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Epigenetics of prostate cancer

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1. ABSTRACT

Prostate cancer is the most common type of cancer other than skin cancer and the second leading cause of cancer death in men in the United States. Its exact causes are unknown. Several risk factors have been associated with prostate cancer including age, race, family history and diet. Epigenetic mechanisms including DNA methylation and histone modifications are important means of gene regulation and play essential roles in diverse biological and disease processes. Recently, frequent epigenetic aberrations such as DNA hypo- and hypermethylation and altered histone acetylation and methylation have been observed in prostate cancer affecting the expression and function of a large array of genes, leading to tumorigenesis, tumor progression and metastasis. In this chapter, we examined the current literature regarding epigenetic changes in prostate cancer and discuss the clinical potential of cancer epigenetics for the diagnosis and treatment of this disease.

2. INTRODUCTION

Prostate cancer is a common malignancy and a leading cause of cancer death among men in Western countries. The molecular mechanisms underlying its development and progression remain poorly understood. It has become evident that genetic alterations such as mutations and epigenetic changes, defined as heritable changes in gene expression that occur without changes in DNA sequence (1), contribute to the malignant transformation and progression of prostate cancer. One of the first identified hallmarks of epigenetic alterations is DNA methylation—the addition of a methyl group to the 5'-carbon of cytosine in CpG sequences— catalyzed by three active DNA methyltransferases (DNMTs), DNMT1, DNMT3a and DNMT3b**.** Methylcytosine residues are often found in short stretches of CpG-rich regions (i.e., CpG islands) that are 0.5 – 2 kb long and found in the 5' region of approximately 60% of genes (2). Most CpG islands are

Table 1. Genes affected by epigenetic aberrations in prostate cancer

Epigenetic aberration	Gene symbol[1]	References
DNA hypermethylation		
Hormonal response	AR, ESR1, ESR2, RARB, RARRES1	12, 14, 19-21, 35, 36, 39, 58, 78, 184
Cell cycle control	CCND2, CDKN2A, CDKN1A, SFN	52, 56, 58, 59, 61-63, 78, 242
Tumor cell invasion/tumor architecture	APC, CAV1, CD44, CDH1, CDH13, LAMA3, LAMB3, LAMC2	55, 58, 66, 69, 70, 72-76, 78-80, 243
Repair of DNA damage	GSTP1, MGMT	85, 86, 84, 87, 88, 90, 203-205, 208, 244, 21, 70, 78, 206, 210, 245, 55, 57, 58, 243, 246
Signal transduction	DAB2IP, DAPK1, EDNRB, RASSF1	55, 58, 71, 78, 98, 103, 247, 248
Inflammatory response	PTGS2	58
Others	ALDH1A2, HIC1, MDR1, PXMP4	21, 58, 243, 249-251
DNA hypomethylation	CAGE, HPSE, PLAU, XIST	131, 133, 135, 136
Histone hypoacetylation	CAR, CPA3, RARB, VDR	172, 179, 184, 252, 253
Histone methylation	DAB2IP, GSTP1, PSA	10, 167, 170, 191

[1] Genes are listed alphabetically in each category

unmethylated, with the exception of certain imprinted genes and genes on the inactive X chromosomes of females (3). Changes in DNA methylation can occur as either hypo- or hypermethylation, leading to chromosomal instability and transcriptional gene silencing respectively. Both have been implicated in a variety of human malignancies, including prostate cancer (4).

DNA is organized into a nucleoprotein complex termed chromatin. The basic chromatin unit is the nucleosome, consisting of 146 base pairs of DNA wrapped around an octamer of four pairs of histone proteins (H2A, H2B, H3, and H4) (5). The N-terminal tails of histones, positioned peripheral to the nucleosome core, are subject to various covalent modifications, such as acetylation, methylation, phosphorylation, and ubiquitination by specific chromatin-modifying enzymes (6). The pattern of these modifications has been referred to as 'the histone code' and acts as a second level of epigenetic regulation of gene expression affecting chromatin structure and remodeling (7). Acetylation and deacetylation of histone tails are catalyzed by histone aceyltransferases (HATs) and deacetylases (HDACs), respectively (8). HATs have been shown to increase the activity of several transcription factors, including nuclear hormone receptors, by eliciting histone acetylation, which facilitates promoter access to the transcriptional machinery (9). Conversely, HDACs reduce levels of histone acetylation and are associated with transcriptional repression. Histone methylation occurs on lysine (K) and arginine (R) residues of H3 and H4 and is carried out by histone methyltransferases (HMTs) which uses S-adenosyl-methionine as the methyl group donor. Similar to acetylation, histone methylation has been recently found to be reversible. Active demethylation of histones can be carried out by at least two enzymes, lysine specific demethylase 1 (LSD1) (10) and JmjC domain-containing histone demethylase1 (JHDM1) (11).

