PCa was hypermethylated, while the expression level of CD24 was increased, mainly because there was an ERG binding site between the methylation sites of CD24 promoter. Overexpression of ERG could upregulate CD24 expression, thereby promoting CD24 expression initiating the development of PCa.

Application of DNA methylation in early diagnosis and prognosis of PCa

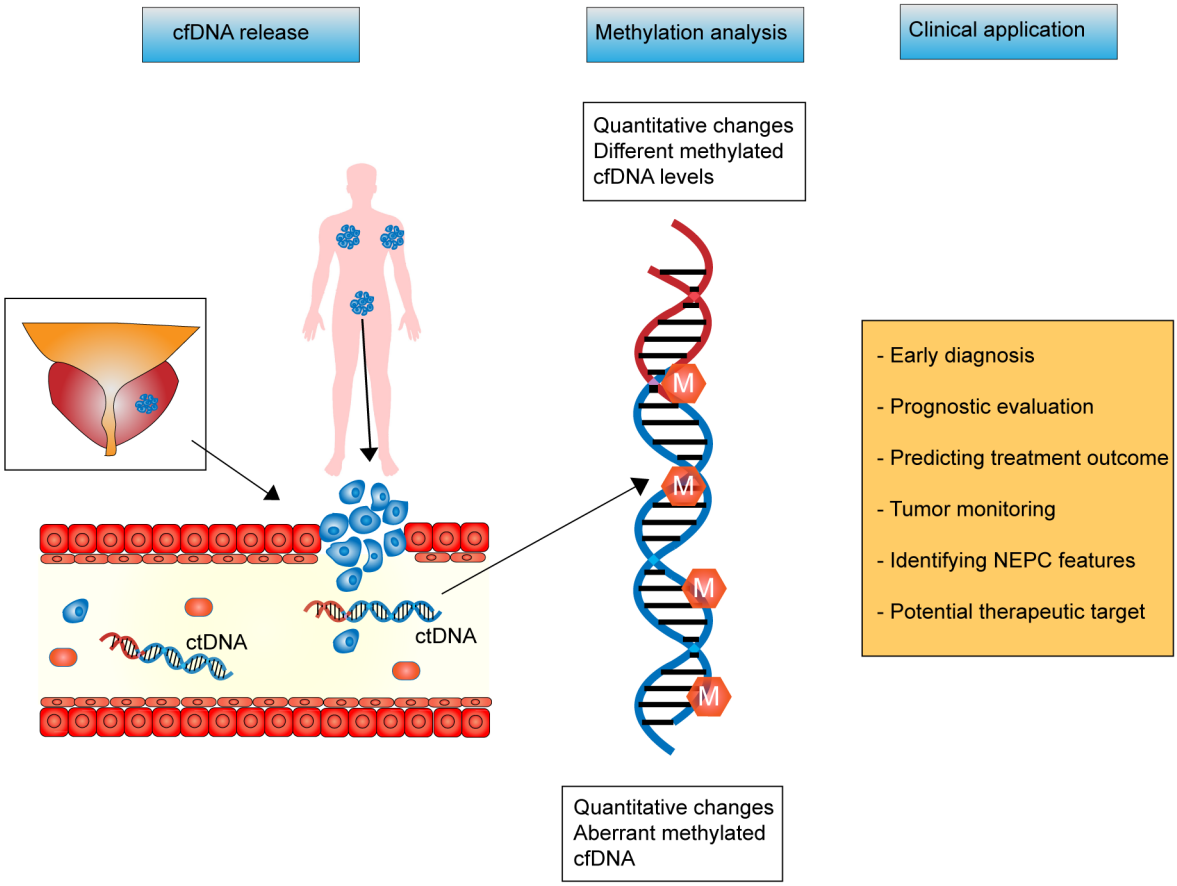

Figure 2. Potential clinical application of cell free DNA and abnormal DNA methylation in prostate cancer. Circulating cell free DNA (cfDNA) can be released in the bloodstream from tumor sites in prostate cancer patients. cfDNA form the bloodstream in prostate cancer patients can undergo both quantitative (e.g., changes in levels of methylated DNA) and qualitative (e.g., specific patterns of aberrant methylation) changes. Its evaluation of the methylation in cfDNA can help manage prostate cancer patients resulting in early diagnosis, a prognostic and predictive evaluation, a better tumor monitoring, and may aid in the identification of resistance such as neuroendocrine prostate cancer (NEPC).

