DNA methylation and histone modifications as epigenetic regulation in prostate cancer (Review)

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Abstract. Prostate cancer is the second most commonly diagnosed cancer in men in Poland after lung cancer and the third leading cause of cancer-related mortality after lung and colon cancer. The etiology of most cases of prostate cancer are not fully known, and therefore it is essential to search for the molecular basis of prostate cancer and markers for the early diagnosis of this type of cancer. Epigenetics deals with changes in gene expression that are not determined by changes in the DNA sequence. Epigenetic changes refer to changes in the structure of DNA, which are the result of DNA modification after replication and/or post-translational modification of proteins associated with DNA. In contrast to mutations, epigenetic changes are reversible and occur very rapidly. The major epigenetic mechanisms include DNA methylation, modification of histone proteins, chemical modification and chromatin remodeling changes in gene expression caused by microRNAs (miRNAs). Epigenetic changes play an important role in malignant transformation and can be specific to types of cancers including prostate cancer.

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1. Introduction

Prostate cancer (PC) is one of the most frequently diagnosed cancers in men. One cause for neoplastic transformation is abnormal gene expression, which is not determined by nucleotide sequence changes in DNA, but by disturbances in epigenetic mechanisms. The epigenetic changes in DNA structure are the result of post-replication modification in DNA and/or post-translational modification of proteins associated with DNA. In contrast to mutations, epigenetic modifications are reversible and dynamically occur. These epigenetic mechanisms include aberrant DNA methylation (hypermethylation and hypomethylation) and modifications of histones, chromatin remodeling and changes in gene expression caused by non-coding RNAs (ncRNAs). Epigenetic mechanisms lead to genomic instability and inappropriate gene expression and are the best-characterized alteration in PC. Epigenetic mechanisms play an important role in the initiation and development of PC. Global and gene-specific hypermethylation and hypomethylation as well as histone modifications have been found to be associated with PC (1,2).

2. DNA methylation and prostate cancer

DNA methylation plays an important role in DNA repair, recombination and replication, as well as regulation of gene activity. DNA methylation involves the addition of a methyl group to the 5'-carbon of cytosine in CpG dinucleotide sequences. This process is catalyzed by a family of DNA methyltransferases (DNMTs). CpG islands are CpG-rich areas of 200 bp to several kilobases in length, usually located near the promoters of highly expressed genes, and are the sites of common methylation in human tumors, including the prostate. CpG islands, in more than 55% of cases, form clusters and their methylation/demethylation results in the inhibition/activation of transcription. Thus, methylation of CpG islands in promoter regions of genes may prevent or deregulate the synthesis of gene products (3-5).

Three active DNMTs have been identified (DNMT1, DNMT3A and DNMT3B). A fourth enzyme previously known as DNMT2 is not a DNA methyltransferase. DNMT2 or TRDMT1 has strong sequence similarities with 5-methylcytosine methyltransferases, but the enzyme was shown to methylate position 38 in aspartic acid transfer RNA and does not methylate DNA. The smallest mammalian DNMT2

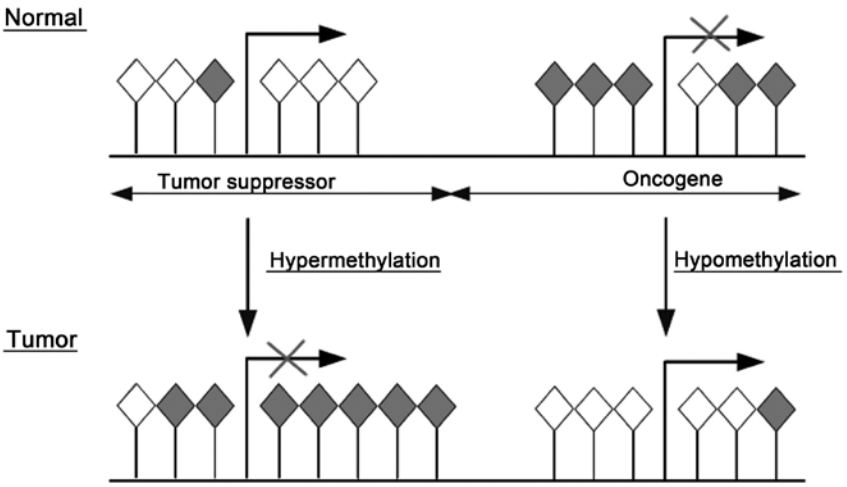

Figure 1. Role of DNA methylation in cancer (9).

is believed to participate in the recognition of DNA damage, DNA recombination and mutation repair. DNMT1, the major enzyme responsible for maintenance of the DNA methylation pattern is located at the replication fork and methylates newly biosynthesized DNA. DNMT3A and DNMT3B cannot differentiate between unmethylated and hemi-methylated CpG sites, and they cannot copy a specific pattern of methylation or contribute to the maintenance of the methylation pattern (6).