DNA methylation and histone modifications are closely related epigenetic mechanisms. Epigenetic control of gene expression often requires the cooperation and interaction of both mechanisms and disruption of any of these events will lead to aberrant gene expression as observed in almost all types of human cancer. Here, we have reviewed the current knowledge and developments regarding epigenetic changes in prostate cancer including DNA methylation and aberrant histone modifications and discuss their implication for understanding the molecular basis of this disease and for its clinical diagnosis and treatment.

3. DNA METHYLATION IN PROSTATE CANCER

3.1. DNA hypermethylation

DNA hypermethylation is the most common and best characterized epigenetic abnormality in human malignancies including prostate cancer. Aberrant hypermethylation of more than 30 genes has been reported in prostate cancer (Table 1). These genes include classic and putative tumor-suppressor genes and genes involved in a number of cellular pathways such as hormonal responses, tumor-cell invasion/tumor architecture, cell cycle control, and DNA damage repair. For many of these genes, promoter hypermethylation is often the main mechanism underlying their functional loss in prostate cancer. Inappropriate silencing of these genes can contribute to cancer initiation, progression, invasion, and metastasis (Figure 1). Hypermethylation in prostate cancer can correlate with pathologic grade or clinical stage and with androgen independence. Some frequently hypermethylated genes constitute a potential prostate cancer-specific methylation signature and are discussed below.

3.1.1. Hormonal response genes

The prostate is an endocrine gland that responds to sex hormones such as androgens, estrogens and progesterones through their specific receptors. Epigenetic modifications such as DNA methylation and histone acetylation participate in the transcriptional regulation of steroid/thyroid receptors in prostate cancer (12, 13).

The androgen receptor (AR) mediates androgen activity, which is essential for the development of both normal prostate and prostate cancer. Prostate cancer is

