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Mini-review

Epigenetic biomarkers in prostate cancer: Current and future uses

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ABSTRACT

Epigenome alterations are characteristic of nearly all human malignancies and include changes in DNA methylation, histone modifications and microRNAs (miRNAs). However, what induces these epigenetic alterations in cancer is largely unknown and their mechanistic role in prostate tumorigenesis is just beginning to be evaluated. Identification of the epigenetic modifications involved in the development and progression of prostate cancer will not only identify novel therapeutic targets but also prognostic and diagnostic markers. This review will focus on the use of epigenetic modifications as biomarkers for prostate cancer.

1. Introduction

Prostate cancer is one of the most commonly diagnosed cancers in men of developed Western countries. Globally, it is the 2nd most commonly diagnosed and 6th leading cause of cancer death in men [1]. Several risk factors such as family history, race, obesity, diet and other environmental factors have been associated with prostate cancer. The best established risk factor for prostate cancer is age, whereby there is an estimated incidence of 80% in men by 80 years of age [2]. Hence, prostate cancer is globally a major health and economic burden in our current aging population.

When diagnosed at an early organ-confined stage of the disease, prostate cancer is potentially curable by radical prostatectomy, which involves the removal of the prostate gland, and/or radiotherapy. However, it has been estimated that approximately 30% of patients relapse after the initial treatment. Since the discovery in the 1940s that prostate cancer is dependent on the male sex hormones androgens [3], the main therapy for patients diagnosed with metastatic disease or progressive disease, targets androgen production and its mediator, the androgen receptor (AR). These therapies, called hormonal or androgen ablation therapy, refers to the administration of anti-androgens that block the functional action of AR [4]. After an initial period of tumor regression, prostate cancers become unresponsive to these therapies and eventually progress to the "castrate-resistant" state. Currently, there is no curative treatment available for castrate-resistant prostate cancer, and chemotherapy has limited benefits in improving survival.

2. Current prostate cancer biomarkers: PSA

Because of the limitations of current treatments, one of the major clinical problems for prostate cancer is to decide what treatment options may be the best for individual patients at the time of diagnosis. Prostate cancer is extremely heterogeneous and can present either as indolent or aggressive disease. Since most prostate cancer occurs in elderly men, patients with indolent disease will die with prostate cancer rather than die from the disease. Therefore, it is important to consider whether it is actually beneficial for these men to go through "unnecessary" treatments that may cause complications and affect their quality of life without contributing to any survival benefits. Unfortunately, there is no biomarker available for prostate cancer to predict disease progression at the time of diagnosis. The only biomarker currently used for the detection and monitoring of treatment efficacy for prostate cancer is the measurement of serum prostate specific antigen (PSA) levels and there is constant debate as to whether PSA actually aids in the management of prostate cancer for the following reasons [5,6]:

(1) There are no distinct cut-off serum PSA levels that absolutely define if a patient does have prostate cancer. Although a high serum PSA level is indicative of the presence of prostate cancer cells, studies have shown that a proportion of men without prostate cancer have high levels of

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serum PSA [7] and about 22% of men with prostate cancer have been found to have low serum PSA levels [8]. This means that a proportion of men will undergo the unnecessary invasive procedure of a needle biopsy, while a proportion of men will have their prostate cancer undetected.

(2) PSA is not a prostate cancer specific marker. An increase in serum PSA level may indicate the presence of other prostatic diseases such as benign prostatic hyperplasia (BPH), which is also common in elderly men (75–90% incidence in men by the age of 80 years) [9,10] and prostatitis.

(3) Serum PSA levels are not able to distinguish patients with indolent disease from those with aggressive prostate cancer at the time of diagnosis. In addition, the current early detection of prostate cancer results in most patients presenting with a low stage/grade prostate cancer, making the clinical decision about whether and how to treat the patient difficult. Particularly in the case for elderly men with an expected life expectancy of less than 10–15 years, clinicians have to decide whether these patients will have a survival benefit from treatment or if watchful waiting is the best option.

(4) Using serum PSA levels to determine treatment efficacy requires monitoring over a period of time before a clinician can decide if the treatment is suitable for a patient. For instance in the case of chemotherapy, the clinician is not able to predict if a patient is responsive to the treatment until after a prolonged treatment period that may be accompanied by unpleasant side-effects.

