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Epigenetics as a Key Factor in Prostate Cancer

Kadriia Enikeeva, Guzel Rafikova, Yuliya Sharifyanova, Diana Mulyukova, Alexandr Vanzin, and Valentin Pavlov*

Nowadays, prostate cancer is one of the most common forms of malignant neoplasms in men all over the world. Against the background of increasing incidence, there is a high mortality rate from prostate cancer, which is associated with an inadequate treatment strategy. Such a high prevalence of prostate cancer requires the development of methods that can ensure early detection of the disease, improve the effectiveness of treatment, and predict the therapeutic effect. Under these circumstances, it becomes crucial to focus on the development of effective diagnostic and therapeutic approaches. Due to the development of molecular genetic methods, a large number of studies have been accumulated on the role of epigenetic regulation of gene activity in cancer development, since it is epigenetic changes that can be detected at the earliest stages of cancer development. The presence of epigenetic aberrations in tumor tissue and correlations with drug resistance suggest new therapeutic approaches. Detection of epigenetic alterations such as CpG island methylation, histone modification, and microRNAs as biomarkers will improve the diagnosis of the disease, and the use of these strategies as targets for therapy will allow for greater personalization of prostate cancer treatment.

1. Introduction

Cancer remains one of the most significant challenges in the field of medicine, and its study continues to be a priority area for scientific research. According to the World Health Organization (WHO), cancer is projected to cause ≈10 million deaths in 2020, accounting for nearly one in six deaths.[1] Substantial progress has been made in recent decades in understanding the mechanisms underlying cancer development, and one area that is receiving increasing attention is epigenetics.

The relevance of epigenetics in cancer research lies in its ability to unravel complex molecular processes involved in oncogenesis. Epigenetics investigates changes in gene expression that are not related to alterations in DNA sequence but significantly impact cellular functions and contribute to the development of cancerous changes.[2]

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adbi.202300520

DOI: 10.1002/adbi.202300520

Prostate cancer ranks third in incidence and second in the number of cancer cases affecting men worldwide. It is among the most commonly diagnosed cancers and a leading cause of cancer-related deaths in men globally.[3] Given its high prevalence and serious health consequences, a thorough understanding of the molecular mechanisms underlying the development of prostate cancer is crucial for the development of effective diagnostic and therapeutic approaches.

A key aspect of prostate cancer research is the substantial association between contemporary epigenetic factors and the development of this disease. Epigenetic alterations, such as DNA methylation changes and chromatin modifications, play a pivotal role in gene expression regulation and can significantly influence the development of prostate cancer.[4]

There is a wide range of therapeutic strategies available for the treatment of prostate cancer, and a key question remains the precision and selection of the therapeutic approach for individual patients. Existing studies indicate the potential use of epigenetic changes as prognostic and predictive markers, aiding in determining disease prognosis and the effectiveness of specific therapeutic approaches.[5,6] This opens opportunities for a personalized approach to prostate cancer treatment based on the consideration of epigenetic factors, optimizing therapy outcomes, and improving patient survival.

2. Treatment and Management of Prostate Cancer

Current approaches to the treatment of localized prostate cancer include surveillance, prostatectomy, and radiation therapy. The selection of prostate cancer treatment relies on various factors, including the prostate-specific antigen (PSA) level, TNM clinical stage, and Gleason score. Additionally, considerations such as the individual's baseline urinary function, existing medical conditions, and age play a significant role in determining the most suitable course of action.[7,8] The occurrence and fatality of prostate cancer globally are associated with advancing age, with an average diagnosis age of 66 years. Age-standardized rates were notably higher in Oceania (79.1 per 100 000 people) and North America (73.7), followed by Europe (62.1). In contrast, Africa and Asia have lower incidence rates compared to developed countries (26.6 and 11.5, respectively).[9] While only 1 in 350 men under 50

will be diagnosed with prostate cancer, the diagnosis rate surges to 1 in 52 men aged 50 to 59. For men over 65, the incidence rate is nearly 60%.[10] Prostate cancer mortality increases with age, with almost 55% of all deaths occurring after the age of 65.[9] Pettersson et al. illustrated in their study that the five-year mortality rate due to prostate cancer rises from 7% at 65 years of age to 25% at 80 years.[11] Bernard et al. delineated the impact of advancing age on the survival rates of individuals with metastatic prostate cancer.[12] This effect was particularly pronounced among men diagnosed at 75 years and older. Hall et al., in their investigation, demonstrated how the presence of additional health conditions elucidates the diminished relative survival of elderly men with prostate cancer.[13] Their findings concluded that while comorbidity is a contributing factor to poorer survival among older men, those with severe additional health issues were less likely to be offered potentially curative treatments.

Variations in PSA values for diagnostic purposes across different global regions have garnered significant attention in several studies. For instance, in Western populations, the positive predictive value (PPV) of PSA within the 4–10 ng mL^{-1} range stands at ≈32%, rising to over 60% when the PSA level exceeds 10 ng mL^{-1}.[14] In one investigation, the PPV of PSA within the 4–10 ng mL^{-1} range was found to be 15.2%, and 24% in patients with PSA levels ranging from 4 to 20 ng mL^{-1}. Comparable low PPV rates have been observed in the Asian population; a study conducted on symptomatic men from Korea revealed a PPV within the PSA range of 4–10 ng mL^{-1} at 15.95%.[15] These observations suggest that racial disparities, genetic variations, and dietary patterns play crucial roles, contributing to substantial differences in the PPV for cancer detection within the PSA range of 4–20 ng mL^{-1}.[16]

In cases where the cancer has progressed to metastatic or locally advanced stage, removal of androgens by surgical or pharmacological castration can lead to a sustained remission. However, stage IV castration resistance, characterized by genetic mutations in the androgen receptor (AR), inevitably leads to a poor prognosis.[17] Depending on the stage and Gleason score of prostate cancer, treatment may involve a combination of different approaches.[18] For localized prostate cancer with low Gleason score and low risk of tumor spread, patients may undergo active surveillance or an "expectant" approach, where immediate treatment is not administered but regular monitoring of disease indicators such as PSA levels is conducted. For higher Gleason scores and/or advanced stages of cancer, combined treatment approaches may include radical prostatectomy (surgical removal of the prostate gland), radiation therapy, and/or hormonal therapy. Hormonal therapy can involve the use of androgen deprivation therapy (surgical or pharmacological castration) to lower the levels of androgens, which fuel the growth of prostate cancer cells.[18] This combined approach aims to target the cancer from multiple angles and improve treatment outcomes.

2.1. Active Surveillance

Active surveillance (AS), recommended for low-risk localized prostate cancer (Gleason score ≤ 6), considers factors like age, cancer volume, patient preferences, and ethnicity. Monitoring PSA levels, digital rectal examination, and biopsies are key within active surveillance protocols.[24–26]

In one study, 1.433 patients were under active surveillance (AS), revealing that those ≤60 years were less likely to require treatment after 5 years.[32] Analyzing the SEER database, a rising trend of AS use in younger men was observed, increasing from 22% in 2010 to 58% in 2015.[33] Prostate cancer-specific mortality rates at 5 years were <0.30% across all age groups regardless of initial treatment strategies.[33] AS is increasingly preferred in younger patients due to their better urinary and sexual function. Previously, younger patients with favorable prostate cancer typically underwent definitive local therapy, but recent trends show greater acceptance of AS in this group.[33] Studies indicate that younger men managed under AS do not exhibit increased progression risks.[34] Druskin et al. reported a 5-year reclassification rate of biopsy grade to group 3 or higher: 4% in men <60. 7% in those 60–69, and 14% in those ≥70 years old ($p < 0.001$), which is consistent with therapeutic intervention rates in our series.[34]

Its benefits include preserving erectile function, reducing treatment costs, and maintaining life quality. However, it may lead to cancer metastasis before treatment, missed treatment opportunities, complex therapy for larger cancers, reduced potency post-surgery, increased patient anxiety, and frequent medical check-ups.[27]