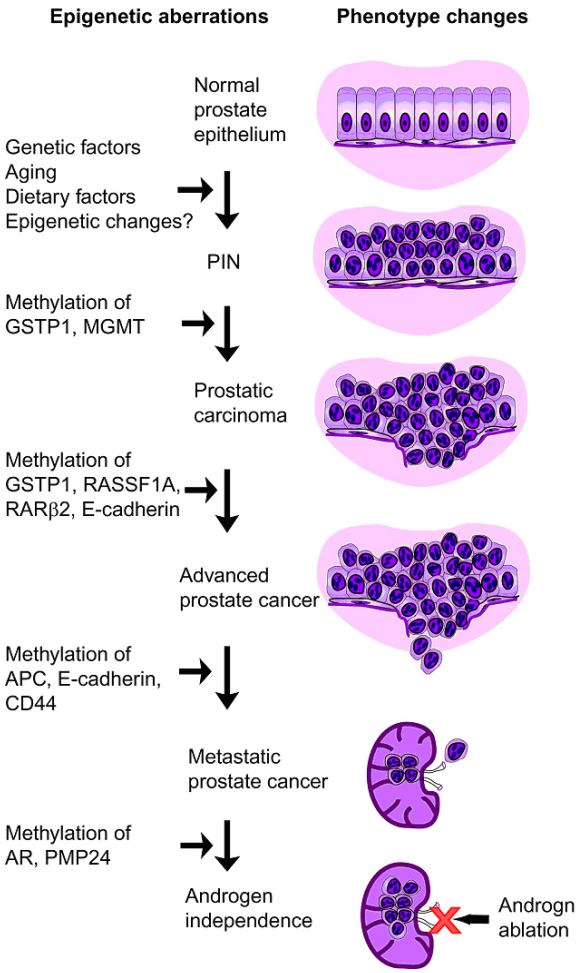

Figure 1. Epigenetic contribution to prostate cancer initiation, progression and metastasis. A number of factors such as genetic defects, aging, dietary and environmental factors are known to contribute to prostate cancer carcinogenesis. Since almost all these factors have been found to be able to modify epigenetic composition of human genome, it is most likely that DNA methylation plays the same critical role as genetic factors in initiating prostate cancer, which is supported by the observation that methylation-mediated inactivation of several genes, especially genes involved in carcinogen metabolism and DNA damage repair such as GSTP1 and MGMT frequently occurs in premalignant lesions of the prostate, which, in turn, causes genetic changes in affected cells. All these events are selective for increased growth rate and lead to irreversible phenotype changes of carcinoma through clone expansion. Subsequently, concurrently epigenetic inactivation of more genes involved in cell cycle control, signal transduction and hormonal response provides a further growth advantage, leading to locally advanced prostate cancer. Further function loss of genes in tumor invasion/metastasis pathway such as APC, CDH1 and CD44 causes cancer cells to dislodge and from subclones in distant sites. Additional inactivation of AR and possibly PMP24 through DNA hypermethylation eventually renders cancer cells androgen independent state. Reproduced from with permission from Li LC, *et al* (18). Elsevier

initially androgen dependent, but can eventually become androgen-independent after androgen deprivation therapy. Androgen-independent prostate cancers are characterized by a heterogeneous loss of AR expression (14-16). Jarrard *et al* (14) first reported aberrant promoter methylation in AR-negative prostate cancer cell lines. Consistent with these results, Izbicka *et al.* (17) showed that 5, 6-dihydro-5'-azacytidine, an inhibitor of cytosine DNA methyltransferase, could restore androgen sensitivity in androgen-insensitive human prostate carcinoma cell lines, which then became sensitive to growth inhibition by anti-androgens. Furthermore, the incidence of methylation-mediated AR inactivation in prostate cancer tissue ranged from 0% to 20% in primary prostate cancers (12, 19-21) and from 13% to 28% in androgen-independent cancers (19, 20).

Genetic alterations, including AR gene mutation (22) and amplification (23) without loss of AR expression, that alter the sensitivity of the AR to androgen are thought to play a key role in the development of androgen-independent advanced prostate cancer. However, there are still 20%-30% of androgen-independent cancers that do not express AR (24). The loss of AR expression in these cases cannot be explained as the result of AR amplification. Indeed, AR promoter methylation in androgen-independent prostate cancer is more prevalent than in primary prostate cancer (19, 20). This suggests that epigenetic silencing of AR by DNA hypermethylation could be an alternative mechanism leading to androgen independence in a subset of patients with advanced prostate cancer.

Although estrogens have been historically used for the treatment of prostate cancer, their function in the prostate remains unclear (25). The action of estrogens was thought to be mediated via a blockade of the pituitary-testicular axis (26). However, estrogens have been shown to exert direct effects on prostatic cancer cells via their own receptors (27, 28).

The prostate expresses two types of estrogen receptors (ERs): ERα (ESR1) and ERβ (ESR2) (29). Lost or decreased expression of ESR1 and ESR2 in prostate cancer has been documented (30-33). Low ESR1 expression is also associated with poor prognosis for effective endocrine therapy using estrogens (34). We observed that, in prostate cancer, the ESR1 gene was frequently methylated and the severity of methylation was positively associated with tumor progression (35).

To study the potential epigenetic inactivation of ESR2, we cloned its promoter sequence and identified a typical CpG island within the promoter (35). Subsequent studies from our laboratory (12, 36) and others (37-39) support the concept that hypermethylation is the main known mechanism responsible for inactivation of ER expression in prostate cancer. It is interesting to note that metastatic prostate cancer cells may regain ESR2 expression (29), which is accompanied by loss of promoter methylation (38). This observation provides further

evidence that ESR2 inactivation in primary prostate cancer is epigenetic by nature, and thus reversible.