Application of DNA methylation in early diagnosis of PCa

Early screening and diagnosis of PCa in clinic mainly rely on PSA detection.

However, the PSA level is affected by various factors, and the specificity and sensitivity are not optimal. DNA methylation is highly specific and stable in PCa, so it has its unique advantages in tumor diagnosis. Yao et al. [25] selected a methylation site on Y chromosome (cg05163709) as a potential biomarker for PCa diagnosis through cancer tissues and adjacent normal tissues of 66 PCa patients, and its sensitivity and specificity were 94.6% and 78.3% respectively. ROC analysis showed that the diagnostic efficiency of cg05163709 (AUC = 0.915) was significantly higher than that of PSA test (AUC = 0.769). Li et al. [26] identified 359 hypermethylation sites, 3 435 hypomethylation sites, 483 up-regulated genes and 1 341 down-regulated genes in PCa using a public database, and selected 17 hypermethylation sites (covering 13 genes) that could highly distinguish PCa from normal tissues. The prediction accuracy of PCa diagnosis was 88%-94%, respectively. These markers provided important clues for PCa diagnosis and prognosis assessment. Lan et al. [27] detected the methylation pattern of DACT-2 in serum of 64 PCa patients, 22 patients with benign prostatic hyperplasia (BPH) and 47 healthy subjects by methylation-specific PCR, and the results showed that DACT-2 was present in PCa patients. The promoter methylation level was significantly higher than that of BPH patients and healthy subjects, and the methylation rate of *DACT-2* was 0.745. The sensitivity was 81.8% and the specificity was 75.0%. Meanwhile, the ROC curve results showed that DACT-2 was better than PSA

in the diagnosis of PCa and was a potential biomarker for PCa diagnosis.

The combined methylation test of multiple genes is helpful to improve the sensitivity and specificity of PCa diagnosis. Brait et al. [28] found that the sensitivity and specificity of MCAM hypermethylation for the diagnosis of PCa was 66% and 73%, and when MCAM was combined with ERα and ERβ, the sensitivity was effectively increased to 75%, but the specificity was not significantly reduced. Haldrup et al. [25] analyzed the methylation of serum samples from 27 patients with PCa and 10 patients with BPH, and finally identified three hypermethylated genes (*ST6GALNAC3* / *CCDC181* / *HAPLN3* genes) for the construction of PCa diagnostic model, with high sensitivity. The sensitivity was 67% and the specificity was 100%. Constancio et al. [29] found that the accuracy, sensitivity and specificity of the combined detection of *FOXA1*, *GSTP1*, *HOXD3*, *RARβ2*, *RASSF1A*, *SEPT9* and *SOX17* for PCa diagnosis was 72%. These studies indicate that polygene methylation detection is expected to become a new idea for PCa diagnosis. DNA methylation test combined with serum PSA also has a common role in PCa diagnosis. A Mexican cohort study [30] found that methylation tests for *GSTP1* and *RASSF1A* genes had a positive predictive value of 73% and a negative predictive value of 59.6% for PCa diagnosis. When combined with serum PSA, the positive and negative predictive values increased to 81% and 66%, respectively. Reis et al. [31] compared serum *GADD45* for a methylation levels in 22 PCa patients and 22 control patients, and found that the sensitivity and specificity of *GADD45* methylation in serum samples were 38% and 98%. However, as a PCa biomarker combination consisting of PSA, circulating cell free DNA (cfDNA) level and GADD45a methylation was established, although the specificity was slightly decreased (87.5%), the diagnostic sensitivity was significantly improved (94.1%). ROC analysis showed that the combination had good diagnostic efficacy for PCa (AUC = 0.937). **Figure 2** shows that cfDNA can be released in the bloodstream from tumor sites in prostate cancer patients.

Taken together, these studies suggest that abnormal DNA methylation may be a potential biomarker for early diagnosis of PCa. At the same time, detection of methylation sites based on body fluids or tissues has the characteristics of non-invasive, economical and convenient, which sets a new direction of cancer diagnosis in the future. However, whether DNA methylation is consistent in blood and tumor tissues needs further research.

Application of DNA methylation in PCa prognostic assessment

DNA methylation is not only involved in the initiation and development of PCa, but also closely related to poor prognosis of PCa. It can be used as an important marker for the assessment of PCa prognosis. In a prospective study, Wang et al. [32] found that the methylation pattern of serum cadherin-13 (*CDH13*) gene not only significantly correlated with Gleason score, tumor stage and PSA level, but also strongly correlated with survival outcomes and relative mortality risk (HR = 6.132, 95%CI: 3.160-12.187). Gao et al. [33] found that the promoter methylation level of *CRMP4* gene could be used as a factor.

