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Review – Prostate Cancer

Epigenetics in Prostate Cancer: Biologic and Clinical Relevance

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Abstract

Context: Prostate cancer (PCa) is one of the most common human malignancies and arises through genetic and epigenetic alterations. Epigenetic modifications include DNA methylation, histone modifications, and microRNAs (miRNA) and produce heritable changes in gene expression without altering the DNA coding sequence.
Objective: To review progress in the understanding of PCa epigenetics and to focus upon translational applications of this knowledge.
Evidence acquisition: PubMed was searched for publications regarding PCa and DNA methylation, histone modifications, and miRNAs. Reports were selected based on the detail of analysis, mechanistic support of data, novelty, and potential clinical applications.
Evidence synthesis: Aberrant DNA methylation (hypo- and hypermethylation) is the best-characterized alteration in PCa and leads to genomic instability and inappropriate gene expression. Global and locus-specific changes in chromatin remodeling are implicated in PCa, with evidence suggesting a causative dysfunction of histone-modifying enzymes. MicroRNA deregulation also contributes to prostate carcinogenesis, including interference with androgen receptor signaling and apoptosis. There are important connections between common genetic alterations (eg, E twenty-six fusion genes) and the altered epigenetic landscape. Owing to the ubiquitous nature of epigenetic alterations, they provide potential biomarkers for PCa detection, diagnosis, assessment of prognosis, and post-treatment surveillance.
Conclusions: Altered epigenetic gene regulation is involved in the genesis and progression of PCa. Epigenetic alterations may provide valuable tools for the management of PCa patients and be targeted by pharmacologic compounds that reverse their nature. The potential for epigenetic changes in PCa requires further exploration and validation to enable translation to the clinic.

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1. Introduction

1.1. Epigenetic regulation: a brief overview

Epigenetics refers to modifications of the DNA or associated proteins, other than the DNA sequence itself, that carry information content regarding gene expression during cell division [1]. At present, three main epigenetic mechanisms are recognized: DNA methylation, chromatin remodeling, and microRNA (miRNA) regulation.

1.1.1. DNA methylation

DNA methylation is the best-studied epigenetic mechanism [2] and occurs in mammals mostly at cytosines within CpG dinucleotides (cytosine followed by a guanine nucleotide) [3,4]. 5-methylcytosine (m5C) is created through the addition of a methyl group to the fifth carbon of the cytosine residue ring by DNA methyltransferases (DNMT), which use S-adenosylmethionine (SAM) as the methyl donor [3,4]. CpG dinucleotides are commonly found in clusters called *CpG islands*, which are preferentially found at the 5′ end (promoter, untranslated regions and exon 1) of human genes. In normal cells, CpG island promoters are commonly unmethylated and are associated with active gene expression. In contrast, hypermethylation of CpG islands associated with promoters occurs on the silenced copy of the X chromosome in females [5] and in a tissue-specific manner [6]. In addition, CpG dinucleotides within repetitive sequences and retrotransposons are also methylated in normal cells, and this process is proposed to prevent ectopic transcription during development and differentiation.

DNA promoter methylation is thought to promote epigenetic gene silencing, either directly through the obstruction of transcriptional activators in or near the promoter or indirectly through the recruitment of methylcytosine-binding proteins (MBP) [6]. MBPs recruit large protein complexes, including DNMTs and histone deacetylases (HDAC), and lead to chromatin conformation changes that also repress gene transcription [5,7–9]. Nonprotein coding regions, such as those around the centrosome, transposons, and inserted viral sequences are densely methylated, which is thought to maintain genomic integrity by preventing recombination events that may lead to gene disruption, translocations, and chromosomal instability [9,10].

DNA methylation is critical for the regulation of multiple cellular events and so has been implicated at a global and local level in carcinogenesis [2,11]. The nature of these events depends on the epigenetic change and its extent. DNA hypomethylation is proposed to cause activation of oncogenes and genetic instability, whilst hypermethylation is associated with inappropriate gene silencing [2,12].

1.1.2. Histone modifications and chromatin remodeling

Chromatin is the higher order of organization of nuclear DNA, and its basic unit is the nucleosome. This organization is composed of a protein core (eight histones) around which 147 bp of DNA is wrapped [13,14]. *Histones* are dynamic biomolecules that provide physical support to DNA and are involved in regulating its transcription, repair, and replication [13,15,16]. Structurally, histones possess a flexible "tail" that is susceptible to post-translation biochemical modification (such as acetylation, methylation, and phosphorylation) [13–17]. The combination of biochemical modifications on specific amino acids produces various structural and translational changes—the so-called *histone code*. Acetylation and methylation are the most characterized modifications. In general, acetylation diminishes the affinity of histones for DNA and creates an "open" chromatin conformation to enable gene transcription, and histone deacetylation is associated with closed, or repressive, chromatin. Histone acetyltransferases (HAT) and HDACs balance acetylation to create an equilibrium. Histone methylation may be associated either with transcriptional activation or repression. For instance, methylation of lysines 4, 36, and 79 of histone 3 (H3K4me3, H3K36me, and H3K79me) are marks for active transcription, whilst methylation of lysines 9 and 27 of histone 3 (H3K9 and H3K27) and of lysine 20 of histone 4 (H4K20) are usually found in silent heterochromatin regions and inactive promoters. Histone methylation–modifying enzymes include histone methyltransferases (HMT) and histone demethylases (HDM). These enzymes display high substrate specificity [13,15,16].