Proteins that bind to methylated CpG islands (MBP, methyl-CpG binding proteins) which include: MBP1, MBP2, MBP3 and methyl CpG-binding protein-2 (MeCP2) recognize methylated CpG islands in the promoter regions of the genes. These proteins at the N-terminal contain methyl-CpG-binding domain (MBD) and in the central portion transcriptional repression domain (TRD). MBP proteins can activate histone deacetylase (HDAC), and/or co-repressors of transcription, after recognizing methylated DNA. Then, chromatin undergoes strong condensation, preventing access of transcription factors to the promoter's genes (7).

Disturbances of DNA methylation can lead to malignant transformation. In PC, hypermethylation and hypomethylation of DNA can be observed. In normal cells, CpG islands are protective against excessive methylation by active transcription, DNA demethylation, regulation of replication and the establishment of appropriate local chromatin structure, impeding access to methyl-DNA transferase. In tumor cells, the mechanisms as described above are altered, resulting in abnormal DNA methylation regulation (2,8).

A common molecular feature associated with tumorigenesis including PC is hypermethylation of cytosines 5' in CpG islands within the regulatory (promoter) region of suppressor genes resulting in gene silencing. In contrast, hypomethylation in the promoter region of oncogenes in tumors reactivates transcription (Fig. 1). Moreover, 5-methyl cytosine is unstable and mutates to thymine and methylated CpG sites degrade to TpG/CpA (9).

DNA hypermethylation. In PC, many CpG islands exhibit aberrant hypermethylation. Genes undergoing methylation in PC include genes involved in key cellular functions such as DNA repair (*GSTP1* and *MGMT*), cell adhesion (*CDH1* and *CD44*), cell cycle control (*CDKN2A*), apoptosis (*PYCARD*) and also are related to suppressor genes (*APC*, *RARβ*, *RARRES1* and *RASSF1*) (5,8,10). Specific genes implicated within each category are summarized in Table I.

The glutathione S-transferase π 1 gene (*GSTP1*) encodes glutathione S-transferase belonging to the π class. It is responsible for protecting cells from oxidative stress and chemical attacks. GSTP1 is involved in the metabolism, detoxification and elimination of compounds potentially genotoxic, and prevents DNA damage and initiation of neoplastic transformation. *GSTP1* is one of the earliest identified genes which is hypermethylated in PC. *GSTP1* hypermethylation has been found in 13.3-100% of PC cases, whereas methylation of *GSTP1* within CpG dinucleotides has not been noted in normal cells. *GSTP1* hypermethylation is present at all stages of PC development, and the assessment of the methylation profile of this gene allows the differentiation of types and subtypes of PC which may facilitate early diagnosis. *GSTP1* gene methylation profile can be assessed not only in serum but also in other body fluids. In 39-83.2% of PC patients, *GSTP1* DNA methylation was detected in urine (11,12). Hypermethylation of *GSTP1* DNA was detected in plasma samples from 27 of 31 (92.86%) patients with PC (13). Methylated *GSTP1* DNA in serum is present in 28-32% of men with metastatic PC (14,15). Mahon *et al* (16) identified plasma methylated *GSTP1* DNA as a potential prognostic marker in men with castration-resistant PC as well as a potential surrogate therapeutic efficacy marker for chemotherapy.

The O-6-methylguanine-DNA methyltransferase gene (*MGMT*) encoding enzyme removes alkyl adducts from the O6 position of guanine. Methylation of the *MGMT* gene promoter region has been detected in PC patients and cell lines (17-19). DNA hypermethylation of CpG dinucleotides of the *MGMT* gene has been associated with decrease and/or total loss of expression of this gene, and results in the development of carcinoma. A study conducted by Sidhu *et al* (18) indicated that development of prostate carcinoma is correlated with modification of the MGMT methylation pattern.

PC tumor cell invasion and progression have been found to be associated with E-cadherin-1 (*CDH1*) gene promoter hypermethylation. The effect of increased methylation of

Table I. Genes hypermethylated in prostate cancer.