Active surveillance has been acknowledged as a safe approach to managing low-risk prostate cancer patients for a duration of 5–10 years.[18] The feasibility assessment of the active surveillance protocol was conducted within a study encompassing 993 patients, ranging in age from 41 to 89 years (median age of 67.8 years), who were diagnosed with prostate cancer and exhibited low risk (Gleason score 6 – grade 1, PSA <10 ng mL^{-1}).[35] The median duration of observation from the time of diagnosis was 6.4 years (ranging from 0.2 to 19.8 years). Among the participants, 149 patients (15%) succumbed to mortality, with 15 (1.5%) attributed to prostate cancer. The 10- and 15-year overall survival rates were determined to be 80% and 62%, respectively. Notably, 13 patients developed metastatic disease. PSA monitoring was performed at 3-month intervals for the initial 2 years, followed by semiannual assessments for stable patients. Confirmatory biopsies were administered within 12 months of the initial biopsy and subsequently, every 3–4 years until the patients reached 80 years of age. The investigation identified an erroneous determination of disease stage based on the Gleason score as a primary limitation of active surveillance. The potential for earlier and more accurate identification of patients classified within Gleason grades 4 and 5 is anticipated to enhance the outcomes associated with active surveillance.[35]

2.2. Radical Prostatectomy

The goal of Radical prostatectomy (RP) by any approach is the eradication of cancer while, whenever possible, preserving pelvic organ function.[36] Age is a significant factor affecting postoperative outcomes, including urinary continence and sexual potency. Numerous studies suggest that while long-term continence rates following RP remain relatively consistent across different age groups, older patients tend to face more severe challenges with

postoperative erectile dysfunction.[37,38] It is important to note that these findings are often based on comparisons that overlook baseline differences; for example, older patients frequently experience poorer erectile function even before undergoing surgery.[39] It is highlighted that although younger patients initially exhibited better potency scores, they were more susceptible to a significant decline in erectile function.

The procedure involves removing the entire prostate with its capsule intact and SVs, followed by vesico-urethral anastomosis. Surgical approaches have expanded from perineal and retropubic open approaches to laparoscopic and robotic-assisted techniques; anastomoses have evolved from Vest approximation sutures to continuous suture watertight anastomoses under direct vision and mapping of the anatomy of the dorsal venous complex and cavernous nerves has led to excellent visualization and potential for preservation of erectile function. However, there are a few complications associated with its use. These complications include incontinence and erectile dysfunction arising from surgical damage to the urinary sphincter and erectile.[40]

Despite the range of complications (**Table 1**), radical prostatectomy remains the established standard for treating prostate cancer.[10] A study of 6.485 men diagnosed with localized prostate cancer in Missouri and Ohio showed a 10-year survival rate of 88.9%, with a 1.8% prostate cancer-specific mortality rate.[31]

In a retrospective study of 22.033 men who had radical prostatectomy between 2005 and 2015 (without additional radiation or hormone therapy), 27% experienced biochemical recurrence (BCR). Among those with BCR, 11% developed metastases. The 5-year and 10-year survival rates without metastases after BCR were 91% and 77%, respectively. Of those who developed metastases, 35% died from the disease. The likelihood of biochemical recurrence post-prostatectomy is ≈1 in 4.[32] Presently, no predictive or prognostic markers significantly affect overall patient survival.

2.3. External Beam Radiation Therapy

External Beam Radiation Therapy (EBRT) is a widely used treatment for prostate cancer.[10] This method delivers high-energy X-rays or protons from an external machine to the specific prostate area needing treatment.[33]

EBRT has shown favorable outcomes in cancer control and long-term survival rates. Studies have reported high rates of biochemical control, with many patients achieving undetectable PSA levels post-EBRT. Treatment outcomes following EBRT in men of various age groups have produced conflicting findings. Some studies suggest a decline in sexual, bowel, and urinary functions among older individuals post-treatment, while others have reported no significant impact on these parameters.[41,42] However, distinguishing between age-related changes and treatment-related deterioration in sexual function can be challenging. Research indicates that younger age and better pre-treatment sexual function are linked to a higher likelihood of functional erections 2 years after EBRT.[43] Conversely, earlier research has suggested that older men may experience a more rapid decline in functional outcomes compared to their younger counterparts.[44] It has demonstrated similar effectiveness to other treatments like surgery or brachytherapy in disease-free and overall survival. For instance, a study of 265 patients with high-risk localized prostate cancer showed an 88% 10-year cancer-specific survival rate after EBRT alone. High-risk disease criteria included PSA ≥ 20 ng mL^{-1}, clinical stage ≥ T3N0M0, or a biopsy Gleason score from 8 to 10.[34]

However, similar to any medical intervention, EBRT is associated with potential side effects. The severity of these side effects can vary depending on individual factors, treatment techniques, and dose levels. Common side effects of EBRT for prostate cancer include urinary symptoms (such as increased frequency, urgency, or temporary irritation), bowel changes (such as diarrhea or rectal bleeding), fatigue, and erectile dysfunction.[45,46] It is important to consider these potential complications when assessing the overall benefits and risks of EBRT as a treatment option for prostate cancer.

2.4. Brachytherapy

Brachytherapy involves the placement of radioactive sources, such as seeds, into the prostate gland with the guidance of ultrasound or computed tomography. There are two types of brachytherapy: low-dose rate (LDR) and high-dose rate (HDR). LDR brachytherapy entails the permanent implantation of radioactive seeds in the prostate. It is generally agreed that patients with the best outcomes after LDR monotherapy are those with low or favorable intermediate-risk prostate cancer and good urinary function, as defined by an International Prostatic Symptom Score (IPSS) of <12 and a maximum flow rate exceeding 15 mL min^{-1} on urinary flow tests, according to the National Comprehensive Cancer Network (NCCN) definition.[47] This method offers advantages such as a single implantation and targeted delivery of a maximum dose to the prostate tissue. However, it has limitations in cases of large prostate volume, urinary impairment, and aggressive forms of cancer. Persistent irritant urinary symptoms are often observed as a disadvantage.[48]

The rates of 5-year freedom from biochemical failure in patients with low, intermediate, and high-risk diseases are reported to be >85%, ranging from 69% to 97%, and ranging from 63% to 80%, respectively.[49,50] Both low-dose-rate (LDR) and high-dose-rate (HDR) brachytherapy are also employed as salvage treatments following primary definitive therapy for prostate cancer. The rates of biochemical control in these series vary between 34% and 89.5% over a duration of 3 to 10 years.[50] The following was found when considering age-related aspects in the use of brachytherapy. In a long-term study conducted on younger men (<60 years) who underwent brachytherapy, it was noted that age did not predict the outcome.[51] They observed similar recurrence rates across age groups, suggesting that brachytherapy is an appropriate primary treatment option for all ages. Recent studies also support these findings.[52]

2.5. Cryotherapy

Cryotherapy is a treatment option for prostate cancer that involves freezing the prostate tissue to induce cell death in cancerous cells. The procedure involves the insertion of thin metal probes through the skin and into the prostate. These probes are

Table 1. Prostate cancer treatment, potential adverse effects, and epigenetic biomarkers.

Treatment	Disease Progression	Potential Adverse Effects	Efficacy	The frequency of occurrence of side effects [%]	Reference
Active surveillance	Localized	Risk of clinical progression	Moderately effective (recommended as a treatment option for patients with low-risk PCa)	0,2–5%	[19,20]
Radical prostatectomy	Localized	Erectile dysfunction urinary incontinence	Highly effective	4-46%	[21]
External beam radiation	Localized and advanced disease	Urinary urgency and frequency, dysuria, diarrhea Urinary incontinence and proctitis Erectile dysfunction	Highly effective	7,2–14,4%	[22]
Brachytherapy	Localized	Urinary urgency and frequency, dysuria, diarrhea Erectile dysfunction Urinary incontinence and proctitis	Highly effective	2,2–40,9%	[23,24]
Cryotherapy	Localized	Erectile dysfunction Urinary incontinence and retention Rectal pain and fistula bladder neck stricture/stenosis	Not applicable (data variability)	0–88%	[25,26]
Hormone therapy	Advanced	Fatigue Hot flashes and flare effect Hyperlipidemia Insulin resistance Cardiovascular disease Anemia Osteoporosis Erectile dysfunction Cognitive deficits	Highly effective	1,4–90%	[27]
Chemotherapy	Advanced	Myelosuppression Anemia Fatigue Vomiting Constipation Hypersensitivity reaction Gastrointestinal upset Peripheral neuropathy	Moderately effective	1–94%	[28]
HIFU		Urinary retention, erectile disfunction, uretheral stricture, rectal pain or bleeding, rectoureteral fistula and urinary incontinence	Highly effective	10–23%	[29]
Drugs targeting to epigenetics	Localized	Disseminated intravascular coagulation, anemia, diarrhea, nausea, febrile neutropenia, CNS cerebrovascular ischemia, hyperbilirubinaemia	Not applicable	5–50%	[30]
Potential epigenetic biomarkers	Localized and advanced disease	Not applicable	Moderately effective	Not applicable	[31]

filled with a gas that freezes the surrounding prostate tissue. However, the use of cryotherapy has been associated with various complications, including urinary incontinence, urinary retention, erectile dysfunction, fistula formation, and rectal pain.[53]