In normal cells, histone modifications are implicated in genomic imprinting, X chromosome inactivation, embryonic stem cell (ESC) development, and differentiation [13,18]. In malignant cells, genome-wide histone modification is also altered in concert with changes in DNA methylation [2,11,19]. For example, reduced lysine 16 acetylation (H4K16ac) and lysine 20 trimethylation (H4K20me3) of H4 are associated with hypomethylation of DNA repetitive sequences, a hallmark of human cancer [20]. In contrast, methylation of lysines 9 and 27 of histone 3 (H3K9me and H3K27me) and loss of acetylation of histone 3 (H3ac) as well as monomethylation of H3K4me are associated with DNA hypermethylation of silenced genes [2,18]. Interestingly, the same repressive histone marks were identified in certain genes with tumor-suppressor behavior that are not silenced by DNA methylation [20]. Accordingly, alterations in the expression of key histone modulating enzymes (HDACs, HATs, HMTs, and HDMs) have been associated with cancer development and progression [2,11,13,14].

1.1.3. MicroRNAs

MicroRNAs (miRNA/miR) are a class of small (18–25 nucleotides in length), noncoding RNA. They are synthesized (pri-miR) and processed in the nucleus (pre-miR) before being exported to the cytoplasm (mature miR). They bind mRNAs with complementary sequences and alter their expression through a RNA-induced silencing complex [21–23]. Each miRNA often regulates multiple mRNAs, and each mRNA can be targeted by multiple miRNAs [24]. Nearly 30% of human genes are regulated by miRNAs, and this regulation occurs in a temporal and tissue-specific manner [21].

MicroRNA expression is frequently altered in cancer and can act as either oncogenes (when overexpressed) or tumor suppressors (via downregulation) [21,22]. The role of miRNAs in cancer depends upon the specific target genes [21,22]. As for coding genes, miRNA expression is altered following gene

amplification, deletion, mutation, chromosomal abnormalities, changes in expression of transcription factors, as well as through epigenetic mechanisms [21,22,25]. Interestingly, miRNAs are also involved in the control of chromatin structure by targeting the post-transcriptional regulation of key chromatin-modifying enzymes. In addition, miRNA genes are common targets for epigenetic regulation through DNA methylation or chromatin modifications of their promoters, thus establishing a cross-talk between the major epigenetic pathways [21,22].

2. Evidence acquisition

We searched PubMed for publications on prostate cancer (PCa) epigenetics using the keywords *prostate cancer, DNA methylation, histone modifications*, and *miRNAs* on March 31, 2011. Only articles written in English were retrieved. Original reports were selected based on the detail of analysis, mechanistic support of data, novelty, and potential clinical usefulness of the findings.

3. Evidence synthesis

A total of 153 publications were selected for the purposes of this review.

3.1. Aberrant DNA methylation and prostate cancer

Although DNA hypomethylation was the first cancer-related epigenetic alteration reported, few reports describe it in PCa. Exceptions have shown that metastatic PCa has global cytosine hypomethylation [26] and have linked hypomethylation to chromosome instability and disease progression [27]. Repetitive DNA regions, like LINE1, are hypomethylated in around 50% of PCa samples, which increases in cases with lymphatic metastases [28]. Loss of imprinting (gene-specific hypomethylation) with consequent biallelic expression of *IGF2* was demonstrated in PCa and in nonmalignant adjacent tissues from the peripheral zone of matched specimens [29]. This suggests a regional and tissue-specific pattern of gene expression, which might predispose subjects to neoplastic transformation over a long period of time. Various other genes are found to be upregulated through promoter hypomethylation in PCa, including *CAGE* [30], *CYP1B1* [31], *HPSE* [32], *PLAU* [33], *CRIP1, S100P,* and *WNT5A* [34]. Of interest, *PLAU* expression, which encodes the urokinase plasminogen activator, is associated with the acquisition of castration resistance and increases tumorigenesis in both in vitro and in vivo models [33]. It is likely that other proto-oncogenes transcriptionally controlled by methylation might be activated by inappropriate promoter hypomethylation.

The best-characterized epigenetic alteration in PCa is DNA hypermethylation. More than 50 genes with common aberrant hypermethylation have now been described (the most relevant are displayed in Table 1 [35–66]). These genes are involved in key cellular pathways, including cell cycle control, hormone response, DNA repair and damage prevention, signal transduction, tumor invasion and architecture, and apoptosis. Epigenetic changes in PCa are also reported to commonly occur in domains [19,67] Genetic alterations (point mutations, deletions, loss of heterozygosity) are seldom demonstrated in these genes, suggesting that promoter hypermethylation is the main mechanism associated with gene silencing. The rates of promoter methylation vary between genes and between reports (because of population-methodologic and biologic variability). Frequent promoter methylation of some genes is also found in high-grade prostatic intraepithelial neoplasia (HGPIN) and morphologically normal prostate tissue (eg, *APC, CCND2, GSTP1, RARB2, RASSF1A, PTGS2*). These data suggest that epigenetic alterations are early events in prostate carcinogenesis [55,68,69]. Several factors appear important for promoter hypermethylation in the prostate, including age, diet, and environmental factors. Of these, aging is the single most important risk factor for the development of PCa [70], and aberrant promoter methylation at several loci appears to increase with advancing age [71,72].