Gene	Chromosomal location	Function	Hypermethylation in prostate cancer (%)	Refs.
GSTP1	11q13	Detoxification, DNA repair	13-100	(13,16,72-82)
MGMT	10q26	Detoxification, DNA repair	0-76	(17-19,35)
CDH1	16q22.1	Cell adhesion	0-72	(22,29,83)
CD44	11p13	Cell-cell interactions, cell adhesion and migration	20-78	(21,23,84)
CCND2	12p13	Regulation of cyclin-dependent protein serine/threonine kinase activity	8.4-99	(19,73,75)
APC	5q21-q22	Tumor suppressor; antagonist of the Wnt signaling pathway; regulator of cell migration, adhesion, transcriptional activation and apoptosis	14.5-100	(19,27,75,76, 80,82,85-87)
RARβ	17q21	Tumor suppressor; regulation of development, differentiation, apoptosis, granulopoeisis, and transcription of clock genes	32.6-100	(19,73,75-77)
RASSF1A	3p21.3	Tumor suppressor; Ras protein signal transduction	19.2-100	(19,72,73,86)

GSTP1, glutathione-S-transferase π 1; *MGMT*, O-6-methylguanine-DNA methyltransferase; *CDH1*, E-cadherin-1; *CD44*, CD44 molecule; *CCND2*, cyclin D2; *APC*, adenomatous polyposis coli; *RARβ*, retinoic acid receptor β; *RASSF1A*, Ras-associated domain family member 1.

CDH1 is a decrease in E-cadherin expression, which is one of the main factors associated with dysfunction of intercellular adhesion, inhibition of cell adhesion and promotion of neoplastic transformation and metastasis (5,20).

The *CD44* gene which encodes cluster of differentiation CD44 may be another important mediator of prostate carcinogenesis, since it is hypermethylated in 78% of patients with PC in compared to only 10% of patients without cancer. Based on the morphology of fibroblasts, increased *CD44* gene promoter hypermethylation was found to be a characteristic feature of epithelial-to-mesenchymal transition (EMT) in non-prostate malignant tumors (8,21-23).

The cyclin D2 (*CCND2*) gene encodes a protein belonging to the conserved family of cyclin D, which are characterized by cyclic occurrence in the cell. Cyclins D form complexes with cyclin-dependent kinases CDK4 and CDK6. These complexes regulate cell cycle transition from G1 to S phase. *CCND2* gene hypermethylation was found in 32% of PC cases which was significantly higher as compared to the hypermethylation noted in 6% of nonmalignant prostate tissues. In addition, high levels of *CCND2* methylation were found to correlate with tumor aggressiveness (8,24). In a study by Henrique *et al* (25), cyclin D2 promoter methylation was found in 80% of benign prostatic hyperplasia (BPH), in 99.2% of PC and in 100% of high-grade prostatic intraepithelial neoplasia.

PYCARD also known as the target of methylation induced silencing 1 (*TMS1*) gene, encodes a proapoptotic protein, which contains a pyrin domain (PYD) in the N-terminus and caspase recruitment domain (CARD) in the C-terminus. Both domains are members of the death domain-fold superfamily. PYCARD plays an important role in the development of many diseases, including cancer. It is considered that PYCARD induces apoptosis via caspase 9. Hypermethylation of *PYCARD* is a common event in PC, and decreased PYCARD expression has been associated with complete methylation of the promoter region in human prostate carcinoma cell line LNCaP (26).

The adenomatosis polyposis coli (*APC*) gene encodes a protein that is involved in the regulation of many cellular processes including division, migration, adhesion and differentiation of cells. Promoter methylation in the *APC* gene has been associated with high grade and advanced stages of PC (27,28).

The retinoic acid receptor β (*RARβ*) gene encodes β retinoid acid (RA) receptor, which forms complexes with two nuclear receptor families i.e. retinoid acid receptors (RARα, β and γ) and X retinoid receptors (RXRα, β and γ). Retinoid acid belongs to the retinoids which induce numerous cellular pathways involving kinases responsible for activation or inhibition of transcription of many genes. A study conducted by Jerónimo *et al* (29) showed that *RARβ*2 is hypermethylated in 97.5% of PC and in 94.7% of prostate intraepithelial neoplasia (PIN), while only in 23.3% of BPH. Thus, hypermethylation of genes encoding retinoid receptors may cause changes in their expression, which can affect PC progression.

The retinoic acid receptor responder gene 1 (*RARRES1*) also known as tazarotene-induced gene 1 (*TIG1*) was first identified as a gene responsive to retinoic acid. Decreased levels of *RARRES1* expression have been demonstrated in PC. Furthermore, it has been suggested that *RARRES1* gene silencing may be a consequence of receptor binding retinoid RARβ methylation and is correlated with PC progression. Decreased *RARRES1* expression affects cell-cell interactions and results in increased proliferation and invasiveness of tumor cells. Loss of *RARRES1* expression may explain the low sensitivity to retinoid-induced growth regulation (30,31).