Despite being one of the treatment options for prostate cancer,[18] studies evaluating the effectiveness of cryotherapy have yielded inconsistent results, suggesting a lack of consensus regarding its efficacy. A thorough systematic review and meta-analysis[54] revealed that there were no statistically significant differences observed in terms of time to death from prostate cancer and overall mortality when comparing whole gland cryotherapy to radiotherapy and whole gland cryotherapy to radical prostatectomy. However, it should be noted that the available studies do not provide evidence supporting the superiority of cryotherapy over radiotherapy in men with clinical stage T2 and T3 prostate cancer, without locoregional or distant metastases, and with PSA

levels below 25 ng mL^{-1}.[55] A meta-analysis involving 1595 men with localized prostate cancer, aged between 60.5 and 69.5 years, and with a mean PSA level ranging from 5.1 to 7.8 ng mL^{-1}, was conducted. The study included men from all risk groups, and the median follow-up duration varied from 13 to 63 months. Among the cohorts that required biopsies 6 to 12 months after treatment, 216 out of 272 men (79%) underwent biopsy, with 47 of them (21.8%) showing positive results. Among these, 10 men had an elevated Gleason level of 7 or higher. Overall, two men had developed metastatic disease, and none of them died as a result of prostate cancer.[25] The overall patient survival rate for localized prostate cancer treated with cryotherapy varies across different studies, ranging from 61.3% to 100%. The overall recurrence rate has also shown variability, ranging from 15.4% to 62%.[26]

The observed variability in data could potentially be attributed to several factors, including the heterogeneity of patient populations, variations in patient management algorithms, and the lack of reliable predictive markers for patient selection in cryotherapy. These factors can contribute to differences in treatment outcomes and result in a wide range of reported survival rates and recurrence rates in studies evaluating cryotherapy for localized prostate cancer.

2.6. Hormonal Therapy

Hormone therapy, also known as androgen deprivation therapy (ADT), is a commonly employed approach for treating advanced and/or metastatic prostate cancer. By blocking testosterone production, the fuel source for tumors is disrupted. Consequently, the substantial reduction in male hormone levels plays a critical role in inhibiting androgen action at the AR, forming the foundation of this therapeutic strategy.[56]

Bilateral orchiectomy, which involves surgical removal of the testes, and medical castration, achieved through the administration of analogs or antagonists of luteinizing hormone-releasing hormone (LHRH), are both equally effective methods.[57] LHRH analogs primarily stimulate pituitary receptors, leading to increased luteinizing hormone and follicle-stimulating hormone levels. This initial stimulation is then followed by suppression of pituitary receptors, resulting in decreased levels of luteinizing hormone and follicle-stimulating hormone, thereby inhibiting testosterone production. Common LHRH agonists include leuprolide, goserelin, triptorelin, and histrelin. On the other hand, antagonists act by blocking pituitary receptors, leading to immediate inhibition of testosterone synthesis.[58] Clinical studies demonstrate an increase in overall survival among patients undergoing androgen deprivation therapy compared to those who do not receive such treatment, with survival rates ranging from 53–80% and 38–78% respectively.[59] Hormone therapy becomes increasingly indicated as patients age, with metastatic prostate cancer being the unequivocal indication for its use.[60] In cases of stage T3,4 or in stages T1–4, monotherapy using antiandrogens might be beneficial for patients aged over 70 years as an alternative to gonadotropin-releasing hormone analogs.[61] One study revealed no difference in tumor-specific survival among patients aged 60 to 80 years with locally advanced prostate cancer, provided they received hormone therapy solely for prostate cancer progression.

Despite its effectiveness, ADT is associated with both acute and long-term side effects. These include hyperlipidemia, fatigue, hot flashes, the flare effect (initial transient tumor growth), osteoporosis, insulin resistance, cardiovascular disease, anemia, and sexual dysfunction.[60]

When utilizing androgen deprivation therapy (ADT) as a treatment option for prostate cancer, it is of utmost importance to carefully consider and effectively manage the potential complications associated with this approach. Furthermore, to advance the current standard of care, it is crucial to take into account an individual's unique androgen physiology, along with the recently recognized genetic factors that influence peripheral androgen metabolism, including the inheritance of prostate cancer.

2.7. Chemotherapy

Chemotherapy plays a limited role in the treatment of prostate cancer and is typically reserved for advanced or metastatic cases. Chemotherapy is often employed when the cancer has spread beyond the prostate gland and into other parts of the body, or when other treatment options have failed to control the disease.[61]

In summary, chemotherapy is not a primary treatment option for localized prostate cancer but is commonly utilized in advanced or metastatic cases. Within a cohort of 4295 patients diagnosed with metastatic prostate cancer, a subgroup of 905 individuals (21.1%) received chemotherapy while the remaining 3390 patients (78.9%) did not. Notably, the group of patients who underwent chemotherapy exhibited significantly improved overall survival rates (61.6% vs 54.3%) at the 30-month mark. These findings suggest that chemotherapy administration is associated with a noteworthy survival benefit in patients with metastatic prostate cancer compared to those who did not receive chemotherapy.[62] Also, chemotherapy can be combined with hormone therapy to enhance treatment efficacy.[61]

Currently, there is not substantial evidence indicating a considerable decrease in the efficacy of chemotherapy among older individuals. For instance, a study investigated the impact of docetaxel combined with ADT on both older and younger patients in terms of the time taken for the onset of castration-resistant prostate cancer (CRPC) and clinical progression. Specifically, among older men, the median duration to develop CRPC was 29.2 months for the group receiving ADT + Docetaxel, compared to 14.7 months for the ADT alone group. In younger men, the median duration to develop CRPC was 18.1 months for the ADT + Docetaxel group and 11.4 months for the ADT alone group.[65] A study report highlighted that the hazard ratios (HR) for mortality in those treated with docetaxel + ADT versus ADT alone showed the benefits of docetaxel in both age groups (hazard ratios of 0.45 for older men and HRs ranging from 0.71 to 0.95 for younger men), as mentioned in a manuscript.[66] It is important to note that chemotherapy for prostate cancer can be associated with various side effects. These can include fatigue, nausea, vomiting, hair loss, decreased blood cell counts, and increased susceptibility to infections.[28] However, advancements in supportive care and the development of newer chemotherapy agents with improved tolerability profiles have helped mitigate these side effects to some extent.