3.2. Altered patterns of chromatin remodeling in prostate cancer

Evidence indicates that chromatin remodeling and histone post-translational modifications are important for the deregulation of gene expression in PCa. In fact, gene repression in PCa commonly occurs in domains of inactive chromatin [19]. In addition, several histone-modifying enzymes, including HDACs (eg, HDAC1), HMTs (eg, EZH2), and HDMs (eg, LSD1) are altered in this tumor. Among these, the best studied is EZH2 (encoded by the enhancer of the zeste homolog 2 gene), a histone methyltransferase polycomb protein that catalyses the trimethylation of histone H3K27 and, occasionally, dimethylates H3K9 [73]. Moreover, EZH2 may be also directly involved in DNA methylation through physical contact with DNA methyltransferase [74]. EZH2 overexpression is correlated with promoter hypermethylation and repression of some genes [75], including *DAB2IP,* which is involved in epithelial-mesenchymal transition [76], *MSMB,* encoding the PSP94 protein that functions as suppressor of tumor growth and metastasis [77], and *NKX3.1* (through *ERG* overexpression) [78]. EZH2 upregulation is associated with a high proliferation rate and tumor aggressiveness in PCa [79] and can lead to silencing of developmental regulators and tumor suppressor genes. This undermines cancer cells to a stem cell–like epigenetic state and prevents differentiation [80,81]. Another class of histone modifiers, HDACs, are upregulated in PCa [82]. In particular, HDAC1 overexpression is a common finding in PCa and increases in castration-resistant disease [83]. Remarkably, HDAC1 is also overexpressed in PCa types containing *TMPRSS2–ERG* fusion [84]. Finally, the role of lysine-specific demethylase 1 (LSD1, or histone demethylase 1a) has been studied in PCa. This enzyme removes mono- or dimethyl groups from H3K4, thus acting as a transcriptional corepressor [85], although it is also paradoxically involved in androgen receptor (AR)–mediated transcription through H3K9 demethylation, functioning as a coactivator [86,87]. LSD1 overexpression is associated with aggressive

Table 1 – Genes frequently methylated in prostate cancer, according with their function and pathway

Pathway	Gene	Designation	Frequency, %	References
Hormonal response				
	AR	Androgen receptor	15–39	[35–37]
	ESR1	Estrogen receptor 1	19–95	[37–39]
	ESR2	Estrogen receptor 2	83–92	[37,40]
	RARβ2	Retinoic acid receptor β2	68–95	[35,41–43,57]
	RARRES1	Retinoic acid receptor responder 1 *(TIG1)*	55–96	[44,45]
Cell cycle control				
	CCND2	Cyclin D2	32–99	[46,47]
	CDKN2A	Cyclin-dependent kinase inhibitor 2A (*p16*)	3–77	[41,42,48]
	RPRM	Reprimo	–	[45]
	SFN	Stratifin (14-3-3 sigma)	99	[49]
Signal transduction				
	DKK3	Dickkopf 3	68	[50]
	EDNRB	Endothelin receptor type B	15–100	[38,45,51,52,57]
	RASSF1A	Ras association domain family protein 1 isoform A	53–99	[41,42,48,53]
	RUNX3	Runt-related transcription factor 3	27–44	[54,55]
	SFRP1	Secreted frizzled-related protein 1	83	[50]
Tumor invasion				
	APC	Familial adenomatous polyposis	27–100	[38,48,55–57,87]
	CAV 1	Caveolin 1	90	[58]
	CDH1	E-cadherin	27–69	[41,59]
	CDH13	Cadherin 13	45–54	[41,54,60]
	CD44	Cluster differentiation antigen 44	19–72	[54,61]
	LAMA 3	α-3 laminin	44	[62]
	LAM C2	γ-3 laminin	41	[62]
	TIMP3	TIMP metallopeptidase inhibitor 3	0–97	[35,38,48]
DNA damage repair				
	GSTM1	Glutathione S-transferase M1	58	[50]
	GSTP1	Glutathione S-transferase P1	79–95	[35,38,41,45,48,50,55,57,63]
	GPX3	Glutathione peroxidase 3	93	[50]
	MGMT	O-6-methylguanine DNA methyltransferase	0–76	[35,38,41,48,54,55]
Apoptosis				
	ASC	Apoptosis-associated Speck-like protein containing a CARD	37–78	[54,65]
	BCL2	B cell lymphoma 2	52–87	[60,65]
	DAPK	Death-associated kinase	0–36	[35,38,41,65]
Others				
	MDR1	Multidrug resistance receptor 1	51–100	[45,60,66]
	PTGS2	Prostaglandin endoperoxidase synthase 2	18–88	[38,55,57]
	HIC	Hypermethylated in cancer	99–100	[35,38]

and hormone-refractory PCa with a propensity for recurrence [86,88] eventually through promotion of cell proliferation [89].

Histone acetylation also seems to be intimately involved in AR activity regulation. Indeed, most AR coactivators and corepressors influence transcriptional activity by regulating the acetylation of either androgen-responsive genes or the androgen receptor itself via their respective HAT or HDAC activities [90]. Moreover, AR activity is downregulated by HDAC1, HDAC2, HDAC3 and siRT1, suggesting that reversal of HAT activity is important for abrogating receptor function [91–94].

3.3. *MicroRNA deregulation in prostate cancer*

Although 50 miRNAs have been reported to be abnormally expressed in PCa, only a few have been experimentally proven to contribute to the disease (a detailed review has recently been published by Catto et al. [23]). In PCa, miRNA deregulation affects epigenetic reprogramming, blockade of apoptosis, promotion of cell cycle, migration, and invasion and is an alternative mechanism sustaining androgen-independent growth [95]. AR signaling aberrations and miRNAs seem to be closely linked with PCa progression, either by miRNA regulation of AR signaling or androgen-independent regulation of miRNAs [95]. Recently, the induced overexpression of miR-221 or miR-222 in androgen-dependent LNCaP cells was shown to dramatically reduce the dihydrotestosterone-induced upregulation of prostate-specific antigen (PSA) expression and increased androgen-independent growth [96]. Conversely, androgens may also play a role in downregulation of miR-221/miR-222 [96]. Other miRNAs are also potential modulators of AR-mediated signaling. Whereas stable overexpression of miR-616 and miR-125b is associated with androgen-independent PCa [97], overexpression of miR-488 represses the transcriptional activity of AR [98], and loss of function of miR-146a is frequent in hormone-refractory PCa [99]. Interestingly, miR-146a is able to suppress *ROCK1* expression, a kinase involved in the activation of hyaluronan-mediated hormone-refractory PCa transition, thus acting as a tumor-suppressor gene [99]. Another illustrative example is provided by miR-331-3p, which is reported to regulate *HER-2* expression and AR signaling in PCa. In normal prostate tissues, this particular miRNA is expressed at higher levels than in malignant prostate tissues [97], and

miR-331-3p transfection in several PCa cell lines reduced *HER-2* mRNA and protein expression as well as blocked downstream PI3K/AKT signaling, suggesting that miR-331-3p is able to regulate signaling circuits critical to the development of PCa [97].