Recently, two large trials investigated the effect of PSA screening test and survival benefits of prostate cancer patients in the US (n = 76,693 men) and Europe (n = 182,000 men) with contradicting results [5,6]. The US study reported no significant difference in prostate cancer mortality between patients who underwent annual PSA screening test compared to the control group, while the European study reported a 20% decrease in prostate cancer mortality due to PSA screening. A meta-analysis on a total of six randomized controlled trials, including the above US and European trials, did not support the usefulness of PSA screening on prostate cancer mortality [11].

Although there are continued efforts to find better biomarkers or improve PSA measurements (i.e. free PSA, total PSA, PSA velocity) for prostate cancer, no biomarkers investigated so far seem to provide any additional diagnostic/prognostic value than serum PSA [12–14]. In this continuous search for new biomarkers for prostate cancer, accumulating evidence for the role of epigenetic modifications in prostate tumorigenesis suggests that they may be candidate biomarkers for prostate cancer. In this review, we shall discuss the previous studies investigating candidate epigenetic biomarkers for prostate cancer, the challenges we face and the latest advancement in this area of research (see Table 1).

3. Epigenetic modifications

Epigenetic modifications are heritable and reversible biochemical changes of the chromatin structure [15–20]. Unlike mutations that involve an alteration in the DNA sequence, epigenetic modifications regulate gene expression via chromatin remodeling [21–23]. Three of the most well studied epigenetic modifications are DNA methylation, histone modifications and microRNAs (miRNAs).

DNA methylation is the addition of a methyl group from methyl donor S-adenosylmethionine to the 5′ carbon of the cytosine predominantly at the cytosine and guanine (CpG) dinucleotides [24,25]. This chemical reaction is catalyzed by a group of enzymes known as DNA methyltransferases (DNMTs) [26,27]. CpG islands, which are clusters of CpGs, are frequently found within gene promoter regions [28]. In contrast to CpG dinucleotides dispersed within the genome or in DNA repetitive elements that are normally methylated, gene-promoter associated CpG islands are usually unmethylated. DNA methylation of promoter-associated CpG islands is associated with gene repression, either through a direct or indirect influence on the chromatin structure that ultimately results in chromatin condensation [25,29–32].

In comparison to DNA methylation, histone modifications are more dynamic and complicated class of epigenetic modifications. In a "closed" and repressed chromatin conformation, the basic amino acid residues (i.e. lysine, arginine and serine) on the N-terminal tails of histones have a high binding affinity to the negatively charged DNA [33]. Histone modifications such as acetylation, phosphorylation and methylation refer to the addition of these specific biochemical groups to the basic amino acid residues on the N-terminal tails of histones [17,19], which alters the affinity of the histone tails to the DNA and results in a conformational change in the chromatin structure that alters gene transcription [17,19]. For example, histone acetylation is associated with active gene transcription while removal of the acetyl groups by histone deacetylases (HDACs) results in subsequent gene repression [21,34,35]. Conversely, histone methylation includes the addition of one or more methyl groups to H3, H4 lysine and arginine residues (mono-, di- or tri-methylation) and has been associated with either activation or repression of gene transcription depending on the target residue and nature of the modification [23,28,36,37]. It is the combination of histone modifications (histone code) and co-operation with DNA methylation that determines the chromatin state and outcome of a gene readout [38].

MicroRNAs (miRNAs) are small non-coding RNAs of approximately 22–25 nucleotides, exist naturally in the genome and are involved in numerous cellular functions like development and differentiation [39]. These miRNAs can bind to complete or partial complementary mRNA targets (a single miRNA is able to target multiple genes), usually at the 3′-untranslated region, to induce gene silencing by mRNA degradation or translational repression [40,41]. miRNAs can induce gene silencing via epigenetic mechanisms, for instance, by targeting a specific gene region for DNA methylation and histone modifications [42,43]. Studies have also identified specific miRNAs that can regulate expression of epigenetic enzymes like the DNMTs, leading to a more global influence on epigenetic regulation [44,45]. Furthermore, the expression of miRNAs themselves may also be regulated by epigenetic mechanisms (i.e. silenced upon DNA methylation), demonstrating the close interactions between miRNAs and other epigenetic mechanisms [43,46,47].