Ras-associated domain family 1 (*RASSF1*) is a putative tumor-suppressor gene (TSG) located in the chromosomal

Table II. Genes hypomethylated in prostate cancer.

Gene	Chromosomal location	Function	Hypermethylation in prostate cancer (%)	Refs.
uPA, PLAU	10q22.2	Blood coagulation, fibrinolysis, hemostasis, plasminogen activation	23.2-96.6	(88,89)
HPSE	4q21.3	Cell adhesion	8.5-30	(43)
CYP1B1	2p22.2	Oxidation	5.7-17.1	(44)
WNT5A	3p21-p14	Cell signaling	65	(45)
S100P	4p16	Cellular calcium signaling	50	(45)
CRIP1	14q32.33	Cellular repair and intracellular zinc transport	65	(45)

uPA, *PLAU*, urokinase plasminogen activator; *HPSE*, heparanase; *CYP1B1*, cytochrome P450 family 1 subfamily B member 1; *WNT5A*, wingless-related MMTV integration site 5A; *S100P*, S100 calcium-binding protein P; *CRIP1*, cysteine-rich protein 1.

region 3p21.3. RASSF1 is functionally associated with cell cycle control, microtubule stabilization, cellular adhesion, motility and apoptosis. Hypermethylation of *RASSF1A* is not specific to PC and has been identified in 35.5-96% of various cancers (32-34). Hypermethylation of *RASSF1A* in 53% of PC cases was found to be associated with a higher level of prostate-specific antigen (PSA) and aggressive PC. The effects of changes in the expression of RASSF1A include disturbances of the cell cycle and cell proliferation (35). A meta-analysis suggested that RASSF1A promoter methylation is significantly associated with PC risk and its clinicopathological variables (Gleason score, serum PSA level and tumor stage) (36).

However, Pellacani *et al* (37) suggested that for a set of genes (including *GSTP1*) that are hypermethylated in PC, gene downregulation appears to be the result of cell differentiation and is not cancer-specific. Hypermethylation is observed in more differentiated cancer cells and is promoted by hyperproliferation. These genes are maintained as actively expressed and methylation-free in undifferentiated PC cells, and their hypermethylation is not essential for either tumor development or expansion.

DNA hypomethylation. The mechanism leading to hypomethylation of DNA in cancer is not fully understood. Many studies have suggested diverse causes for the hypomethylation of DNA, including shortages of S-adenosylmethionine precursors or folic acid in the diet or genetic abnormalities in the metabolic pathway of the donor of methyl groups. DNA hypomethylation can also be attributed to a deficiency in methyltransferases. Hypomethylation in promoter regions of genes can lead to a reduction in genome stability through an increase in the expression of transposons, which under physiological conditions are silenced by methylation. Reduction in methylation may also cause a reduction in chromosomal stability and activation of proto-oncogenes (3,5).

Hypomethylation of DNA is more often observed at metastasis than in the early stages of neoplastic transformation in PC. Hypomethylation in PC is observed among repetitive sequences of LINE-1 (long interspersed nuclear element-1), which is found in 64% of cases of advanced PC with metastases to lymph nodes (38,39).

Reduction in the level of methylation of the genome reduces the stability of chromosomes leading to activation of the proto-oncogene MYC (homolog of viral myelocytomatosis oncogene) and RAS (homolog of viral rat sarcoma oncogene). A strong correlation between overexpression of proto-oncogene MYC in PC, and increased degree of invasiveness of the tumor has been shown. Hypomethylation of promotor regions of proto-oncogenes contributes to the activation and overexpression of their products, and thus excessive cell proliferation (8,40).

In PC, hypomethylation and higher expression of several genes, among them *PLAU*, *HPSE* or *CYP1B1* have been identified (Table II).

Urokinase plasminogen activator (uPA; PLAU) plays a key role in tissue degradation, cell migration, angiogenesis, cancer invasion and metastasis. uPA is a member of the serine protease family and is strongly implicated as a promoter of tumor progression in various human malignancies. It is synthesized and secreted as a pro-enzyme, whose activation is markedly accelerated upon binding to specific membrane-bounded or soluble cell surface uPA receptors (uPARs). Binding to uPAR, uPA efficiently converts the inactive zymogen, plasminogen, into the active serine protease, plasmin, which then directly or indirectly cleaves extracellular matrix components including laminin, fibronectin, fibrin, vitronectin and collagen (41,42). The link between the hypomethylation of the *PLAU* gene and alterations in chromosome 10 and the invasiveness and metastasis of different cancers has been confirmed, including PC (5,8).