In addition to their role in AR signaling, miRNAs are implicated in the avoidance of apoptosis during prostatic carcinogenesis. Whereas miR-21 overexpression antagonizes apoptosis in PCa cells through targeting mRNA of the phosphatase and tensin homolog (*PTEN*) and programmed cell death 4 (*PDCD4*) genes [100,101], the same effect may be accomplished through decreased targeting of mRNA from the silent information regulator 1 (*SIRT1*) and *BCL2* genes owing to miR-34a downregulation [102]. Likewise, miR-34c, which negatively regulates the proto-oncogenes *E2F3* and *BCL2,* is downregulated in PCa, further contributing to apoptosis evasion by neoplastic cells [103]. Furthermore, other studies have shown that several miRNAs (including miR-15a, miR-16-1, miR-125b, miR-145, and let-7c) targeting well-known proto-oncogenes, such as *RAS, BCL2, MCL1,* and *E2F3,* are frequently downregulated in PCa [104,105].

An interesting link between apoptosis and AR signaling has recently emerged from a systematic analysis of miRNAs putatively targeting the AR [106]. In this study, a negative correlation between miR-34a and miR-34c expression and AR levels was found in primary tumors, providing clues to the development of new therapeutic strategies for PCa [106]. Finally, miRNA-altered expression might also foster metastatic spread of PCa through facilitation of epithelial–mesenchymal transition resulting from miR-205 downregulation [107].

3.4. Cross-talk between genetics and epigenetics in prostate cancer

3.4.1. ETS–polycomb group proteins and ETS–miRNA cross-talk

Recently, a link between the polycomb proteins and E twenty-six [*ETS*] fusion genes has been established (Fig. 1) [78,81]. These *ETS* fusion genes are key to prostate carcinogenesis and involve the fusion of an androgen-responsive gene, androgen-regulated transmembrane protease, serine 2 (*TMPRSS2*) to members of the oncogenic *ETS* family, either ETS-related gene (*ERG*) or ETS variant gene 1 (*ETV1*) [108]. Indeed, overexpression of the *TMPRSS2–ERG* fusion gene has been reported in 40–70% of PCa cases and around 25% of HGPIN lesions [109,110]. ETS proteins regulate many target genes that modulate critical biologic processes like cell growth, angiogenesis, migration, proliferation, and differentiation [110].

Interestingly, the histone methyltransferase and polycomb group protein *EZH2* has been identified as an *ERG* target gene, although independent of the effects on AR in PCa [78,81]. Specifically, a high-affinity *ETS* binding site was identified on the promoter of EZH2, and *ERG* binding to this promoter site was documented in multiple cell lines and prostate tumors [78]. *ERG* binding leads to activation of *EZH2,* and this effect can explain the ESC-like dedifferentiation program observed in *ERG*-expressing tumors and cell lines, as mentioned earlier in this review [78,81,111]. The relevance of the ETS transcriptional network in regulating *EZH2* and respective target genes in PCa has been further corroborated, with the demonstration that the epithelial-specific ETS factor ESE3, an ETS member endogenously present in normal prostate, controls the expression of *EZH2*

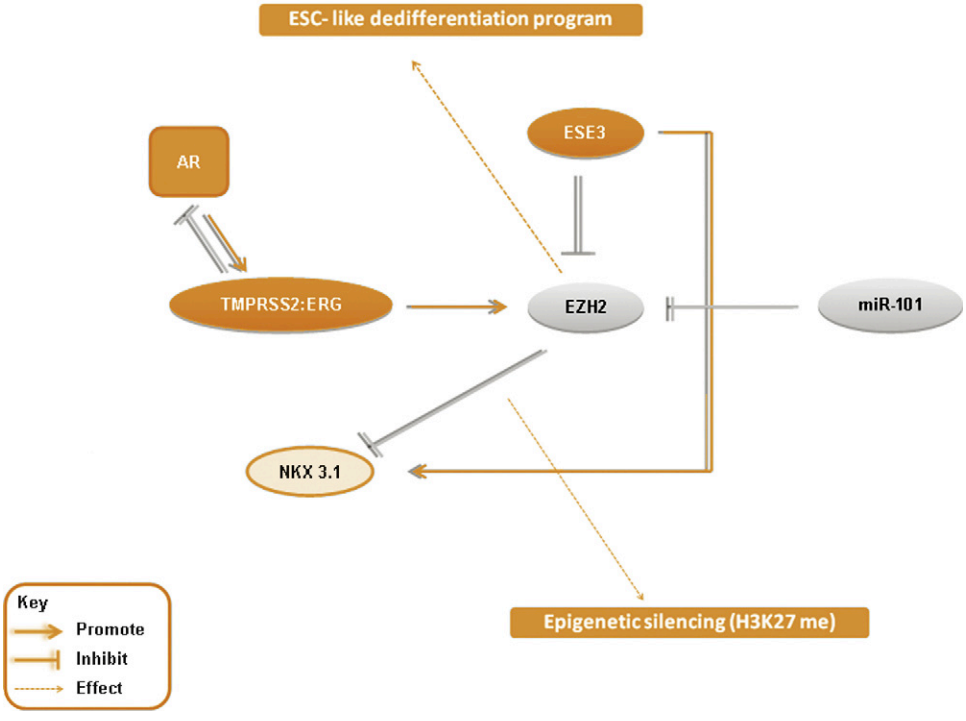

Fig. 1 – Schematic overview of the crosstalk between *ETS*–polycomb group proteins and *ETS*–miRNA in prostate cancer. ESC = embryonic stem cell; AR = androgen receptor