While chemotherapy can be associated with side effects, ongoing advancements in supportive care and personalized medicine hold promise for improving outcomes in prostate cancer patients receiving chemotherapy. Furthermore, ongoing research efforts are focused on identifying specific genetic markers and molecular targets that may aid in tailoring chemotherapy treatments to individual patients of HSD3B1.[63]

2.8. HIFU

High intensity focused ultrasound therapy (HIFU) is also part of the protocol for treating prostate cancer.[18] This non-invasive technique uses focused ultrasound waves to excite thermal energy in the prostate gland, allowing precise and targeted destruction of cancerous tissue while minimizing harm to adjacent healthy structures. A notable advantage of HIFU therapy is its ability to provide highly localized and precise treatment.[64]

HIFU therapy has demonstrated significant efficacy in achieving cancer control, characterized by favorable disease-specific survival rates and low cancer recurrence rates. A prospective study conducted in the United Kingdom from 2005 to 2020 involved 1379 patients diagnosed with nonmetastatic prostate cancer who underwent HIFU therapy. The patients were followed up for a minimum period of 6 months across 13 participating centers. Among these patients, a subgroup of 252 individuals received re-treatment with focal HIFU due to residual or recurrent cancer. The study findings revealed a 7-year freedom from failure rate of 69%.[65] A retrospective study was conducted on a cohort of 032 patients who underwent focal ablation as treatment for stage T1-T3 prostate cancer within the time frame of 2005 to 2017. The study evaluated the overall survival and recurrence-free survival rates over a period of 96 months. The results showed an overall survival rate of 97% and a recurrence-free survival rate of 46%. Notably, no significant difference in outcomes was observed between the two treatment approaches.[29] Moreover, this treatment method has a favorable safety profile, demonstrating lower rates of urinary incontinence and erectile dysfunction compared to surgical interventions such as radical prostatectomy. Common side effects of HIFU therapy for prostate cancer may include urinary symptoms such as frequency and urgency, as well as transient rectal discomfort or irritation.[29]

3. Prostate Cancer Epigenetics for Targeted Therapy

Epigenetic traits are inherited phenotypes due to changes in chromosomes or DNA alterations without changes in DNA sequence.[66] Epigenetic regulation – a number of factors are involved, including those that are posttranslational modifications to DNA or histone proteins, thus determining whether a given gene will be turned on or off. Epigenetic changes can occur in the promoter regions that encircle the transcription initiation site as well as in the enhancer regulatory regions. Thus, for instance, ubiquitination, acetylation, methylation, and phosphorylation, as well as many other epigenetic alterations, play an important role in transcription, DNA repair, and replication.[2] Regulation by DNA methylation has been the most explored mechanism for a number of decades.[67] Several recent studies have revealed that epigenetic upregulation may also involve chemical changes in the histone proteins that constitute chromatin.[68] In exploring the genetic landscape of Clonal Hematopoiesis of Indeterminate Potential (CHIP), researchers have discovered that most mutations occur in pre-leukemic driver genes, which intermittently participate in the pathogenesis of hematologic malignancies and other disorders.[73] The natural acquisition of mutations throughout life leads to the emergence of genetically diverse HSPCs. Among the mutations observed in CHIP, those affecting epigenetic modifiers such as DNMT3A, TET2, ASXL1, and JAK2, with TET2 being the most well-known.[73] It is speculated that one mechanism contributing to treatment resistance is hypermethylation caused by TET2 deletion, inducing a stem cell-like dormant state, and reducing cell sensitivity to chemotherapy agents like cytaraFbine and doxorubicin.[74] Employing hypomethylating agents to specifically target TET2 mutant cells could potentially enhance the sensitivity of these cells to chemotherapy.[75] Despite the widespread presence of mutations linked to CHIP, most individuals with CHIP never develop it, suggesting that environmental factors are critical in determining clonal expansion. Regarding the potential use of epigenetic manipulation on tumor-modulating genes, novel methods for modulating the bone marrow microenvironment deserve consideration to reduce the selective advantage of specific CHIP clones.[73] This highlights the need for a comprehensive approach to prostate cancer therapy, considering individual patient characteristics and innovative disease management strategies.

3.1. DNA Methylation

DNA methylation is the outcome of binding of a methyl group to C5 cytosine residues in CpG dinucleotides, which is linked to gene silencing.[69] The DNA methylation phenomenon was thought to be non-significant, and more strongly related to closed chromatin and transcriptional silencing. However this has been shown to be variable; methylation is highly sensitive to the genomic context, and DNA methylation in promoters and enhancers or in recurrent sequences plays a repressive role, whereas DNA methylation targeting major genes often leads to increased transcriptional reactivity.[70] DNMT (DNA methyltransferase) enzymes catalyzes the methylation of 5-methylcytosine (5mC) within DNA. This processing can be terminated by the DNA demethylase of the TET (ten-eleven translocation) family.[67] Ultimately, several studies that demonstrate a positive correlation between the development of PC and methylation provide an opportunity to inhibit this process and to use epigenetic changes as a promising target in the treatment of malignant neoplasms.[71] Drugs like azacitidine (5-Aza) and decitabine are DNMT inhibitors that have been developed to counteract aberrant DNA hypermethylation (**Figure 1**). In experiments with PC3 xenografts, followed by administration of azacitidine revealed a 36.7% decrease in tumor weight in comparison with the control ($p < 0.001$) and a retardation of tumor growth by an average of 5.5 days.[72] Azacitidine and decitabine (NSC127716) have undergone a series of clinical trials in the treatment of PCa. The possibility of combining DNMT inhibitors with other anti-tumor drugs is also remarkable. Combination therapy of docetaxel and

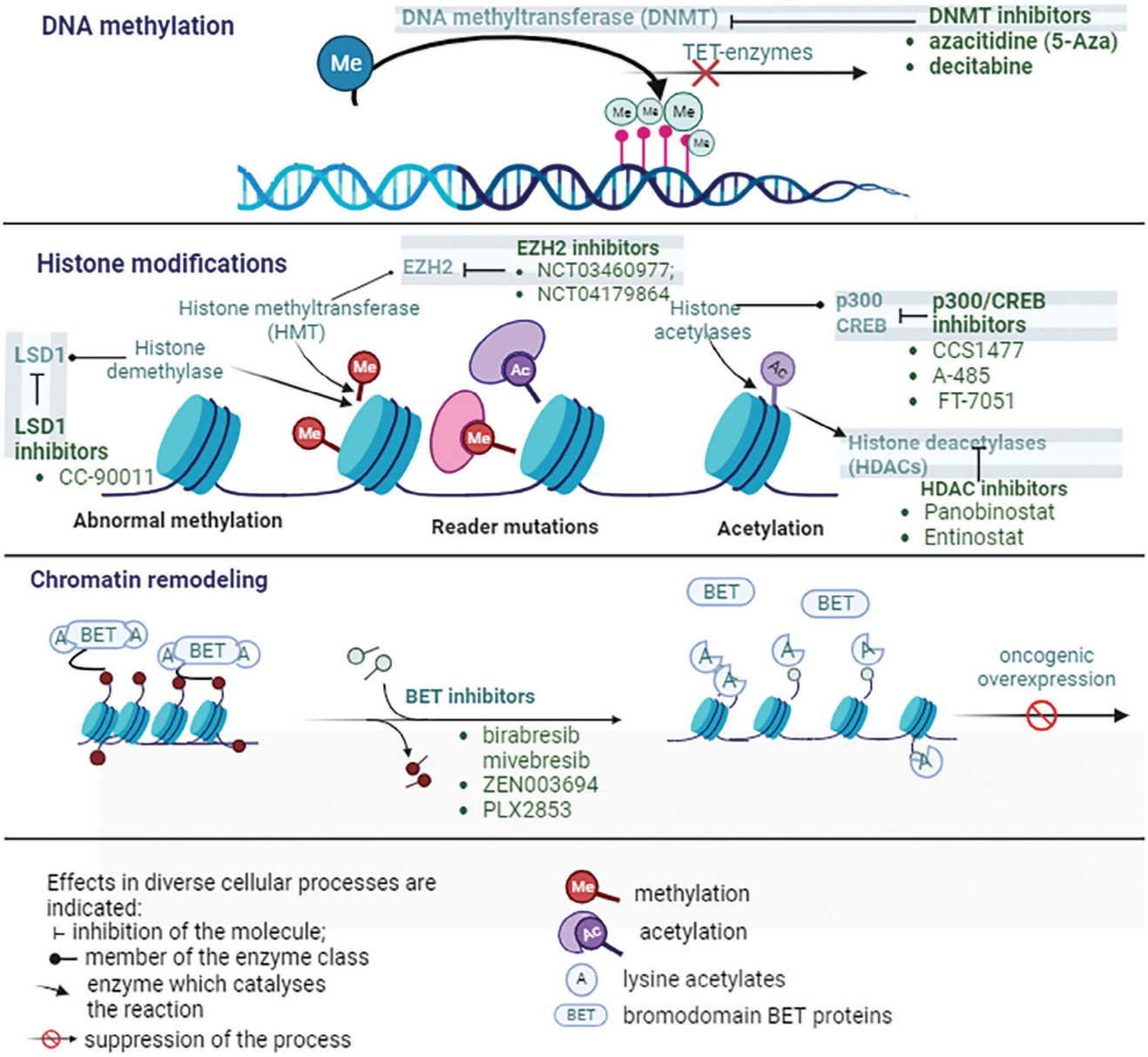

Figure 1. Epigenetic alterations in cancer and therapeutic targeting of key prostate cancer pathways.