Heparanase (HPSE), an endo-β-D-glucuronidase, can cleave the heparan sulfate chain of heparan sulfate proteoglycans, and is actively involved in the process of extracellular matrix degradation. Heparanase activity is detectable in platelets, neutrophils, activated T lymphocytes, and various malignancies including PC. Hypomethylation of the *HPSE* gene has been identified in 8.5-30% of PC cases (43).

Cytochrome P450s are a multigene family of constitutively expressed and inducible enzymes involved in the oxidative metabolic activation and deactivation of carcinogens and cancer therapeutics. Cytochrome P450 1B1 (CYP1B1) is encoded by a member of the CYP1 gene family CYP1B1 and is one of the major enzymes involved in the hydroxylation

of estrogens and activation of potential carcinogens. CYP1B1 has been found to be hypomethylated in 5.7-17.1% of PC cases (44).

Wingless-related MMTV integration site 5A (WNT5A), S100 calcium-binding protein P (S100P) and cysteine-rich protein 1 (CRIP1) genes are also found to be hypomethylated in primary PC tissues. The encoded proteins similarly as other hypomethylated genes are associated with tumorigenesis and metastasis (3,45).

WNT5A activates the WNT/β-catenin-independent pathway and its overexpression is associated with tumor aggressiveness enhancing invasive activity. For this action, WNT5A-induced receptor endocytosis with clathrin is required. WNT5A promotes the aggressiveness of PC and its expression is involved in relapse after prostatectomy (46,47).

S100P protein regulates calcium signal transduction and mediates cytoskeletal interaction, protein phosphorylation and transcriptional control. It is suggested that S100P could be considered as a potential drug target or a chemosensitization target, and could also serve as a biomarker for aggressive, hormone-refractory and metastatic PC (48).

Cysteine-rich intestinal protein 1 (CRIP1) belongs to the LIM/double-zinc finger protein family and has been shown to be overexpressed in several tumor types, including breast, endometrial and prostate (45,49,50).

3. Histone modifications and prostate cancer

Histones are highly conserved alkaline proteins that can become post-translationally modified at amino acid residues located on their N- and C-terminal tails. There are four core histones: histone 2A (H2A), histone 2B (H2B), histone 3 (H3) and histone 4 (H4), and one linker histone, histone 1 (H1). Approximately 146/7 base pairs of DNA are wound around each histone octamer, which consists of two copies of each of the core histones, in left-handed superhelical turns (51).

Modifications involving covalent attachment/detachment of the molecules or functional groups to the N-terminal tails of histone contribute to changes of chromatin structure. The main histone modifications include: methylation, acetylation and phosphorylation. Changes in chromatin structure are the result of the remodeling caused by looseness, shifting nucleosomes along the DNA and/or replacement of histones comprising the nucleosome core. Post-translational modifications of histones play an important role in the organization of chromatin structure, particularly in the homeostasis between euchromatin, transcriptionally active and heterochromatin, transcriptionally inactive. Histone modifications can lead to activation or repression of gene expression, depending on the type and position of attached functional groups and amino acid residues which are modified (2,51).

In tumor cells, changes in the pattern of the modification and genomic location of modified histone occur. These alterations overlap in the early stages of tumorigenesis and accumulate as the disease progresses. Changes in the pattern of histone modification in the promoter sequences may be the cause of altered gene expression by activation of oncogenes and TSG repression. These mechanisms may contribute to oncogenesis through upregulation of transcription, replication, DNA repair or cell cycle progression (51,52).

Histone acetylation. Acetylation is a key histone modification and introduction of an acetyl group to the lysine residues of the histone tail. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are a family of enzymes that acetylate/deacetylate the histone tails of the nucleosome. Linking these reversible modifications with changes in gene expression contribute to the recognition of HATs as co-activators and HDACs as co-repressors of transcription. Mutations in genes encoding the above-mentioned enzymes may result in neoplastic transformation (2).

In prostate tumor cells, increased activity of HDACs, and particularly isoforms HDAC1, HDAC2, and HDAC3, which is correlated with elevated serum PSA levels and with increased invasiveness of tumor cells has been observed. In cancer therapy, HDAC inhibitors of the activity of HDACs are used to inhibit proliferation and induce differentiation or apoptosis of neoplastic cells. Hyperacetylation resulting from treatment with HDAC inhibitors has been observed in both normal and neoplastic cells; however, proapoptotic and antiproliferative activity is increased in tumor cells. In addition, studies have demonstrated a lower level of serum PSA in patients who were treated with HDAC inhibitors. HDAC inhibitors activate transcription of the gene encoding the protein p21, which is an inhibitor of cyclin-dependent kinases, and simultaneously inhibits expression of cyclins D1, A and E. The presence of p21 protein affects cell cycle arrest at the G1 phase or G2/M in tumor cells (1,2,8).