4. Epigenetic modifications as biomarkers for prostate cancer

Epigenetic alterations are frequent in prostate cancer and are thought to contribute both to the disease initiation and progression [22,24,48,49]. Although the exact mechanisms of how these epigenetic alterations arise in prostate cancer are not understood, the fact that they occur at a much higher frequency than mutations and are common in premalignant stages of the disease make them attractive biomarkers for diagnosis, prognosis and treatment response (Fig. 1) [50].

4.1. DNA methylation-based biomarkers: GSTP1

The most frequently studied epigenetic modification in prostate cancer is DNA methylation. Hence, many studies investigating

Table 1
Epigenetic prostate cancer biomarkers.

Biomarker	Function	Significance in prostate cancer	Diagnostic, prognostic or treatment response	References
GSTP1	Involved in detoxification and protects cells from DNA damage	• Hypermethylated in all stages of disease • Detected in body fluids	All	[55,64,65,71,72,75,76,79,81–83,127–135]
APC	Tumor suppressor gene involved in several cellular processes such as the Wnt signaling pathway, cell migration and adhesion	• Hypermethylated in all stages of disease • Commonly assessed with *GSTP1* • Detected in body fluids	Diagnostic and prognostic	[64,71–74,76,77,79,81,128,130,131,135–138]
RASSF1A	Tumor suppressor gene involved in cell cycle and apoptosis	• Hypermethylated in early stages of the disease • Commonly assessed with *GSTP1* • Detected in body fluids	Diagnostic	[64,72,131,132,134,139]
RARβ2	Hormone receptor that regulates cellular processes such as cell growth and differentiation	• Hypermethylated in all stages of disease • Commonly assessed with *GSTP1* • Detected in body fluids	Diagnostic and prognostic	[55,72,77,128,130,133–135,140]
PTGS2	A pro-inflammatory enzyme required for prostaglandin biosynthesis	• Hypermethylated in all stages of disease • Detected in body fluids	Diagnostic and prognostic	[71,77,78,80,131]
CD44	Cell-surface glycoprotein involved in cell-cell interaction, cell migration and adhesion	• Hypermethylated in all stages of disease • Frequently associated with tumor metastasis	Diagnostic and prognostic	[55,78,133,141,142]
EZH2	Histone methyltransferase that methylates H3K27	• Over expressed during prostate cancer progression	Prognostic	[92,97,99–102,143]
Genomic DNA methylation of *LINE-1/Alu*	Retrotransposon elements; DNA methylation status associated with chromosomal stability	• Hypomethylated during prostate cancer progression • Detected in body fluids	Diagnostic and prognostic	[51,55,144–146]
miR-141	Member of miR-200 family that regulates epithelial to mesenchymal transition; multiple gene targets	• Over expressed in metastasis compared to primary cancers • Detected in body fluids	All	[108,115,147–151]
miR-375	Multiple gene targets	• Over expressed in metastasis compared to primary cancers • Detected in body fluids	Prognostic	[115,148,151]
Global levels of H3K18Ac	Histone modification associated with active gene expression	• Aberrant expression during prostate cancer progression	Prognostic	[86,92]
Global levels of H3K4Me2 and H3K27Me3	Histone modifications associated with active (H3K4Me2) and repressed (H3K27Me3) gene expression	• Aberrant expression during prostate cancer progression • H3K27Me3 can be detected in body fluids	Prognostic	[86,92,96,106]

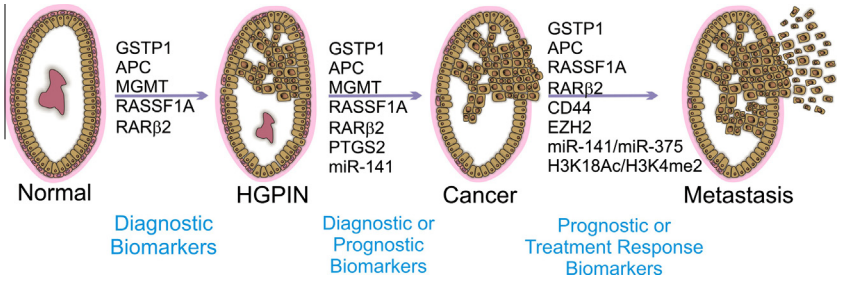

Fig. 1. Epigenetic alterations as diagnostic, prognostic and treatment response biomarkers in prostate cancer. Epigenetic modifications that have been tested as biomarkers and described and cited in the text are depicted on the figure. HGPIN refers to high grade prostatic intraepithelial neoplasia.