cisplatin with azacitidine for 7 days was more effective in PC3 and 22rv1 xenograft models compared to monotherapy. For example, docetaxel and cisplatin in monotherapy reduced PC3 tumor weight by 41.0% ($p < 0.001$) and 22.2% (p 0.01) and caused inhibition of tumor growth by 5.1 and 2.1 days, respectively, compared with controls. For example, a clinical trial of azacitidine and docetaxel was performed, fifteen patients with mCRPS were treated in phase I and seven in phase II. In the phase I study, the azacitidine and docetaxel dosages were successively increased in a routine 3 + 3 regimen. The research showed a response in a reduction of PSA levels in 52% of patients in phase II.[73] The importance of identifying further drug candidates for targeting DNA methylation and performing clinical trials to see whether all patient groups are most likely to provide a therapeutic response to therapy remains unchallenged.

3.2. Histone Modifications

3.2.1. Histone Acetylation

The binding of the acetyl group to histone lysine residues involving both open and active chromatin is a process of acetylation. This process usually correlates with activation of transcription, while deacetylation of histones is usually associated with gene silencing. Cang et al. first reported that in certain areas of prostate

tumor tissue samples, the level of histone H3 acetylation is low compared to the level in normal tissue samples.[74] However, one study found that the stimulating effects of androgens result in alterations in the histone pattern and other AR targets, facilitating their transcriptional activation.[63] The binding of the acetyl group to histone lysine residues involving both open and active chromatin is a process of acetylation. This process usually correlates with activation of transcription, while deacetylation of histones is usually associated with gene silencing. For instance, super-enhancers, a cluster of enhancers characterized by high levels of H3K27ac, play a crucial role as a cancer driver in tumor cells.[62] However, one study found that the stimulating effects of androgens result in alterations in the histone pattern and other AR targets, facilitating their transcriptional activation.[63] The binding of the acetyl group to histone lysine residues involving both open and active chromatin is a process of acetylation. This process usually correlates with activation of transcription, while deacetylation of histones is usually associated with gene silencing. For instance, super-enhancers, a cluster of enhancers characterized by high levels of H3K27ac, play a crucial role as a cancer driver in tumor cells.[62] However, one study found that the stimulating effects of androgens result in alterations in the histone pattern and other AR targets, facilitating their transcriptional activation.[63]

This comes about because the AR recruits HATs (histone acetylases) and coactivators with HAT activity, such as p300 and CREB binding protein, to the promoter and enhancers of the kallikrein 3 (KLK3) gene, (encoding PSA).[63] At the time when HDMT (histone demethyltransferases) can also be recruited to the promoter and enhancers of the kallikrein 3 (KLK3) gene (encoding PSA) target genes are crucial for AR action.[64] It is remarkable that p300 and CBP, including AR target genes, have a crucial role in the up-regulation of key genes, based on which inhibitors of p300 and CBP have been discovered.[65] Thus, p300 and CBP inhibitors (known from clinical trials as CCS1477, A-485, and FT-7051) have been developed (Figure 1). A research study by Lasko et al. on the example of androgen-dependent CRPC xenografts in vivo revealed the application of A-485 to block their signaling and growth, indicating its potential effect on histone acetylation. Based on these advances, A-485, a new catalytic inhibitor of p300/CBP with improved efficacy and specificity above previous histone acetyltransferase inhibitors, has been discovered.[66] An investigation on the effect of A-485 on pituitary adenoma of growth hormone using a subcutaneous xenograft model with GH3 cells in BALB/c nude mice is available. The administration of A-485 significantly reduced the tumor growth in comparison with its volume (by 32.37% decreased in the 50 mg kg^{-1} – Group 1, and by 54.15% in the 100 mg kg^{-1} – Group 2). The decrease in tumor weight was observed in the following proportions (by 43.83% in Group 1 and by 61.41% in Group 2) in comparison with the control.[67] It is also remarkable that a combination of A-485 and an anti-PD-L1 drug, whereby in a syngeneic prostate cancer model an increase in T-cell penetration into the tumor was observed, leading to an enhanced tumor immune response. It was also found that in addition to inhibiting p300/CBP, A-485 also inhibited exosomal PD-L1 secretion by tumor cells. The above data highlights the promise of combining histone acetyltransferase inhibitors with immunotherapy. Clinical trials of p300/CBP histone acetyltransferase inhibitors in prostate cancer (NCT03568656 and NCT04575766) are currently in progress.[66]

3.2.2. Histone Deacetylation

There is another class of proteins that participate in histone acetylation, known as HDACs (histone deacetylases).[68] The expression of numerous genes is regulated by the process of acetylation or deacetylation of histone proteins and transcription factors.[69] For instance, hyperacetylation of lysine residues of histone proteins causes relaxation of chromatin and activates gene expression. Both histone and non-histone proteins are various targets for HDACs, which not only adjust chromatin activity but also manage apoptosis, cell cycle progression, and differentiation. The association of HDACs with regulatory processes indicates their role in cancer phenotypes.[70] Drugs targeting HDACs have been extensively tested in both preclinical and clinical trials in prostate cancer. One of the most well-known drugs from this group is panobinostat, which was approved by the Food and Drug Administration in 2015 for the treatment of myeloma[71] (Figure 1).

In addition, panobinostat has also been studied for the treatment of other types of cancer such as CTCL (cutaneous T-Cell lymphoma), AML (acute myeloid leukemia), MDS (myelodysplastic syndrome), thyroid carcinoma, colorectal cancer, and prostate cancer. Its effectiveness and safety have been validated in more than 50 clinical trials.[72] In clinical research on multiple myeloma, patients who had received at least two lines of treatment and were relapsed/refractory to bortezomib were studied. The results showed a combination therapy of panobinostat with bortezomib and dexamethasone produced at least a partial response in 34.5% of patients in one clinical trial, and the median progression-free survival (PFS) was 5.4 months.[73] For example, in a phase 2 study including 38 patients, only two achieved a clinical response (1 partial response, 1 minimal response); both patients had a long-term response of 19 and 28 months, respectively.[74]

Nevertheless, information on the effectiveness of panobinostat in the treatment of solid tumors is also limited. The best clinical responses have been observed for myeloma and leukemia therapy, whereas an inadequate response to HDAC inhibitors has been shown in solid tumors.

Clinical trials of HDAC inhibitors as monotherapy have not revealed significant activity.[75] The combinations of bicalutamide with LBH589 in a clinical trial illustrated a conceivably effective strategy to increase tumor sensitivity to AR simultaneously.[76] In the case of CRPC treatment, an overall reduction in PSA from the initial level was found in 12 patients, with a dose of 40 mg (group A) showing a reduction of 44%, and a dose of 20 mg (group B) showing a reduction of 28% out of 55 patients. Notably, 10 (38%) cases of radiographic regrowth were observed in group B compared to 3 (10%) cases in group A at the first checkpoint of the protocol after 12 weeks, in comparison with 3 (10%) cases in group A at the first protocol reference point after 12 weeks of treatment.[76] This is one of the prerequisites for further research into HDAC inhibitors in co-therapy together with AR inhibitors.