3.1.2. Cell cycle control genes

An important characteristic of tumor cells is their increased proliferative ability, which is often associated with impaired regulation of the cell cycle. The cell cycle has multiple checkpoints that are controlled by a number of complex modulation systems, including the retinoblastoma (RB1) protein, cyclins, cyclin dependent kinases (CDKs), and CDK inhibitors (CDKIs) (40).

RB1 was the first tumor suppressor gene to be identified and its alteration has been observed in many tumor types (41). RB1 inactivation resulting from promoter methylation is a common event in retinoblastomas (42, 43) but a rare event in prostate cancer (44). RB1 inactivation in prostate cancer is apparently the result of loss of heterozygosity and mutation (44, 45).

CDKIs are negative regulators of cell cycle progression and thus are considered to be potential tumor suppressor genes. Currently, CDKIs are grouped into two families: the INK4 family, which includes CDKN2A (p16), CDKN2B (p15), CDKN2C (p18), and CDKN2D (p19), specifically inhibits cyclin D-associated kinases (CDKs 4 and 6); and the CIP/KIP (kinase inhibitor protein) family, which includes CDKN1A (p21), CDKN1B (p27), and CDKN1C (p57), inhibits most CDKs (46).

Failure of cell cycle arrest secondary to alterations in CDKI expression has been implicated in prostate cancer (47, 48). CDKN2A can be inactivated by a variety of mechanisms including deletion, point mutation, and silencing by hypermethylation in a number of cancers, including prostate (49-51). Methylation-mediated inactivation of the CDKN2A gene has been reported in prostate cancer cell lines (52, 53) and prostate cancer tissue, with incidence rates ranging from 0%-16% (53-57). Methylation at exon 2 of the CDKN2A gene is more frequent in prostate cancer tissue (66%) than methylation of the promoter region (57); however, exon 2 methylation does not affect gene expression (56, 57) making the functional relevance of this epigenetic event unclear. However, because CDKN2A exon 2 hypermethylation only occurs in cancer tissues, it may serve as a biomarker to detect or confirm a prostate neoplasm. Inactivation of other cell cycle genes such as CDKN2B, CDKN1A and CDKN1B by hypermethylation is rare in prostate cancer (57-59).

Recently, 14-3-3sigma (SFN), a putative cell cycle negative regulator (60), was found to be downregulated in prostate cancer cell lines and tissues by promoter methylation (61, 62). However, SFN methylation also occurs in BPH tissue and thus is not cancer specific (63).

3.1.3. Tumor invasion and tumor architecture genes

The cadherin–catenin adhesion system is critical to the preservation of normal tissue architecture and is regulated by a family of proteins collectively termed cell adhesion molecules (CAMs). Decreased expression of E-cadherin (CDH1) and other CAMs has been reported to have prognostic significance in various human cancers, including prostate cancer (64, 65).

In human prostate tumors, expression of CDH1 is dramatically suppressed and its promoter is methylated to varying degrees (55, 66-68). Graff *et al.* (69) were the first to report that the 5' CpG island of CDH1 was densely methylated in prostate cancer cell lines. A study of prostate cancer tissue samples from our own laboratory showed that methylation of the CDH1 promoter, as detected by bisulfite genomic sequencing of 29 CpG sites within the promoter and first exon, occurred in 30% of low grade and 70% of high grade prostate cancer samples and correlated with absent or reduced CDH1 protein expression, as detected by immunohistochemistry (66). Consistent with our data, Kallakury *et al.* (67) reported an 80% prevalence of CDH1 methylation in prostate cancer samples analyzed by methylation-specific polymerase chain reaction (PCR). In addition, methylation of the CDH1 promoter is increased in advanced prostate tumors, suggesting that it might be a useful biomarker to assess tumor progression (66). There are, however, some discrepancies regarding the prevalence of CDH1 methylation in prostate cancer. A study by Woodson *et al.* (70) reported in 2003 that methylation of the CDH1 promoter (-159 to -51 region) could not be detected in any of 101 prostate cancer samples using real-time PCR. However, the same group (71) reported in 2004 a 22.4% prevalence of CDH1 methylation in prostate cancer using the same assay method but covering a different region (+59 to +140). Thus, different methodologies (methylation-specific polymerase chain reaction vs. bisulfite genomic sequencing) and different genomic regions (promoter vs. exon) examined may contribute to the observed discrepancies.