in opposing direction from *ERG*. ESE3 maintains *EZH2* in a repressed status whilst promoting the expression of tissue-specific differentiation genes like *NKX3.1* in prostate epithelial cells [78]. Thus, when *ERG* is overexpressed, it competes with *ESE3* for promoter occupancy at *EZH2* and *NKX3.1*, thus reversing the effects of *ESE3* [78]. However, it must be kept in mind that *EZH2* upregulation in PCa is not exclusively the result of deregulation of the ETS transcriptional network.

There are scarce data describing ETS–miRNA interactions. A recent study, however, provides a link between miRNA and gene fusion expression, as miR-221 was found to be downregulated and *ERG* oncogene overexpressed in PCa patients, with tumors bearing the *TMPRSS2–ERG* fusion transcripts [112]. It is likely that ongoing research in this field will uncover novel interactions between *ERG*, miRNAs, and other PcG proteins with relevant implications in prostate tumorigenesis.

3.4.2. *Interplay among epigenetic mechanisms*

Epigenetic mechanisms are interrelated. Consequently, epigenetic gene regulation derives from the net result of the several epigenetic influences acting upon a gene. Thus, disruption of the epigenetic homeostasis may be the result of epigenetic deregulation of epigenetic effectors. In other words, epigenetic mechanisms may alter the expression of genes that control epigenetic regulation to create a cycle of aberrant gene expression. This process can be demonstrated in PCa. For example, several miRNAs are known to be downregulated through promoter methylation, including miR-34a [113], miR126 [114], miR-193b [115], miR-145 [116,117], miR-205, miR21, and miR-196b [118], whereas miR615 is upregulated as a result of hypomethylation [118]. Interestingly, up to one-third of transcriptionally deregulated miRNA loci disclosed a concordant pattern of DNA methylation and H3K9 acetylation [118], further emphasizing the intimate cooperation of different epigenetic mechanisms.

Remarkably, miRNAs may also regulate epigenetic processes by controlling the expression of DNA and histone-modifying enzymes such as DNMTs, HDACs, and HMTs [2,119]. However, in PCa, the only reported example is miR-449a, which targets *HDAC1*, and is able to induce tumor growth arrest in vitro [120]. Likewise, miR-101 (which directly represses *EZH2* expression) is downregulated in approximately one-third of prostate carcinomas, resulting in an increase of *EZH2* expression [121], a feature that is associated with aggressive PCa [79].

3.5. *Epigenetic-based markers for prostate cancer detection, management, and risk estimation*

Because of the poor specificity of current methods (eg, serum PSA), new, robust biomarkers are needed to improve PCa detection and management. These should be based on the biology of PCa development and progression. Epigenetic-based biomarkers, especially DNA methylation, appear promising for several reasons. First, epigenetic alterations are highly prevalent and occur early in carcinogenesis [69,122]. Second, genomic DNA is more stable and easier to manipulate than RNA, and some global histone modifications may be detected using widely available methodologies, such as immunohistochemistry. DNA methylation is, in addition, a positive signal that may be identified among normal DNA, even when present in small amounts, making it particularly suited for detection in clinical samples. Finally, standardized high-throughput technologies are now available for simultaneous detection of DNA methylation at several loci in a large number of samples, thus enabling their use in clinical practice. Of note, although high-throughput miRNA analysis is possible, it is currently unclear which method is standard [123]. Figure 2 and Table 2 provide an overview of the most promising epigenetic-based biomarkers for PCa management. Epigenetic factors may also be used in patient risk assessment. Known PCa risk factors include age, diet, and genetic background [70,124]. Hypothetically, ethnic-related patterns of gene methylation might modulate the susceptibility for the development or progression of PCa. Indeed, differences in *GSTP1* and *CD44* gene promoter methylation have been reported among African-Americans, Asians, and Caucasians [125]. In the case of *GSTP1* promoter methylation, it also correlates with pathologic parameters predictive of more aggressive disease (higher stage and Gleason score) [125].

3.5.1. *Cancer detection and diagnosis*

GSTP1 promoter methylation is the best-characterized epigenetic biomarker for PCa [126]. The *GSTP1* gene encodes for an enzyme (GSTpi) that is involved in reactive chemical species and carcinogens detoxification [127]. *GSTP1* is frequently silenced (>90% of the cases) through aberrant promoter methylation in PCa [128] and can be specifically detected by quantitative methylation-specific polymerase chain reaction (PCR) assays [64,129]. However, *GSTP1* promoter hypermethylation is not tumor specific (present in 70% of HGPIN) [130,131]. *GSTP1* promoter hypermethylation testing can be detected in prostate tissue samples and body fluids, mainly urine and blood. Thus, its presence could be used to screen men or stratify the need for biopsy. When evaluated in this context, *GSTP1* hypermethylation appears to have a high specificity (86.8–100%) but low sensitivity in both urine (18.8–38.9%) and serum/plasma (13.0–72.5%) [132–138]. However, 5–20% of PCa cases have little *GSTP1* hypermethylation [126], so multigene promoter methylation testing has been suggested. Gene panels have been evaluated, including *GSTP1/ARF/CDNK2A/MGMT* [137] and *GSTP1/APC/RARB2/RASSF1A* [138] in urine and *GSTP1/PTGS2/RPRM/TIG1* [139] in serum samples. As expected, the detection rate increased significantly (86% for urine and 42–47% for serum), whilst maintaining high specificity (89–100% for urine and 92% for serum) [137–139]. However, the number of genes should be restricted and carefully selected, because the simultaneous use of more than three or four markers is likely to compromise the specificity of the test, with only a marginal gain in sensitivity [38].