potential epigenetic biomarkers for prostate cancer have focused on this particular epigenetic alteration, especially since it is easy to assess. The use of a DNA methylation-based biomarker for prostate cancer is appealing for several reasons – the high stability of DNA, ease of analysis with the current techniques available and the ability to assess the biomarker in body fluids such as blood, urine and saliva.

Global hypomethylation, which is a decrease in genomic DNA methylation level, is commonly linked to activation of proto-oncogenes and chromosomal instability [23,27]. In prostate cancer,

studies have demonstrated global hypomethylation associated with the advanced metastatic stage [51–54]. An immunohistochemical study on human prostate tumor tissues found a significant decrease in the global levels of 5-methylcytosine in patients with recurrent prostate cancer compared to patients without recurrence [52]. Retrotransposons elements such as *LINE-1* and *Alu* repeats, which are repetitive DNA sequences normally methylated in normal tissues, have also been found to be hypomethylated in prostate cancer [53,54]. In a separate study, quantitative methylation-specific PCR (QMSP) carried out on human prostate adenocarcinoma tissues, compared to BPH, showed that hypomethylation of *LINE-1* and *Alu* repeats correlated with PSA levels and tumor stage [55]. However, none of the above studies have investigated if global DNA methylation levels may be of diagnostic or prognostic value for prostate cancer. One recent immunohistochemical study by Yang et al. investigated the potential of global 5-methylcytosine levels to predict survival of patients with prostate cancer [56]. While the authors observed a significant decrease in 5-methylcytosine levels in the prostate tumors compared to the adjacent normal tissues, there was no association between global DNA methylation levels and patient survival [56].

Interestingly, while genomic hypomethylation in cancer was discovered prior to gene-specific hypermethylation, it is the latter that is receiving the "spotlight" in the area of epigenetic therapeutic and biomarker research for cancers. Gene-specific hypermethylation refers to the increase in DNA methylation of specific gene promoter regions, and has been associated with inactivation of genes involved in DNA repair, cell-cycle regulation, apoptosis and tumor-suppression [57,58]. In prostate cancer, a large number of hypermethylated genes have been identified and can be found listed in several reviews. For instance, reviews by Li et al. [24], Perry et al. [25], Park [59], Phe et al. [60] and Jeronimo et al. [49] have together listed approximately 66 hypermethylated genes identified in prostate cancer.

Out of this large number of hypermethylated genes in prostate cancer, the most established epigenetic biomarker for prostate cancer is DNA methylation of the glutathione-S-transferase P1 (*GSTP1*) gene, which encodes an enzyme required for detoxification and protection of DNA from oxidants and electrophilic metabolites [8]. What makes DNA methylation of *GSTP1* an attractive potential epigenetic biomarker for prostate cancer are:

(1) It has a higher specificity (>90%) for prostate cancer compared to serum PSA (~20%) [61].
(2) DNA methylation levels of the promoter region of *GSTP1* can differentiate prostate cancer from other prostatic diseases such as BPH and high-grade prostatic intraepithelial neoplasia (HGPIN) [62,63].
(3) DNA methylation levels of *GSTP1* are associated with different stages of prostate cancer and recurrence of the disease following initial treatments [64–66].
(4) It can be measured via non-invasive procedures in body fluids such as serum, plasma and urine.

Since there are a handful of excellent reviews [8,49,61,67–70] highlighting the significance of hypermethylated *GSTP1* as an epigenetic biomarker in prostate cancer, including comparisons on the techniques and specimens currently used for its analysis (i.e. serum, urine), further discussions on this area will not be provided in this review. Instead, we will focus on the issues or factors that need to be considered for the development of *GSTP1* as an epigenetic biomarker in prostate cancer.