The sensitivity and specificity of this method was more than 90% and served as independent predictors of prognosis in PCa patients. The sensitivity of this method was significantly better than that of MRI in diagnosing lymph node metastasis of PCa ($P < 0.001$). Pellacani et al. [34] utilized a detection method based on 17 methylation markers for stratification of PCa prognosis based on whole-genome DNA methylation analysis in PCa tissues and normal tissues, and the diagnostic accuracy rate of this method was as high as 92%. Zhang et al. [35] divided PCa patients into hypermethylated and hypomethylated types based on DNA methylation patterns at CpG sites. The two subtypes showed significant differences in the epigenome, genome, transcriptome, disease state, immune cell infiltration and function, among which 8 were associated with high-risk subtypes. The PCa prognostic evaluation model constructed by *AURKA*, *DLGAP5*, *FOXD1*, *KIF4A*, *MELK*, *MYBL2*, *SPAG5* and *TPX2* showed that a higher risk score was associated with poor prognosis in PCa ($P < 0.05$). The validity of the model was 66%-84% in both internal and external validation sets. In conclusion, high specificitysensitivity and diagnostic accuracy of DNA methylation detection in PCa is applicable for prognosis assessment. In addition, a large number of studies have found that the level of DNA methylation was closely related to the biochemical recurrence of PCa. Goltz et al. [36] found that *CXCL12* methylation significantly correlated with Gleason grade and biochemical relape-free survival after radical prostatectomy, and could be used as an active indicator of postoperative biochemical relapse in PCa patients.

Norgaard et al. [37] found that *MEIS2* gene was significantly hypermethylated in PCa, and in 3 independent radical anterior adenectomy cohorts (700 patients in total), The expression level of *MEIS2* significantly correlated with postoperative biochemical recurrence (P.0084, 0.001 and 0.01,). Holmes et al. [38] found that promoter methylation of *PITX3* could effectively predict biochemical recurrence of PCa (training set: HR = 1.83, 95%CI: 1.07 ~ 3.11, P = 0.027; Validation set: HR = 2.56, 95%CI: 1.44 ~ 4.54, P = 0.001), and the combination of *PITX2* detection could also perform risk stratification in PCa patients to better assess the risk of biochemical recurrence. In conclusion, detection of methylation markers can predict the risk of biochemical recurrence in PCa patients after surgery, which is of great significance for the prognosis and PCa monitoring.

Relationship between DNA methylation and PCa treatment

At present, DNMTs inhibitors (DNMTi) are widely used in the treatment of disease with abnormal DNA methylation. DNMTi is mostly used in the treatment of myelodysplastic syndrome, leukemia, and other hematological diseases, but in recent years, it has been found that 5-Aza-2 '-deoxycytidine, 5-Aza-CdR, as a nucleoside analogue of DNMT, also has therapeutic effects on PCa, and its mechanism is shown in **Figure 3**. Wang et al. [39] observed through in vivo and in vitro experiments that 5-Aza-CdR restored the expression of miR-146a through demethylation, thereby inhibiting cell proliferation and promoting apoptosis. Similarly, Lynch et al. [40] found that 5-Aza-CdR treatment could increase the expression of miR-200c and miR-141, inhibited the expression of downstream *DNMT3A* and *TET1* / *TET3* genes, thereby negatively regulating proliferation of PCa cells and inducing apoptosis. This indicated that there were great prospects in the development of targeted drugs for differential methylation sites in PCa. With the deepening of research, the current study also found that DNMTi can enhance the chemotherapeutic sensitivity of PCa. Fang et al. [41] found that 5-Aza-CdR combined with cisplatin can play a synergistic anti-tumor effect and induce apoptosis of PCa cells. Ramachandran et al. [42] found that the use of 5-Aza-CdR could enhance the cytotoxicity of cabazitaxel in PCa cells, effectively improving the result of second-line therapy in mCRPC patients. Another in vitro test confirmed [43] that 5-Aza-CdR could enhance the expression of tumor suppressant gene *TSPYL5* through demethylation, thus enhancing the sensitivity of PCa cells to docetaxel and paclitaxel, which provided a new means to solve the problem of chemotherapeutic resistance in PCa.