Few studies have evaluated the diagnostic or predictive roles of histone modifications in PCa. Recently, Ellinger and co-workers showed that H3K4me1, H3K9me2, H3K9me3,

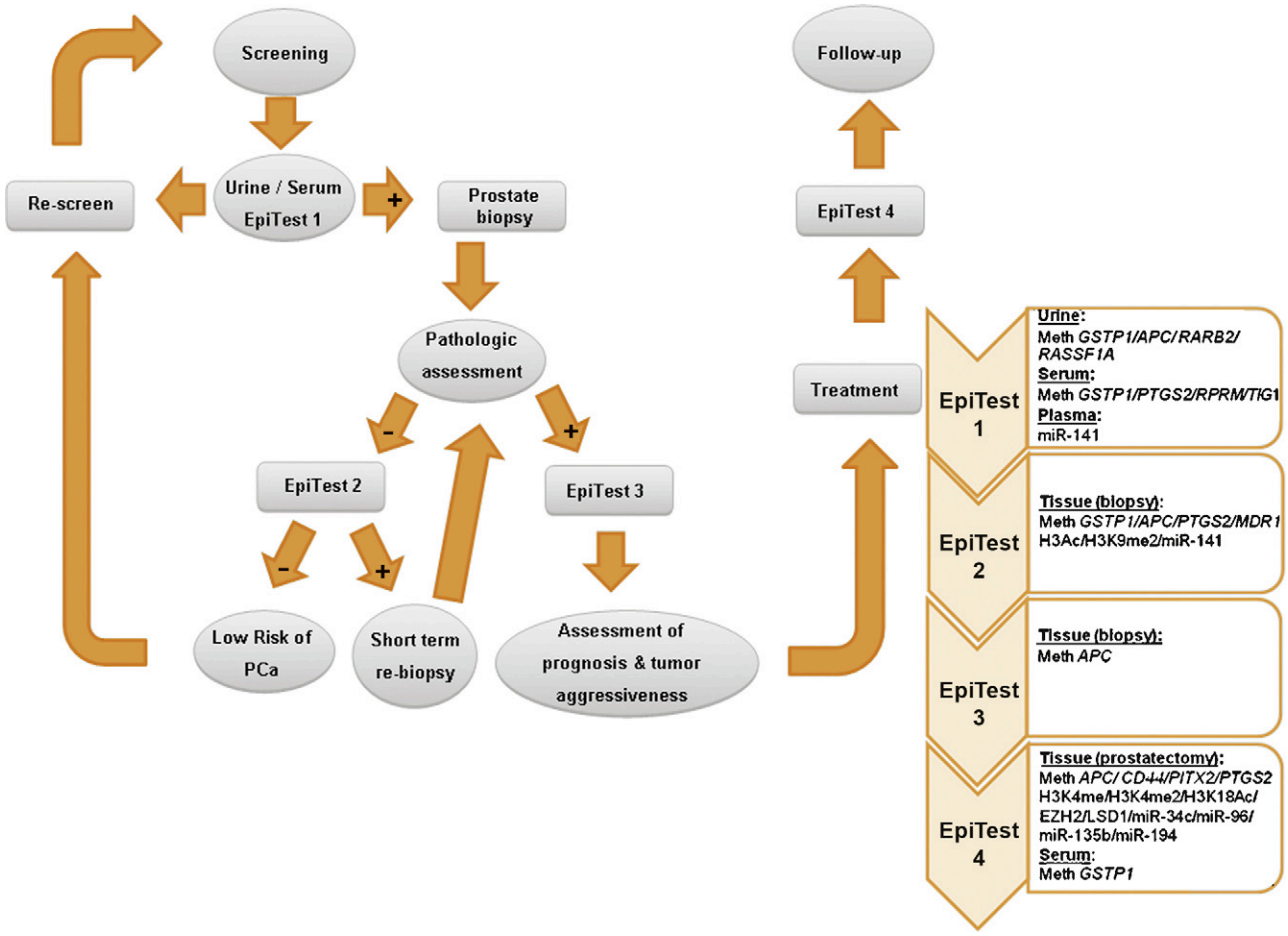

Fig. 2 – Epigenetic biomarkers in prostate cancer (PCa) management. From the published data, specific sets of informative biomarkers were chosen for cancer detection (EpiTest 1), as ancillary tools to histopathologic observation (EpiTest 2), pretherapeutic prediction of prognosis and tumor aggressiveness (EpiTest 3), and prediction of recurrence and progression following radical prostatectomy (EpiTest 4). *Meth* refers to quantitative DNA methylation analysis; histone modifications and expression of histone modifiers are assessed by immunohistochemistry; microRNA expression is assessed by quantitative reverse-transcriptase polymerase chain reaction.
PCa = prostate cancer

H3Ac, and H4Ac were reduced in PCa (vs nontumorous prostate tissue) and that H3Ac and H3K9me2 discriminated between the two tissue samples (80% sensitivity and >90% specificity) [140]. Likewise, the investigation of miRNAs as PCa biomarkers is still in its infancy. In a recent study, Schaefer et al. performed miRNA profiling in 76 patients who underwent radical prostatectomy (RP) and found a signature of 10 downregulated and 5 upregulated miRNAs in cancerous tissue compared to normal prostate tissue as well as 2 miRNAs able to discriminate tumorous from nontumorous prostate tissue in 84% of cases [141]. The validation of these findings in urine or serum might provide additional tools for the identification of PCa patients with aggressive disease. In this regard, miR-141 seems to be a