3.2.3. Histone Methylation

Histone methylation is catalyzed by histone methyltransferase (HMT). This process may be coupled to both activation and repression of gene expression, depending on the position of amino acid residues in the protein and the level of methylation.[77,78]

Interestingly, decreased levels of trimethylation of lysine 4 histone 3 and monoacetylation of lysine 18 histone 3 are noted in prostate tumor tissue. A prerequisite for a negative prognosis and risk of recurrence in patients with PC is a reduced concentration of products resulting from these processes compared to patients with high levels of these modifications.[78,79] Depending on the part of the histone lysine amino acid engaged, histone methylation can both activate or suppress transcription. The enzyme histone demethylase is normally used to remove methyl groups from histones. For instance, LSD1 is engaged in the demethylation of H3K4me1 and H3K4me2,[80] interfering with AR to promote transcription of cell cycle gene expression (Figure 1).[81,82]

Noteworthy is histone demethylase LSD1; in patients with disseminated PC, its overexpression has been observed.[83] A new LSD1 inhibitor, CC-90011, has been initiated in a clinical trial.[84] In one clinical trial, 34 patients with solid tumors were enrolled for dose expansion, including eight patients with low-grade tumors. Consequently, patients with low-grade tumors (grade 1 or 2) who received CC-90011 treatment more frequently had long-term stable disease (≥4 months), particularly in patients with bronchial neuroendocrine tumors ($n = 6$). Long-term stable disease was also detected in one patient with renal neuroendocrine tumor and one patient having paraganglioma.[85] A clinical trial evaluating the effectiveness of CC-90011 is underway in comparison with other drugs in patients with prostate neoplasms in phase 1 (NCT04628988).[86]

The most studied histone methyltransferase in prostate cancer is EZH2, part of the polycomb repressor complex. In one study, it was found that inhibition of EZH2 reactivates AR signaling and increases tumor sensitization to AR inhibition in tumor specimens.[87] Consequently, several companies have developed EZH2 inhibitors. These studies suggest the potential of combination therapy with EZH2 and AR inhibition. Clinical trials have recently begun on EZH2 inhibitors alone (NCT03460977), in combination with AR targeting agents (NCT04179864, NCT03480646, and NCT03741712), or in combination with immunotherapy (NCT04388852) in prostate cancer.

3.3. Chromatin Readers and Remodelers

Alterations in proteins controlling chromatin conformation are associated with prostate cancer progression.[88,89] Consequently, the deletion of the gene encoding the chromatin remodeler CHD1 is frequently observed in prostate cancer.[88] For instance, the BAF complex adjusts the spatial accessibility of chromatin. Several reports have demonstrated dysregulation of this conformation's subunits in some prostate cancer cohorts.[89] Acetylated lysines are identified by a special bromodomain-containing class of BET proteins, categorized into subtypes BRD2, BRD3, BRD4, and BRDT.[90] Acetylated lysine residues in histones can be bonded to BET proteins via several proteins of the mentioned subtypes, which is a key step in the regulation of transcription.[75]

Remarkably, bromodomain BET proteins can contribute to resistance to AR blockers by binding to glucocorticoid receptor expression regulator.[76] BET inhibitors have been shown to resensitize neoplasms to enzalutamide by affecting glucocorticoid receptor expression in glucocorticoid-dependent mouse xenograft model.[76] In clinical trials, birabresib and mivebresib, which are BET inhibitors, have been studied in patients with solid tumors, including CRPC, but these two drugs have not proven significant antitumor activity in patients with CRPC.[77,78] Another phase Ib/IIa study explored the impact on patients with metastatic castration-resistant prostate cancer (mCRPC) who were resistant to enzalutamide and/or abiraterone of a combined therapy of enzalutamide with BETi ZEN003694[79] (Figure 1). The combination of enzalutamide and ZEN003694 was well tolerated without reaching the maximum tolerated dose, and <5% of patients experienced thrombocytopenia of grade ≥3, prolonged progression-free survival rates of PFS in a subgroup of patients was revealed. Among 75 patients on therapy, the median radiographic progression-free survival (rPFS) was estimated to be 9.0 months (95% CI: 4.6, 12.9) and the composite median clinical progression-free survival was 5.5 months (95% CI: 4.0, 7.8).[79] One recent study proved that molibresib (GSK525762) is sufficiently well tolerated by patients.[80] There are clinical trials underway, such as a phase Ib/IIa study that aimed to examine the combination of BETi PLX2853 with abiraterone or olaparib in metastatic CRRS (NCT04556617). The current clinical trials of BET inhibitors are critical to identify the effects on AR and possible combinations of these drugs with other therapies to further validate their safety and effectiveness.

4. Prognostic and Predictive Epigenetic Biomarkers

Epigenetic changes occur with much higher frequency than mutations and are common in precancerous stages of the disease makes them attractive biomarkers for diagnosis, prognosis, and response to treatment.[81] Prostate cancer is closely connected with epigenetic changes, early detection of which can lead to early detection of the tumor and allow selection of a more effective anticancer therapy.

4.1. DNA Methylation

The goals of numerous studies include the identification of changes in DNA methylation, which can be reliable diagnostic, prognostic, or predictive biomarkers. The use of a prostate cancer biomarker based on DNA methylation is attractive for several reasons: high DNA stability, ease of analysis using current available methods, and the ability to assess biomarkers in tissues and body fluids such as blood, urine, and saliva.[68]

4.1.1. Biomarkers for Tissue Biopsy

One of the most widely described manifestations of epigenetic abnormalities in tumor cells (including prostate) is hypermethylation of CpG islets located in the promoter of the GSTP (glutathione-S-transferase GSTP class pi) gene. The GSTP gene

plays a role in DNA detoxification and protection from oxidants and electrophilic metabolites, and inactivation of this gene leads to the growth of additional mutations.[82] In one study, twenty-seven formalin-fixed and paraffin-embedded specimens were randomly selected from a series of patients who had undergone radical retropubic prostatectomy (mean age 59 years, median Gleason score was 6, pathological stage ranged from pT2N0Mx to pT3aN0Mx). GSTP hypermethylation was found to occur in 70% of highly differentiated prostate neoplasias (PIN) and in >90% of adenocarcinomas.[83] A recent meta-analysis of 22 articles showed significant specificity (89%) but moderate sensitivity (52%) of differential GSTP1 methylation for prostate cancer screening.[84]

The MIEN1 gene (migration and invasion enhancer 1, 17q12) has a high level of expression at various stages and degrees of prostate cancer phenotype compared to normal tissues, making it a convenient biomarker.[85] This gene is responsible for a more aggressive and AD-resistant (androgen deprivation) form of prostate cancer.[86] Inactivation of the DNA methyltransferase gene DNMT1 occurs during disease development, which suppresses MIEN1 expression through methylation in the absence of pathology. Demethylation of the gene leads to its expression, which promotes tumor cell migration and invasion.

In a study conducted by Jerónimo et al.,[102] methylation of the RARβ2 (retinoic acid receptor beta) gene was analyzed by quantitative polymerase chain reaction in tumor tissue of 118 patients with prostate cancer (mean age of patients was 64 years, pathological stage was T1c and T2, average Gleason score of 7), 38 paired high-grade intraepithelial neoplasia of the prostate (HG-PIN), and non-tumor prostate tissue from 30 patients with benign prostatic hyperplasia (BPH). RARβ2 was found to be hypermethylated in 97.5% of prostate cancer and 94.7% of prostatic intraepithelial neoplasia (PIN) cases, whereas only 23.3% of prostate adenoma cases ($p \leq 0.05$). Methylation of the promoter region of the RARB2 gene encoding receptor-mediated tumor growth suppression protein leads to prostate cancer progression. Methylation of the CpG site of this gene is not detected in cells of healthy prostate tissue.[103]

Methylation of the classic tumor growth suppressor gene RASSF1 is an early event in prostate cancer carcinogenesis and increases with disease progression.[104] The consequences of changes in RASSF1A expression include disruption of the cell cycle and cell proliferation. A Texas Medical Science Center study analyzed RASSF1A gene promoter methylation in 101 prostate cancer samples and 32 nonmalignant prostate tissue samples (mean age was 63 years, Gleason scores were 4–10, and all pathological stages were present in the sample). RASSF1A hypermethylation was found to be associated with higher PSA levels and aggressive prostate cancer in 53% of cases.[105] The frequency and degree of RASSF1 methylation correlate with tumor aggressiveness and thus allow predicting the course of the disease.[106]

4.1.2. Biomarkers for Liquid Biopsy

A new prognostic biomarker, PLA2G16, was identified in the work of William E. Jarrard for liquid biopsy. The study sample consisted of 90 urine samples from patients with positive prostate cancer biopsy (TA, mean age 65 years, Grade Group GG $\geq$ 1), and 77 samples from patients without prostate cancer (NTA, mean age 64 years). The work revealed that the PLA2G16 gene, which is a class II tumor suppressor, was hypermethylated in the promoter regions in prostate cancer patients. The sensitivity and specificity of the multivariate model were 92% and 35%, respectively.[107]