Firstly, although *GSTP1* has a much higher specificity than serum PSA, it is still not 100% prostate cancer specific (DNA hypermethylation does occur in other cancers). To improve the overall specificity and sensitivity, a panel of hypermethylated genes including *GSTP1* may be a better option as a biomarker for prostate cancer [64–66,71]. For example, the methylation profile of a panel of 10 hypermethylated genes (*GSTP1, RASSF1A, CDH1, APC, DAPK, MGMT, p14, p16INK4a, RARβ2, TIMP3*) was assessed in a single study by Roupret et al. [72], whereby all genes except *p14* and *p16INK4a*, were significantly hypermethylated in the urine sediments of prostate cancer patients ($n = 95$) compared to age-matched controls ($n = 38$). The methylation profile of a 4-gene panel, *GSTP1*, *RASSF1A*, *RARβ2* and *APC*, was able to discriminate cancers from controls at 86% sensitivity and 89% specificity. A separate study also demonstrated that while DNA methylation level of *GSTP1* alone was able to detect and differentiate between BPH, HGPIN and prostate adenocarcinoma, combination of the DNA methylation profiles with the *APC* gene increased the detection sensitivity from 94.3% to 98.3%, and increased specificity from 83.3% to 100% [64]. A multicenter study investigated the use of a 3-gene panel (*GSTP1*, *RARβ2* and *APC*) as a diagnostic marker for prostate cancer [73]. The DNA methylation levels of these three genes were assessed using QMSP in the urine samples of 337 subjects (178 men with prostate cancer) post digital rectal examination (DRE) and prior to needle biopsy. Compared to the serum PSA marker (AUC of 0.52–0.56), the 3-gene panel had a significantly higher accuracy (AUC of 0.57–0.71) in predicting the presence of prostate cancer in a patient [73]. The authors further extended their study recently in a larger cohort of 704 subjects (320 men with prostate cancer) and demonstrated again that the 3-gene panel (AUC of 0.73) performed better than any other risk factors (i.e. age, serum PSA levels, DRE and family history) (AUC of 0.52–0.66) [74].

GSTP1 has also been studied as a potential prognostic biomarker for prostate cancer. For instance, it has been shown that the detection of hypermethylated *GSTP1* in patient serum is associated with a 4.4-fold increased risk of biochemical recurrence (PSA relapse) [75]. DNA methylation levels of the promoters of *GSTP1*, *RASSF1A*, *APC* and *RARβ2* in blood samples were also significantly associated with increased risk of biochemical recurrence, although the definite DNA methylation levels of each specific gene in relation to its prognostic value were not discussed [76]. Conversely, both Bastian et al. [77] and Woodson et al. [78] did not find any correlation between *GSTP1* hypermethylation and biochemical recurrence of prostate cancer. Instead, Woodson et al. reported that hypermethylation of *PTGS2* and *CD44* was associated with a 9-fold increased risk of biochemical recurrence in prostate cancer patients when assessed in DNA extracted from prostate tumor tissues [78]. In addition, several studies have shown the potential in using cell-free serum DNA to measure the levels of hypermethylated *PTGS2* as a diagnostic and prognostic indicator of prostate cancer [79,80]. In a particular study, hypermethylation of *GSTP1* in human prostate tissues was associated with a decreased, rather than an increased risk of biochemical recurrence of prostate cancer [81]. The discrepancies among the different studies are commonly thought to be due to differences in patient cohort, methodologies and sample type.

Indeed, it is critical to consider the sample type, the timing of sample collection (i.e. before or after treatment) and what methods may be the most appropriate for the biomarker that is being studied. For instance, in the case of *GSTP1*, common sample types that have been investigated include those that are tissue-based (biopsies or tumors excised after surgery) or body fluids (blood, serum, plasma or urine), with the latter being the more desirable option for a biomarker. A comprehensive review recently published by Wu et al. [61] performed a meta-analysis to compare and consolidate the specificity and sensitivity of DNA methylation of *GSTP1* as a biomarker in prostate cancer across a total of 22 studies that used a variety of methods and sample types (body fluid-based only). Interestingly, the specificity of *GSTP1* was not significantly different when measured by different methodologies and in different

sample types. The sensitivity of *GSTP1* was reduced when measured in whole blood compared to other body fluid-based sample types (i.e. plasma and serum) and in samples that were collected after treatment, suggesting that serum or plasma and sample collection time following treatment for prostate cancer are not ideal. However, the "timing" of sample collection is dependent on the purpose of the epigenetic biomarker (i.e. as a diagnostic, prognostic or treatment response biomarker) and needs to be more carefully thought about in future study designs.