Table 2 – Diagnostic, prognostic, and predictive information in prostate cancer provided by epigenetic biomarkers in tissue and biologic fluids

	Cancer detection		Prognostic/predictive	
	Biomarker	Sample	Biomarker	Sample
DNA methylation	*GSTP1/APC/ RARB2/RASSF1A*	Urine	*APC*	Biopsy
	GSTP1/PTGS2/RPRM/TIG1	Serum	*APC/CD44/PITX2/PTGS2*	Prostatectomy
	GSTP1/APC/PTGS2/MDR1	Biopsy	*GSTP1*	Serum
Histone modifications and modifiers	H3Ac/H3K9me2	Biopsy	*H3K4me/H3K4me2/H3K18Ac*	Prostatectomy
			EZH2/LSD1	Prostatectomy
miRNA	miR-141	Plasma	miR-34c/miR-96/miR-135b/miR-194	Prostatectomy

miRNA = microRNA.

promising PCa biomarker, because it is highly expressed in prostate carcinoma compared to nontumorous prostate tissues [142,143], and it is detectable in plasma samples from PCa [142].

3.5.2. Prognosis and prediction of response to therapy

There is an urgent clinical need for tests that reliably discriminate aggressive and indolent prostate tumors. Various epigenetic alterations are associated with PCa outcomes and could be used in this prognostic role (eg, *APC, CDH1, EDNRB, GSTP1, MDR1, MT1G, PTGS2, RARβ2, RASSF1A,* and *RUNX3*) [38,43,45,48,55,56,59,68,144,145]. For example, Yegnasubramanian et al. found that *PTGS2* hypermethylation was independently predictive of PCa recurrence after RP [38]. Enokida et al. found a methylation score derived from *GSTP1, APC,* and *MDR1* hypermethylation that discriminated organ-confined and locally advanced disease (72.1% sensitivity and 67.8% specificity) [56]. Serum *GSTP1* promoter methylation was found to be an independent predictor of biochemical recurrence (BCR) in PCa patients with clinically localized disease treated with RP [146]. Furthermore, methylation of *CD44* and *PTGS2* was also predictive of PSA recurrence following RP [147]. Similar findings were reported for the methylation of the *ABHD9* gene promoter and for an expressed sequence tag on chromosome 3 (Chr3-EST) as well as for the *PITX2* gene, especially in patients at intermediate risk (Gleason score 6–7 tumors) [148,149]. Interestingly, high levels of *APC* and *CCND2* methylation predicted time to post-RP recurrence in Gleason score 3 + 4 = 7 carcinomas [150]. The prognostic value of *APC* promoter hypermethylation has also been established using pretreatment prostate biopsies [151].

Once again, few data evaluate histone modifications in this setting. Varambally and co-workers found that *EZH2* was upregulated in castration-resistant PCa, suggesting a link between chromatin modifiers and aggressive disease [152]. Patients with localized carcinomas and *EZH2* overexpression were also at higher risk of postprostatectomy recurrence than matching tumors with low expression [152]. Of note, a polycomb-repression gene signature (13 genes targeted by *EZH2*) is associated with metastatic PCa [80], whilst high *LSD1* expression is associated with shorter progression-free survival in prostatectomy-treated patients [88].

Data relating particular histone modification patterns and clinical behavior of PCa have emerged in recent years. Using immunohistochemistry, Seligson and colleagues analyzed a range of histone modifications, including H3K9Ac, H3K18Ac, H4K12Ac, H4R3me2, and H3K4me2, in 183 primary PCa samples. They found distinctive groups among low-grade tumors (Gleason score $\leq$6). Tumors with high immunostaining for H3K4me2, H3K18Ac, and H3K4me2 had a low recurrence risk [153]. These findings have been challenged by a more recent study of primary and metastatic PCa samples in which high global levels of H3K18Ac and H3K4me2 correlated with a three-fold increased risk of PCa recurrence [154]. Individuals with high H3K4me1 levels are more likely to experience PCa recurrence [140], suggesting that analysis of H3K4 methylation status may provide clinically relevant prognostic information.

Reports on the prognostic significance of alterations in miRNAs in PCa are scarce. Nonetheless, expression profiling has revealed widespread dysregulation of miRNAs that was associated with pathologic features of locally advanced PCa, such as extraprostatic extension [105,155]. A recent study also suggested that a specific miRNA signature (increased expression of miR-135b and miR-194) was associated with earlier BCR in PCa patients submitted to RP [156]. Schaefer and colleagues found a correlation between miRNA expression and the Gleason score (miR-31, miR-96, miR-205) or the pathologic tumor stage (miR-125b, miR-205, miR-222) [141]. Interestingly, miR-96 expression was shown to have prognostic value in PCa patients, being associated with cancer recurrence after RP [141]. Likewise, a study by Hagman et al. showed that miR-34c expression was inversely correlated with tumor aggressiveness, World Health Organization grade, PSA levels, and metastases formation. Remarkably, low miR-34c expression levels could discriminate between patients at high and low risk for PCa progression [103].

Taken together, these data strongly indicate that some epigenetic alterations, either isolated or in combination, might be able to stratify patients in different prognostic groups, adding relevant information to clinical and pathologic parameters that contribute to the definition of current therapeutic strategies.