At present, there are few clinical trials on DNMTi for PCa, and most of them are phase I and II clinical trials. Sonpavde et al. [44] recruited 36 patients with castration-resistant Pca for the phase II trial of azacytidine combined with androgen blocking agent,

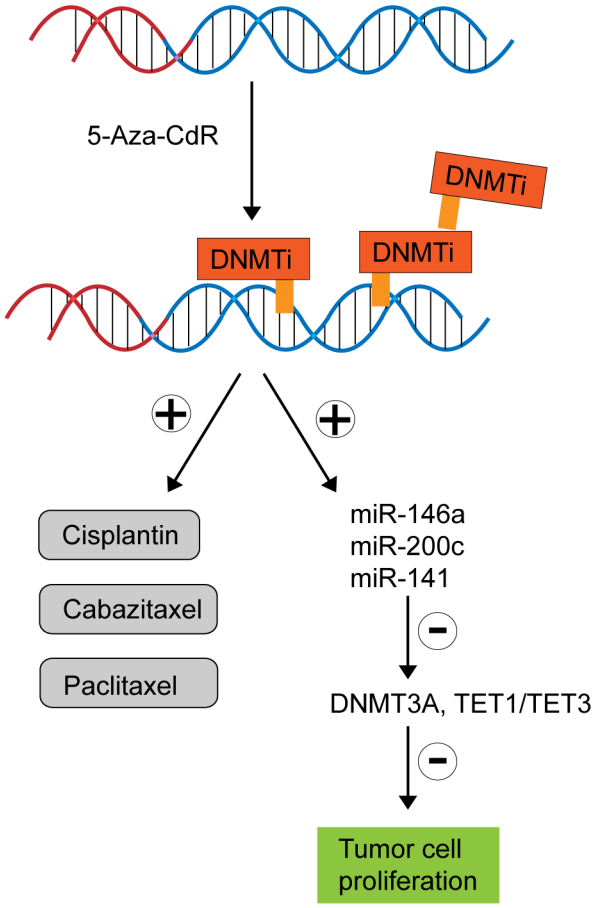

Figure 3. Graph of DNMTi (5 - Aza - CdR) for therapy of prostate cancer.

and results showed that the PSA doubling time of 19 patients was greater than 3 months, and the overall median PSA doubling time of patients was significantly longer than baseline ($P < 0.01$), which is related to the reduction of DNALINE-1 methylation in plasma by azacitidine. A small Phase II clinical trial [45] involving 14 mCRPC patients using three intravenous doses of decitabine 75 mg/m2 every 8 hours, repeated every 5 to 8 weeks, showed that the patients were well tolerated, but only 2 patients had stable disease and disease progression takes longer than 10 weeks. Another phase I/ II study [46] used azacytidine plus docetaxel plus prednisone to treat mCRPC patients, with 15 and 7 patients enrolled in phase I and II trials, ultimately observed a complete response in 1 patient and a partial response in 2 patients out of 10 evaluable patients. There were five patients with stable disease. These clinical trials indicate that the safety and clinical efficacy of DNMTi need to be further studied and improved, and that targeted therapy for abnormal DNA methylation sites should be developed to achieve better results of PCa treatment.

Conclusions

At present, more and more studies have confirmed that DNA methylation plays an important role in the initiation, development, early diagnosis, prognosis assessment and treatment of PCa. Nevertheless, there are many abnormal DNA methylation sites in PCa, and there is more complex in the downstream regulatory pathways and networks involved. How to find the key abnormal DNA methylation sites remains to be studied in the future. In addition, there are other challenges, such as the specific molecular mechanism still needs to be further perfected, whether the detection of abnormal DNA methylation can become an economical and convenient means of detection. In addition, there are few clinical studies on DNMTi in the treatment of PCa. The clinical efficacy and safety of DNMTI need to be verified by multi-center clinical trials with larger sample size. Meanwhile, we also need to explore the coactivity between genome and epigenome in the context of Chinese population, so as to solve the heterogeneity of PCa. With the deepening of research, abnormal

DNA methylation will become a new mode of PCa treatment.

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None.

Ethical policy

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Approval from institutioalethical committee was taken.

Availability of data and materials

All data generated or analysed during this study are included in this publication.

Author contributions

ZR: Conception, design of study and manuscript preparation; MY: Data collection and analysis; YX: Approval for the final version of the manuscript and funding supports.

Competing interests

The authors have no competing interest.

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