Surprisingly, most studies have mainly focused on the use of DNA methylation-based markers such as *GSTP1* as potential diagnostic and prognostic biomarkers, but not as a biomarker of treatment response for prostate cancer. *GSTP1* and other hypermethylated genes are commonly used in studies and clinical drug trials to determine whether epigenetic drugs such as 5-aza-2′-deoxycytidine (5-aza) exhibit their DNA demethylating property. To explore the usage of *GSTP1* as a treatment efficacy biomarker, we have recently demonstrated that both the DNA methylation and protein status of *GSTP1* was associated with the treatment efficacy of 5-aza in human prostate cancer cells [82]. However, *GSTP1* has not been closely investigated as a marker of treatment response in prostate cancer patients in terms of survival benefits. Especially in the case of patients undergoing chemotherapy, an epigenetic biomarker to indicate treatment response in comparison to monitoring serum PSA levels over a period of time, would be of great benefit. An interesting study by Horvath et al. [83] examined methylated *GSTP1* in the plasma of human prostate cancer patients with castrate-resistant disease to investigate if *GSTP1* can predict chemotherapy response and overall survival in these patients. Methylated *GSTP1* levels per patient were measured before and after the 1st chemotherapy cycle using the quantitative methylation-specific head-loop PCR. Patients with decreased methylated *GSTP1* levels after the 1st chemotherapy cycle were more likely to present a >50% decrease in PSA levels prior to the 4th chemotherapy cycle ($n = 40$). Patients with detectable methylated *GSTP1* had a poorer overall survival (23% survival rate) compared to patients with undetectable methylated *GSTP1* (71% survival rate) ($n = 75$), supporting the use of DNA methylation of *GSTP1* as a potential chemotherapy efficacy biomarker for prostate cancer.

4.2. Histone modifications as biomarkers in prostate cancer

Global loss of specific histone modifications such as H4K16Ac and H4K20Me3 have been demonstrated across several different human cancer cell lines and primary tumors and are predictive of prognosis and survival in several cancers [84–93]. But in comparison to DNA methylation-based biomarkers, the number of studies is limited. To our knowledge, there are only a total of four studies that have demonstrated the significance of global levels of specific histone modifications as prognostic markers in prostate cancer [86,89,91,92]. In addition, unlike the DNA methylation-based biomarkers that have been tested as both diagnostic and prognostic tools for prostate cancer, no study has demonstrated that specific histone modifications may be potentially used as an early detection biomarker for prostate cancer.

The pioneer study providing evidence that specific histone modifications may be potential prognostic markers in cancers was in a prostate cancer cohort [86]. The global levels of specific histone modifications, H3K9Ac, H3K18Ac, H4K12Ac, H3K4Me2 and H4R3Me2 were analyzed using immunohistochemistry in human primary prostate tumor tissues and a correlation between levels of all the histone modifications, except H3K9Ac, with prostate tumor stage was shown [86]. In addition, statistical analyses found that combinations of specific histone modifications, H3K18Ac and H3K4Me2, were indicative of the recurrence of prostate tumors in patients with low grade prostate cancer [86]. However, our study [92] examining the same specific histone modifications as Seligson et al. [86,92] found that global levels of H3K18Ac and H3K4Me2 were both independent predictors of prostate cancer progression regardless of tumor grade. H3K27Me3 is also frequently altered in cancers such as renal, pancreatic, breast and ovarian cancers, whereby a global loss of H3K27Me3 has been shown to be associated with a poor prognosis [94,95]. In prostate cancer, only a single immunohistochemical study has so far investigated and found an overexpression of H3K27Me3 global levels in metastatic prostate tumors compared to non-malignant prostate tissues [96]. Nevertheless, results from this study corresponded with previous findings that the epigenetic enzyme *EZH2* responsible for H3K27 methylation, is overexpressed during prostate tumorigenesis and is associated with biochemical recurrence in prostate cancer patients [97–103].