Histone methylation. Histone methylation is a modification catalyzed by histone methyltransferase (HMT) carrying a methyl group ($-CH_3$) derived from S-adenosylmethionine (SAM) on a lysine or arginine residue. Methyl groups from lysine or arginine residues are removed by histone demethylase (HDM). For the lysine residue in histone mono-, di- and trimethyl groups may be attached. The methylation of histones is associated with activation, as well as repression of gene expression, depending on the position of amino acid residues in a protein and the level of methylation (53,54).

In PC cells, reduced levels of histone 3 lysine 4 trimethylation (H3K4me3), and histone 3 lysine 18 monoacetylation (H3K18Ac) are observed. Low levels of these two modifications in patients with PC are negative prognostic indicators as well as indicators of an increased risk of relapse in comparison with patients with high levels of these modifications. Elevated levels of histone 3 lysine 9 and 18 monoacetylation (H3K9Ac, H3K18Ac) and histone 3 lysine 4 dimethylation (H3K4me2) allow discrimination between normal and PC cells (51,53).

Phosphorylation of histones. Phosphorylation of histones is catalyzed by a number of kinases such as the cyclin-dependent kinases, called mitogen- and stress-activated protein kinase (MSK) and the kinase Aurora B. Phosphorylation is closely associated with the cell cycle. Most phosphorylation take place on histone 3 serine residues at position 10 and 28, as well as threonine residue at positions 3, 6 and 11 (55,56).

DNA double-strand breaks which may lead to chromosomal aberrations and/or apoptosis contribute to the phosphorylation of serine residue at position 139 of histone subtype 2A, called H2AX. The phosphorylated form of histone H2AX is termed γH2AX. Histone H2AX phosphorylation is catalyzed

by protein kinase ataxia teleangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK) which belong to the family of phosphatidylinositol 3-kinase-related protein kinases (PI3KK/PIKK kinases). Histone γH2AX causes relaxation of chromatin and thereby facilitates the accumulation of repair factors at the site of damage. Nanni *et al* (57) demonstrated the presence of histone γH2AX at different stages of PC. Detailed knowledge of the function of histone H2AX may be important for the diagnosis of cancers, including PC and tumor therapy (2,55,58,59). In PC, overexpression of histone H2AZ, which is a variant of histone 2A was observed (60). H2A.Z is also associated with androgen receptor (AR) gene transactivation and progression of PC. In PC histone 2AZ is ubiquitinated in K120, K121 and K125 and is associated with transcriptional silencing (61).

Recent studies have attempted to determine the relationship between the activity of the proliferation marker Ki-67 (Kiel) in PC, and the level of phosphorylation of histone H3. The expression of Ki-67 protein was found in all cell cycle phases except G0 phase, whereas the phosphorylation of histone H3 was identified only in G2 phase and mitosis. One study showed a higher frequency of phosphorylation of histone H3 in PC, compared to BPH. Furthermore, histone H3 phosphorylation in PC was found to correlate with the proliferation index and expression of the Ki-67 protein and the level of serum PSA (62).

4. Role of histone modification in *MDR1* gene silencing in prostate cancer

Multidrug resistance receptor 1 (*MDR1*) gene encodes a transmembrane P-glycoprotein (P-gp), which is an ATP-dependent transporter. P-glycoprotein plays an important role in the removal of xenobiotics captured in the cell membrane and their removal outside of the cell, which in the case of tumor cells determines their resistance to the chemotherapeutic agents used. *MDR1* promoter methylation is common in prostate carcinoma. However, studies indicate no correlation between *MDR1* gene promoter methylation and reduced transcription of the gene in the early stages of carcinogenesis while post-translational modifications of histones appear to be the primary mechanism for suppression of the *MDR1* gene. *MDR1* promoter methylation and P-gp expression in 121 PC, 39 high-grade prostatic intraepithelial neoplasia (HGPIN), 28 BPH and 10 morphologically normal prostate tissue (NPT) samples were studied, using quantitative methylation-specific PCR and immunohistochemistry, respectively. PC cell lines were exposed to a DNMT inhibitor, 5-aza-2'deoxycytidine (DAC), and histone deacetylase inhibitor trichostatin A (TSA). Methylation and histone post-transcriptional modification statuses were characterized and correlated with mRNA and protein expression. *MDR1* promoter methylation levels and frequency were significantly increased from NPTs, to HGPIN and to PC. Conversely, decreased or absent P-gp immunoexpression was observed in HGPIN and PC, inversely correlating with methylation levels. Exposure to DAC alone did not significantly alter methylation levels, although increased expression was apparent. However, P-gp mRNA and protein re-expression were higher in cell lines exposed to TSA alone or combined with DAC. Accordingly, histone active markers