3.6. Epigenetic silencing as a therapeutic target in prostate cancer

In contrast to genetic alterations, epigenetic changes are chemically reversible, making them potential therapeutic targets. The re-expression of epigenetically silenced genes has been accomplished in vitro through the use of inhibitors of DNMTs and HDACs. The anticancer properties of some of these compounds led to US Food and Drug Administration approval for the elective treatment of myelodysplastic syndromes (5-azacytidine and 5-aza-2′-deoxycytidine [DNMT inhibitors]) and cutaneous T-cell lymphomas (suberoylanilide hydroxamic acid [SAHA] and romidepsin, which are HDAC inhibitors) [157–161]. Although these compounds might provide therapeutically useful tools for other malignancies, including PCa, it should be recalled that among the side effects, the promotion of malignant transformation or progression is a major concern [162]. Finally, the expanding knowledge of the role of miRNAs in tumorigenesis is also expected to provide relevant targets for the development of specific anticancer molecules that may simultaneously target several key pathways.

3.6.1. Pharmacologic reversal of DNA methylation

Inhibition of DNMTs may reactivate genes silenced predominantly through aberrant promoter methylation. DNMT inhibitors can be divided in two main categories: nucleoside (cytidine) and non-nucleoside analogs. The former includes 5-azacytidine and 5-aza-2′-deoxycytidine, which become

incorporated into DNA for the period of replication and sequester DNMTs, resulting in decreased methylation of cytosines incorporated de novo at each replication cycle. Although there is substantial experimental evidence sustaining the efficacy of these compounds in reversing DNA methylation in PCa cell lines, clinical trials demonstrated a limited benefit in PCa patients with advanced-stage, hormone-refractory disease, expanding in only a few weeks the progression-free interval [163,164]. Owing to the cytotoxic effects and mutagenic potential of the nucleoside analogs, the nonanalog class of DNMT inhibitors might be a safer therapeutic alternative. Although procaine and procainamide have been tested in PCa cells lines, there is no available data concerning their effectiveness in PCa patients. Because these compounds are also less potent in the inhibition of DNMTs, there is clearly a need for novel DNMT inhibitors with improved pharmacologic and clinical profiles.

3.6.2. Therapeutic use of histone deacetylase and methyltransferase inhibitors

HDAC inhibitors are a promising group of agents for epigenetic therapy of cancer, which may be divided in four classes: hydroxamic acids, cyclic tetrapeptides, short-chain fatty acids, and benzamides [165]. This discussion will be restricted to the former two classes, because they were the only compounds that have been clinically tested in PCa patients, despite extensive in vitro evidence of potential therapeutic benefit. The mechanism of action of those compounds is based on the occupancy of the catalytic domain of HDACs, thus blocking substrate recognition, leading to restoration of the expression of relevant genes involved in cell cycle arrest, induction of differentiation, and apoptosis [165]. Furthermore, inhibition of HDACs also sensitizes tumor cells to chemotherapy and inhibits angiogenesis [165]. Interestingly, the hydroxamic acids panobinostat and SAHA also interfere directly with AR-mediated signaling, as these two compounds inhibit AR gene transcription and interfere with the assembly of RNA polymerase II complex at the promoter of AR target genes [166].

Panobinostat and SAHA have been tested in PCa patients with advanced-stage disease. Response to treatment (partial) was only observed in patients treated with a combination of the HDAC inhibitor and a conventional chemotherapeutic drug (doxorubicin for SAHA and docetaxel for panobinostat) but not when given alone (although the efficacy of isolated SAHA has not been assessed) [167,168]. Concerning the cyclic tetrapeptide romidepsin, a phase 2 clinical trial enrolling 35 patients with chemonaive, hormone-refractory PCa patients, demonstrated minimal antitumor activity and significant toxicity [169]. There is no available information concerning the efficacy of combination with conventional chemotherapy.

Histone methyltransferases are additional therapeutic targets that deserve further testing in a clinical setting. In contrast to HDAC inhibitors, no therapies that directly target histone methylation are clinically available despite the fact that there is experimental evidence for a potential therapeutic benefit for this approach. Indeed, 3-deazaneplanocin A (DZNep), an S-adenosylhomocysteine hydrolase inhibitor, was found to globally inhibit both repressive and active histone methylation marks as well as to induce apoptosis in cancer cells [170,171]. Furthermore, and contrarily to what was previously thought, the effects of DZNep on cancer cells are not *EZH2* specific [170,171].

As previously mentioned for histone methyltransferase inhibitors, the development of histone demethylating agents is also of great interest, although currently no compounds have entered into clinical trials or been approved for treatment. Finally, concerning histone demethylase inhibitors, monoamine oxidase inhibitors, such as pargyline, have been tested in vitro, taking advantage of the amine oxidase properties of *LSD1*. Although pargyline efficiently inhibited demethylation of mono- and dimethyl H3K9 during androgen-induced transcription in the LNCaP cell line, the drug concentration required to achieve such effect in vivo is toxic, and alternative inhibitors are required [86].

4. Conclusions

Epigenetic alterations are a common feature of PCa and play an important role in prostate carcinogenesis as well as in disease progression. Although aberrant DNA methylation is the best-studied cancer-related epigenetic alteration in PCa, the study of changes in chromatin remodeling and miRNA regulation constitute a growing research field that will provide a more global view of the PCa epigenome as well as of the interplay between epigenetic and genetic mechanism involved in prostate carcinogenesis.

Deriving from this knowledge, there is handful of biomarkers based on cancer-specific epigenetic alterations that constitute promising tools for PCa detection and screening, diagnosis, assessment of prognosis, and follow-up. Finally, owing to the reversible nature of epigenetic alterations, specific therapeutic interventions based upon the reversal of those alterations is likely to provide innovative tools for PCa treatment and contribute towards improved PCa patient care in the future.

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Acquisition of data: Jerónimo, Catto, Henrique.
Analysis and interpretation of data: Jerónimo, Henrique.
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