A comparative quantitative analysis of methylated DNA of a few biomarkers in urine samples from 90 patients with confirmed prostate cancer biopsy (TA, median age 65 years, Grade Group GG $\geq$ 1), and 77 patients with an unconfirmed diagnosis (NTA, median age 64 years). Methylation levels of islet CpG caveolin 1 (CAV1), homeobox 1 with an even gap (EVX1), fibroblast growth factor 1 (FGF1), gene acyltransferase 3 (PLA2G16) were significantly higher in patients with detectable prostate cancer.[108]

Methylation of the GSTP gene, which was mentioned earlier, can also be used as a biomarker for liquid biopsy.[99]

4.2. Histone Modification

There is increasing evidence for the involvement of histone modifications in the initiation and progression of prostate cancer. Different types of modifications, especially methylation and acetylation, show varying correlations between normal and cancerous samples.[109]

4.2.1. Acetylation of Histones

One of the most common local modifications of histones is the acetylation of histone 3 at the K18 position (H3K18Ac). Abnormal levels of H3K18Ac have been found in breast, colorectal, lung, hepatocellular, pancreatic, thyroid, and prostate cancers. The expression of histone H3K18Ac acetylation and proteins regulating its acetylation (P300) and deacetylation (SIRT2) were evaluated in BPH, highly differentiated prostatic intraepithelial neoplasia (HGPIN), prostate cancer, and metastases. H3K18Ac levels were found to be higher in primary cancer and metastases compared to benign tissues, and elevated H3K18Ac levels identified patients with an increased risk of BPH recurrence ($p \leq 0.05$).[87]

This prognostic significance of H3K18As was confirmed in a cohort study of 279 (pathologic stage pT2 and pT3) prostate cancer cases using Kaplan–Meier analysis, which showed a significant association between levels of acetylated H3K18As and with a 1.71-fold ($p < 0.0001$) increased risk of tumor recurrence.[88]

In another study, it was demonstrated that SIRT7 plays an important role in the aggressiveness of prostate cancer, which means that it is a promising prognostic marker of aggressive prostate cancer. SIRT7 expression was evaluated by immunohistochemistry in malignant and adjacent healthy prostate tissues of 57 patients (sample included all pathological stages, mean age 68 years, GS $\geq$ 6). SIRT7 levels were significantly elevated in tumors, and its expression was positively related to the degree of malignancy. It was also shown that suppression of SIRT7 decreased migration of DU145 and PC3 cells (two androgen-independent prostate cancer cell lines), whereas overexpression of the native protein, but not the mutated form, increased cell migration and invasion of the low-aggressive prostate cancer cell line LNCaP.[89]

Deacetylation of H3K18Ac SIRT7 is critical for maintaining key properties of cancer cells, including exit from contact inhibition and anchorage-independent growth.[90]

Decreased levels of acetylated histone H3K9ac have been associated with tumor progression, histological grade, and clinical stage in prostate tumor, hence associated with a poor prognosis for these patients.[91]

4.2.2. Histone Methylation

The effect of histone methylation on chromatin function depends on the position of the modified amino acid and the degree of its methylation, that is, on the number of attached methyl groups. For example, methylation of lysine 4, 36, and 79 in histone 3 (H3K4me3, H3K36me, and H3K79me) leads to activation of transcription, while conversely, methylation of lysine 9 and 27 in histone 3 (H3K9 and H3K27) causes chromatin to assume a form that blocks gene transcription.[92,93]

Global changes in histone H3 play a role in tumor development and progression by deactivating the expression of specific genes, which makes them an effective biomarker of malignant cell transformation.[94]

Hypermethylation of the histone marker H3K27me3 was detected in 34 prostate malignancies (11 patients with a GS score > 7, 10 with a GS score ≤ 7), compared with 13 morphologically normal prostate specimens. The number of enriched genes with the hypermethylated H3K27me3 tag had a direct correlation with the Gleason number: 386 enriched genes in the control group, 545 genes in the GS ≤ 7 group, and 748 genes in the GS > 7 group.[95]

Similarly, in a study of 71 morphologically healthy tissues and 66 prostate cancer tissues, it was found that upregulation of histone methyltransferase EZH2 correlates with deregulation of H3K27me3 and poor prostate tumor prognosis. The repressive H3K27me3 tag has been found on many gene promoters that are silenced during tumor development.[96]

Another study evaluated H3K4 and H3K9 methylation by immunohistochemistry in a tissue microarray containing 23 benign tumor samples and 113 adenocarcinoma samples (cohort included all pathological stages of disease, 48 patients had GS < 7, 65 had GS ≥ 7). Di- and trimethylation of H3K9 were significantly reduced in cancer tissues. In contrast, all three H3K4 methylation states were elevated in androgen-independent tumors and correlated with clinicopathological parameters. The findings suggest that changes in the overall intensity of certain histone modifications may be closely related to cancer and may have prognostic value for clinical outcomes.[97]

In Seligson D.B., et all, in an analysis of 183 primary prostate cancer tissues (79 patients had a Gleason score ≥ 7, 104 < 7), among which 171 cases had confirmed recurrence data, there was found a positive correlation of H4R3me2 demethylation with tumor malignancy level and predictive of tumor recurrence risk.[91]

The field of research on other types of modifications as predictive and prognostic biomarkers of prostate cancer is less developed currently. Of the work that has been done in this area, we can mention the study of histone yH2AX, which is a phosphorylated form of histone H2AX. yH2AX causes chromatin relaxation and thereby promotes the accumulation of repair factors at the site of damage. Nanni et al.[98] demonstrated the presence of histone yH2AX at different stages of PC and that histone modification is an early factor in disease development. To confirm the prognostic value of yH2AX, immunohistochemical analysis was performed on tissue samples, a group of 20 patients (17 with a diagnosis of prostate cancer and 3 with BPH. Characteristics of the sample: patients ranged in age from 41 to 73 years; range of Gleason score from 6 (3 + 3) to 10 (5 + 5); range of tumor node-metastases pT2b-pT3b. It was recorded that yH2AX was expressed in samples with prostate cancer as opposed to normal/hyperplastic tissue.

Wang, S.-Y., et all demonstrated that H2A.Z ubiquitination is associated with AR gene transactivation and prostate cancer progression.[99]

4.3. MicroRNAs

MicroRNAs in prostate cancer play an important role as biomarkers, exhibit a specific profile, and are used as therapeutic targets. The use of microRNAs as potential prognostic biomarkers of prostate cancer has several advantages: they are detectable in biological fluids such as blood and serum, have high stability and tissue specificity.[88,100,101]

4.3.1. Biomarkers for Liquid Biopsy

The prognostic value of 669 microRNAs was studied by Nguyen et all in 84 serum samples from patients with prostate cancer, including 28 patients with localized low-risk disease, 30 patients with localized high-risk disease, and 26 patients with mCRPC. The authors demonstrated that miR-375, -378, and -141 expression tended to increase with disease progression and was overexpressed in the group of patients with mCRPC. While miR-409-3p expression increased in the high-risk group compared to that in the low-risk group, but significantly decreased in the metastatic form of the disease. Similarly, when comparing samples of primary prostate tumor and normal prostate tissue, the expression of miR-375 and miR-141 was higher in the former variant.[102]

Another study compared the expression levels of 742 microRNAs circulating in the plasma of 25 patients with localized prostate cancer and 25 patients withmCRPC). Analysis showed that miR-141, miR-151-3p, miR-152, and miR-423-3p were associated with poor prognosis and/or higher Gleason score. In addition, miR-141 and miR-152 identified patients with a high probability of developing recurrence after radical prostatectomy, and miR-205 (down-regulated in mCRPC)) was associated with a lower Gleason score and a lower probability of both biochemical recurrence and clinically significant metastatic events.[103]