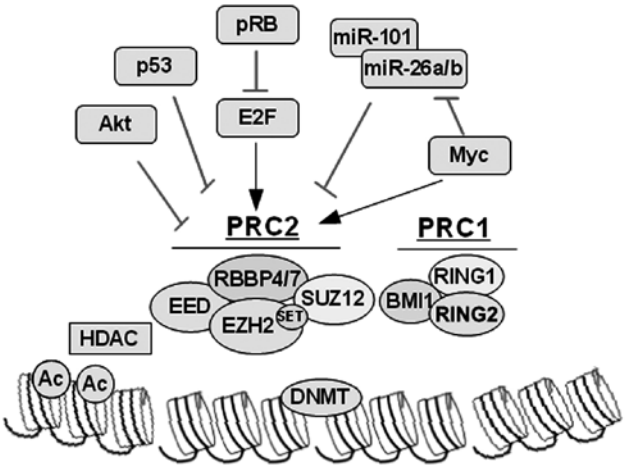

Figure 2. EZH2 histone methyltransferase as an epigenetic silencer of tumor-suppressor genes in prostate cancer (64).

H3Ac, H3K4me2, H3K4me3, H3K9Ac and H4Ac were increased at the *MDR1* promoter after exposure to TSA alone or combined with DAC (63).

5. Modifications of histones and proteins polycomb and Trithorax in the development of prostate cancer

Polycomb group (PcG) proteins as well as trithorax group (TrxG) proteins have been discovered to cooperatively maintain the desirable histone patterns by methylating the histone tails for precise gene expression in various cellular processes. Playing opposing roles although both act to modify lysine residues within histone tails, activities of the PcG proteins are associated with repression of transcription while those of Trx proteins are associated with enhancement (64).

PcG proteins form repressive complexes (PRCs). One of them is the mammalian PRC1 complex consisting of the following proteins: three Ph homologues [polyhomeotic-like protein 1 (PHC1), PHC2 and PHC3], five Pc homologues [chromobox protein homologue 2 (CBX2), CBX4, CBX6, CBX7 and CBX8], two Psc homologues (BMI1 and MEL18) and four other polycomb group RING finger proteins (PCGFs) (65). Mammalian PRC2 complexes contain the direct homologues enhancer of zeste called homologue 2 (EZH2) (or, in some cases, EZH1), embryonic ectoderm development (EED), suppressor of zeste called 12 proteins (SUZ12) and RBBP4 and RBBP7 (histone-binding protein, also known as retinoblastoma associated protein 46 and 48 RBAP48 and RBAP46). PcG repressor complexes inactivate specific regions of chromatin, inhibiting transcription by specific modification of histone tails, then recognized by specific effector proteins (65-67).

EZH2 catalyzes trimethylation of the histone H3 at lysine 27 and is the enzymatic member of the polycomb repressor complex PRC2. In addition, EZH2 induces chromatin compaction and epigenetic silencing of key TSGs in cooperation with PRC1, HDAC and DNMT, which subsequently result in tumorigenesis and metastasis. EZH2 itself can be regulated post-translationally (by Akt-mediated phosphorylation), post-transcriptionally (by microRNAs), and through multiple