The discrepancies of previous studies and modest development of histone modifications as biomarkers in prostate cancer are likely to be due to the limitations of the technology available for the analysis of histone modifications. Immunohistochemistry is the only method used to investigate the global expression of specific histone modifications in the above-mentioned studies. The level of sensitivity of QMSP used for DNA methylation analysis cannot be achieved with immunohistochemistry. Variations are also easily introduced. For example, different antigen-retrieval methods and antibodies used may affect the immunostaining for a single antigen. Moreover, techniques to measure specific histone modifications in body fluids such as blood or plasma for diagnostic and prognostic purposes are not established, which make these less desirable as early detection biomarkers for prostate cancer. Theoretically, specific histone modifications may be measured in DNA extracted from serum, plasma or circulating cell-free DNA via methods and commercial assay kits such as ELISA [104,105]. However, only one study so far has investigated the global levels of a specific histone marker H3K27Me3 in the plasma of prostate cancer patients using ELISA and demonstrated a significant decrease in H3K27Me3 in metastatic disease ($n = 28$) compared to localized disease ($n = 33$) with an AUC of 0.68 [106]. While this study serves as a "proof of concept" for the ability to measure a histone marker in body fluids, more follow-up studies are required using larger patient cohorts with age-matched controls, using plasma samples collected at diagnosis and following treatment and comparing PSA specificity and sensitivity with the H3K27Me3 histone marker.

4.3. miRNAs as biomarkers in prostate cancer

An emerging and exciting field of biomarker discovery in prostate cancer is miRNAs. Studies have provided evidence that miRNAs may be potential diagnostic and prognostic biomarkers for prostate cancer and possess advantages over DNA methylation and histone modifications as biomarkers. Some of the traits of miRNAs that make them attractive as biomarkers for prostate cancer are: they are detectable in body fluids like blood and serum, they are highly stable and are thought to be tissue- and tumor-specific [92,107,108]. The identification of critical miRNAs also allows discovery of interesting, novel candidate genes (target genes of the miRNA) and biological pathways that may be implicated in prostate tumorigenesis.

Several miRNAs have been identified to be altered in prostate cancer, as listed in several reviews [109–112]. Differential miRNA expression profiles are able to distinguish between non-malignant and prostate tumors, suggesting the potential of such miRNA profiles as diagnostic and prognostic biomarkers for prostate cancer [113–120]. Using a miRNA microarray analysis and validation by RT-PCR, Schaefer et al. identified 15 miRNAs differentially expressed between prostate tumor and adjacent normal tissues

(n = 76) that were able to discriminate the two tissue types with an accuracy of 82% [113]. Furthermore, 5/15 miRNAs were found to be significantly associated with Gleason score (miR-31, miR-96 and miR-205) and tumor stage (miR-125b, miR-205, miR-222) in a second separate prostate cancer cohort (n = 79). Expression of miR-96 alone was able to predict biochemical recurrence, whereby high expression of miR-96 was associated with a poor prognosis. In a recent microarray study, a miRNA expression profile consisting of 22 miRNAs was able to discriminate between normal (91% prediction rate) and tumor (100% prediction rate) prostate tissues [114]. The authors further modeled two miRNA expression profiles as a diagnostic and prognostic biomarker respectively and validated the potential of these biomarkers in the same patient cohort used by Schaefer et al. [113]. The miRNA diagnostic expression profile (54 miRNAs included) demonstrated a better AUC of 0.949 compared to that of Schaefer et al. [113]. Most importantly, the miRNA prognostic expression profile (25 miRNAs included) demonstrated an AUC of 0.991 and was the best predictor compared to Gleason score, pathological stage and serum PSA level [114].