Docetaxel is the first-line chemotherapy for CRPC Resistance to docetaxel therapy is ≈50% and is determined quite late, so patients who do not respond to treatment are subject to unnecessary toxicity.[104] The purpose of the Lin, H.-M. study was to identify circulating microRNAs as early biomarkers of response to docetaxel in patients with CRPC. As a predictor in a series of 97 patients (mean age 68 years; 7 patients had GS < 7, 69 GS ≥ 7, 21 unknown) with CRPC, the profile of microRNAs, whose levels were measured before and after treatment with docetaxel, was evaluated. It was found that elevated miR-200b levels before treatment

were an independent predictor of overall survival ($p = 0.001$). Unchanged or decreased miR-20a levels after docetaxel treatment was another independent predictor ($p < 0.0005$).[105]

A comparative analysis of exosomal microRNAs isolated from morning urine of 60 patients diagnosed with PC, 37 diagnosed with BPH, and 24 healthy men (age ranged from 49 to 78 years, GS < 6 – 10 patients, GS ≥ 7 – 50, clinical stage T1/T2 – 27, T3/T4 – 33) was performed. The expression levels of miR-145-5p, miR141-5p, miR-1290, and miR-572 were significantly elevated in patients with prostate cancer, and miR-145-5p was directly related to the Gleason scale.[106]

S.Y. Wang et al. showed that miR-19, −345, and −519c-5p levels in tissue and serum are independent prognostic biomarkers that predict unfavorable tissue pathomorphological changes. In patients with prostate cancer in whom dynamic surveillance tactics may have been chosen. Discriminatory ability to distinguish patients with adverse pathology increased from 0.77 to 0.94 when these microRNAs were added to a model including age, PSA level, clinical stage, and number of positive biopsy columns. The study sample consisted of 48 patients with a postoperative pathologic Gleason score of 7 or higher (follow-up group) and 48 patients with a pathologic Gleason score of 6 (control group). The follow-up cohort consisted of 25 cases and 35 controls. The study of the plasma microRNA profile, according to the authors, can optimize the selection of prostate cancer patients for dynamic follow-up.[107]

4.3.2. Biomarkers for Tissue Biopsy

Schaefer et al. identified 15 microRNAs differentially expressed between prostate tumors and adjacent normal tissue from 76 specimens after radical prostatectomy (mean age 63 years, 32 patients GS < 6, 44 GS ≥ 7, pathological stages included T2 and T3). It was found that high miR-96 expression predicted biochemical recurrence after radical prostatectomy and was associated with poor prognosis.[108]

It has been reported that miR221 is one of the most strongly and frequently suppressed microRNAs in primary prostate cancer.[109,110] Kneitz, B. and et all proved the prognostic significance of miR-221 as a biomarker in high-risk prostate cancer. Analysis of two independent cohorts of 134 and 89 high-risk prostate cancer patients showed that dichotomized expression of miR-221 was multifactorial significant in predicting cancer-induced mortality ($p < 0.001$). The suppressor function of miR-221 in prostate cancer was also demonstrated for the first time, in an analysis of the mechanism by which miR-221 promotes tumor cell growth, invasiveness and development, and apoptosis in prostate cancer.[111]

Avgeris et al. evaluated the clinical utility of miR-145 for diagnosis and prognosis of prostate cancer. Prostate tissue samples from 73 patients with PC after radical prostatectomy (24 patients with GS ≤ 6, 39 with GS = 7, 10 GS ≥ 8, pathological stages: pT2a, pT2b, pT2c, pT3a, and pT3b) and 64 patients with BPH after transurethral or open prostatectomy were studied. Decreased expression of miR-145 was recorded in prostate cancer compared with BPH patients. Decreased miR-145 levels in PC correlated with higher Gleason score, advanced clinical stage, larger tumor diameter, and higher PSA levels, with a higher risk of biochemical recurrence and significantly shorter recurrence-free survival.[112]

A recent study measured miRNA expression in prostate tissue samples from 49 patients with prostate cancer and 25 without cancer to determine early prognostic markers of prostate cancer with aggressive progression characteristics. The sample consisted of five patients with WHO grade I, 19 with grade II, and 25 with grade III; clinical stages ranged from T1 to T4. Based on the results obtained in this study, a combination of four microRNAs (mir-96-5p, mir-145-5p, mir-183-5p, and mir-221-5p) was proposed as a prognostic tool in prostate cancer to predict tumor invasion, metastasis, and overall survival. The microRNAs in the panel have a similar expression pattern in prostate cancer; their combined use increases prognostic accuracy and specificity, which is important in heterogeneous prostate cancer.[113]

Mir-96 can also be used as a marker of tumor aggressiveness because the association of Mir-96 with tumor aggressiveness, postoperative outcome, and disease recurrence was revealed.[114] Tissue samples of 50 patients with adenocarcinoma of the prostate and 25 men with BPH) (the mean age of patients with prostate cancer was 76 years, BPH was 71, WHO grade I-III) were examined. 93 additional cases with PC were studied to confirm the result. It was found that miR-96 expression was significantly higher in patients with prostate cancer and correlated with WHO grade, and with overall life expectancy: patients with low miR-96 levels lived 1.5 years longer than patients with high miR-96 levels.

Mir-21 is known to be associated with prostate cancer recurrence in many groups. For example, Leite, K. R. M. et all identified microRNAs in 53 samples of prostate cancer from patients who had undergone radical prostatectomy for localized cancer. A comparison of microRNA profiles between patients who had biochemical recurrence and those who did not found that miR-21 overexpression was associated with increased biochemical recurrence after surgical treatment of prostate cancer.[115,116]

When the 1435 microRNA profile of a randomly selected 30 patients from a total cohort of 535 patients undergoing radical prostatectomy was evaluated, only the Mir-21 expression level was significantly increased and further studied as a prognostic marker for the entire cohort. Mir-21 expression was found to be associated with poor biochemical recurrence-free survival after radical prostatectomy.[103]

One study examined the expression of miR-153 in tissue samples obtained through prostatectomy in 29 patients with metastatic cancer and 32 patients with localized (stages I, II) PCA. Researchers identified the prognostic ability of miR-153 with respect to the development of metastases (AUC = 0.85; 95% CI 0.75–0.95; sensitivity = 0.72, specificity = 0.86)).[117]

5. Conclusion

Epigenetic changes are considered to be one of the first genomic aberrations that occur during the development of cancer and are closely related to its progression, despite the fact that carcinogenesis is extremely heterogeneous by nature and is caused by multifactorial influences. Numerous evidence of the contribution of epigenetics to the pathogenesis of prostate cancer, as well as the reversible nature of epigenetic modifications, allow them to be used as promising targets for targeted therapy at different stages of the disease. Modern developments of epigenetic an-

ticancer drugs include regulators of epigenetic transcription of DNA and RNA, aimed either at DNA methylation or at certain histone-modifying enzymes. It is interesting to note that DNA methylation, apart from its role as a biomarker, contributes to cancer development by fostering hypermethylation of tumor suppressor genes and hypomethylation of oncogenes. In the progression of common diseases, DNA methylation may act as a mediator, modifier, or even a consequence of the disease. Considering all these complexities, it is crucial to understand that the experimental design is highly significant for any epigenetic study. At this stage, the implementation of epigenetics into clinical practice is promising, despite the limited research in this area, which indicates the need for further efforts to fully understand the molecular genetic profile of epigenetic mechanisms in the development of prostate cancer. The development and use of test systems based on prognostic and predictive epigenetic biomarkers can potentially increase the effectiveness of therapy and the choice of further tactics for the treatment of prostate cancer. Research and implementation of such non-invasive genetic methods in clinical practice will expand and improve the range of diagnostic procedures for early detection of prostate cancer. Considering epigenetics as a key factor influencing carcinogenesis opens up new opportunities for the discovery of biomarkers and therapeutic targeting.

Acknowledgements

The study was funded by the state grant of the Ministry of Science and Higher Education of the Russian Federation "Genetic and epigenetic editing of tumor cells the microenvironment to block metastasis" No. 075-15-2021-1073 (data collecting) and BSMU Strategic Academic Leadership Program PRIORITY-2030 (data analysis).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

predictive epigenetic biomarkers, prostate cancer epigenetic, prostate cancer treatment

Received: September 27, 2023
Revised: January 1, 2024
Published online:

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