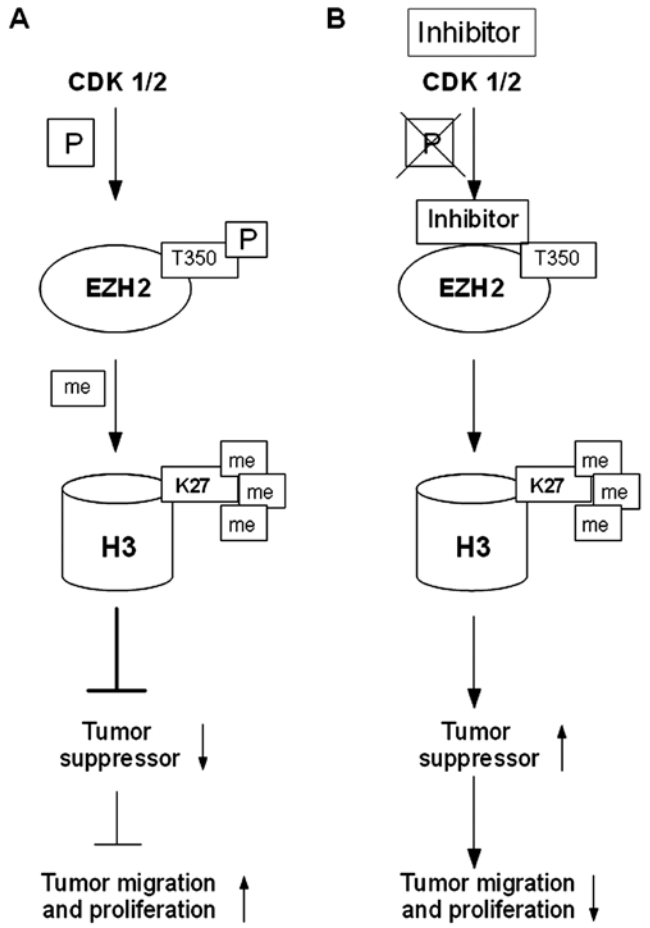

Figure 3. Invasiveness of tumor cells in the case of (A) activation or (B) inactivation of EZH2 histone methyltransferase (8).

pathways transcriptionally (by E2F, p53 and Myc). Molecular targeting of EZH2, as well as HDAC and DNMT, provides important lines for the development of therapeutic strategies with which to target EZH2-high tumors such as late-stage metastatic PC (Fig. 2) (64).

Moison *et al* (68) found that RARβ, a TSG frequently silenced in PC, was hypermethylated in all studied prostate tumors and methylation levels were positively correlated with H3K27me3 enrichments. Thus, using bisulfite conversion and pyrosequencing of immunoprecipitated H3K27me3 chromatin, they demonstrated that DNA methylation and polycomb repression co-exist *in vivo* at this locus. They found this repressive association in 6/6 patient tumor samples of different Gleason score, suggesting a strong interplay of DNA methylation and EZH2 to silence RARβ during prostate tumorigenesis.

Cyclin-dependent kinases 1 and 2 (CDK1 and CDK2) phosphorylate threonine at position 350 (T350) of the EZH2 histone methyltransferase, which via SET domain (histone methyltransferase domain), catalyzes trimethylation of lysine at position 27 of histone H3 (H3K27me3). EZH2 phosphorylation at T350 neither affects the assembly of the PRC2 complex nor the intrinsic HMTase activity of PRC2. Phosphorylation at T350 is important for the recruitment of EZH2 to the promoters of its target genes. Trimethylated lysine residue at position 27 of histone H3 (H3K27me3) carried by the EZH2 protein, correlates with silencing of expression of the *SLIT2* gene (Slit homolog 2; SLIT2) in most cases of metastatic PC (69,70).

In PC, enhanced transcription of the *EZH2* gene was observed, resulting in an increased level of methylation of the lysine residue at position 27 of histone H3 (H3K27me), which inhibits the expression of TSGs, such as DAB2 protein interaction (*DAB2IP*), adrenoceptor β2 (*ADRβ2*) and *CDH1*. Epigenetic silencing of uppressor genes was found to lead to the activation of the RAS proto-oncogene and the gene encoding the nuclear transcription factor-κB (NF-κB), which contributes to increased proliferation and migration of tumor cells. An increase in the EZH2 expression level was found to correlate with the development of PC and may indicate an aggressive nature of cancer. In the presence of an inhibitor of CDK1/2 and EZH2/T350, 3-dezaneplanocin A (DZNeP), a decrease in H4K20 followed by an increase in expression of TSGs, and subsequent blocking of the growth and invasion of PC cells were observed (Fig. 3) (8,69).

Antagonists of polycomb proteins are trithorax group (TrxG) proteins, which prevent the inhibition of transcription, resulting in the relaxation of chromatin and a consequent increase in access of transcriptional activators. TrxGs include both ATP-dependent chromatin remodeling factors and histone modifiers. Histone modifier TrxGs in mammals are grouped into 3 major complexes: complex protein associated with SET domain (COMPASS), COMPASS-like and ASH (absent small and homeotic discs). COMPASS contains a histone methyltransferase domain, which is shared with PRC2. COMPASS mediates H3K4me, unlike PRC2. COMPASS-like complexes also display the SET domain, which is used to silence a more restricted group of genes. Through H4K16 acetylation, COMPASS-like can also activate gene expression. This complex is also able to demethylate H3K27me3, depending on subunit composition thereby directly counteracting PRC2. ASH1 protein can catalyze H3K36me3 giving a signal for activation (66,71).

6. Conclusion

Epigenetics is the study of the modification of DNA and associated proteins without changing the nucleotide sequence. DNA methylation, histone modification, nucleosome remodeling, and RNA-mediated targeting regulate many biological processes that play an important role in normal cell function, and trends occurring in the process of carcinogenesis. These steps result in changes in gene expression and disruption of cellular processes, initiating the promotion of the development of cancer, including PC. The most common epigenetic changes used as biomarkers of cancer are DNA methylation and histone modifications.

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