Brase et al. [115] performed a Taqman miRNA microarray analysis to profile the expression of 667 miRNAs in serum samples from 21 prostate cancer patients (14 with primary prostate cancer and 7 with metastatic disease). The top five most significantly overexpressed miRNAs (miR-375, miR-9*, miR-141, miR-200b and miR-516-3p) in the metastatic compared to the primary cancers, were further validated in serum samples collected from a second prostate cancer patient cohort (n = 45), and found miR-375, miR-141 and miR-200b to be significantly associated with pathological stage and Gleason score. In a final validation patient cohort (n = 71), high expression of miR-375 and miR-141 remained significantly associated with pathological stage and Gleason score. Most importantly, the association of high serum miR-141 level with a more aggressive prostate cancer was in consensus with previous findings [108].

In a recent study by Selth et al. [121], four miRNAs, miR-298, miR-346, miR-141 and miR-375, identified as significantly altered in the serum of a mouse model of prostate cancer (TRAMP), were also significantly altered in serum from patients with castrate-resistant prostate cancer (n = 25) compared to their healthy counterparts. The intra-tumoral over-expression of miR-141 (HR = 2.32) and miR-375 (HR = 3.49) were significantly associated with increased risk of PSA relapse and miR-375 (HR = 5.70) was an independent predictor of PSA relapse in multivariate analysis.

5. Future directions for the development of epigenetic biomarkers in prostate cancer

Altogether, there is ample evidence that an epigenetic biomarker for both the early detection and prognosis of prostate cancer is promising (Fig. 1), but currently, there are only a few clinical trials investigating the use of epigenetic biomarkers for such purposes. From a search in the clinicaltrials.gov database, only three clinical trials were found; two trials investigating a panel of hypermethylated genes in urine and serum as an early detection marker (NCT00340717 and NCT01441687) and a single trial aiming to investigate the association of a miRNA expression profile as a prognostic biomarker (NCT01220427). There are a few reasons that may contribute to the impediment of translating the epigenetic biomarkers for prostate cancer into clinical trials. It may be due to the lack of understanding of the significance of these candidate epigenetic biomarkers in prostate tumorigenesis, the inconsistency of experimental designs to test the biomarkers and until recently the limitation of technology available for analysis. In addition, there are a few important factors that should be taken into consideration but have often been overlooked in previous studies investigating the use of epigenetic biomarkers in prostate cancer. For instance, since epigenetic alterations arise normally during aging, we need to consider whether the epigenetic biomarker of interest may also undergo such age-related epigenetic alteration especially in an aging-associated disease like prostate cancer.

Nevertheless, there are constantly new discoveries in the field of epigenetics that present themselves as more opportunities in the development of potential epigenetic biomarkers for prostate cancer. For example, the identification of a new DNA modification 5-hydroxymethylcytosine converted from 5-methylcytosine provides an additional mechanism of DNA demethylation [122]. Global levels of 5-hydroxymethylcytosine was significantly reduced in prostate cancer when analyzed in an immunohistochemical study [123]. A combination of the global levels of 5-methylcytosine and 5-hydroxymethylcytosine may be a better representation of the genomic DNA methylation status and may be tested as a possible epigenetic biomarker for prostate cancer. Besides 5-hydroxymethlycytosine, a very recent study has discovered two other new DNA modifications converted from 5-methylcytosine, 5-carboxylcytosine and 5-formylcytosine, and requires further investigation of their significance in prostate cancer [124].

An exciting evolution of the development of epigenetic biomarkers is the improvement of the technology, which now allows us to profile epigenetic alterations at a much higher sensitivity and genomic scale previously not possible. One classic example is the limiting analysis tool to investigate the expression of specific histone modifications in prostate cancer. At present, methods such as ChiP-sequencing may be utilized to profile several specific histone modifications concurrently at a more global and sensitive level to investigate if they may be potential epigenetic biomarkers for prostate cancer. The power of current technology also led to a recent discovery of CpG "shores", which are non-CpG islands found outside of promoter regions and has been shown to be tissue-specific and differentially methylated between normal and colon tumor samples [125,126]. Further studies are required to determine if such differential CpG "shores" profile may occur in prostate cancer and be a potential epigenetic diagnostic or prognostic biomarker as well.

6. Concluding remarks

With the advancement of new technologies, such as next-generation sequencing and with the development of platforms for global epigenome analyses the critical epigenetic alterations involved in prostate tumorigenesis will be identified. These epigenetic biomarkers will be powerful tools for determining patient diagnosis, prognosis and therapy response in prostate cancer.

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