CD44 is an integral membrane protein that is involved in matrix adhesion, and signal transduction. In prostate cancer, loss of CD44 expression correlates with methylation of its gene promoter (70, 72-76) CD44 expression and promoter methylation also correlate with prostate cancer stage and patient prognosis (75, 77). Other genes involved in the cadherin–catenin adhesion system have also shown methylation-mediated inactivation in prostate cancer such as H-cadherin (CDH13) (78), adenomatous polyposis coli (APC) (78), caveolin-1 (CAV1) (79), laminin alpha 3 (LAMA3), laminin beta3 (LAMB3) and laminin gamma 2 (LAMC2) (80) (Table 1).

3.1.4. DNA damage repair genes

DNA repair is a correcting mechanism that maintains genome stability during replication or following DNA damage. Cells defective in components of DNA repair pathways exhibit higher rates of spontaneous DNA mutations, which can lead to cancer (81). Hypermethylation of two genes involved in DNA damage repair has so far been reported in prostate cancer, they are the detoxifier gene glutathione S-transferase Pi (GSTP1) and the DNA alkyl-repair gene O^6-Methylguanine DNA methyltransferase (MGMT).

GSTP1, located at chromosome 11q13, belongs to a supergene family of glutathione S-transferases (GSTs) that play an important role in the detoxification of

electrophilic compounds (such as carcinogens and cytotoxic drugs) by glutathione conjugation (82). GSTP1 inactivation may lead to increased cell vulnerability to oxidative DNA damage and to the accumulation of DNA base adducts, which can precede other relevant genetic alterations in carcinogenesis (83).

In prostate cancer, methylation of the GSTP1 gene promoter is the most frequently detected epigenetic alteration, with a frequency ranging from 70%-100% in prostate cancer DNA specimens (84-86). Most notably, GSTP1 methylation could also be detected in 50%-70% of prostatic intraepithelial neoplasia (PIN) (87, 88), a precursor lesion of prostate cancer (89). Hypermethylation of the GSTP1 gene has also been detected in non-malignant prostate tissue, but at a much lower level and frequency compared to malignant tissue (21, 88, 90). The profound and yet cancer-specific hypermethylation of the GSTP1 gene in prostate cancer cell lines and tissues provides a good model for the study of the molecular mechanisms underlying methylation-mediated gene silencing and may also server as a potential tumor biomarker for clinical detection of prostate cancer (to be discussed later in the text).

MGMT is a DNA repair protein that removes mutagenic and cytotoxic alkyl adducts from genomic DNA. Tumors that lack MGMT expression have a higher incidence of point mutations in the genes encoding p53 and K-ras, which may advance the cancerous state (91). In addition, MGMT deficient tumors exhibit high sensitivity to the effects of chemotherapeutic alkylating agents. Moderate to high levels of MGMT promoter methylation have been detected in prostate cancer (55, 57), while others report no significant MGMT promoter methylation (21, 78, 92). Further work will be necessary to resolve this discrepancy.

3.1.5. Putative tumor suppressor genes

Functional loss of classic tumor suppressor genes through DNA hypermethylation is not a common event in prostate cancer. For instance, methylation of the retinoblastoma-1 gene (93), the mismatch repair gene MLH1 (94) and von Hippel–Lindau gene (95) has been frequently detected in other types of cancer, but not in prostate cancer. Instead, some putative tumor suppressor genes are silenced by DNA hypermethylation in prostate cancer, most notably, the Ras association domain family 1 gene (RASSF1).

RASSF1, located at 3p21.3, encodes a protein similar to the RAS effector proteins. The biologic activity of RASSF1 is largely unknown. Both *in vitro* and *in vivo* studies show that overexpression of RASSF1 in cancer cells leads to cell cycle arrest (96), reduced colony formation, and inhibition of tumor growth in nude mice (97). Thus, a tumor suppressor role has been proposed for RASSF1 (98, 99).

In prostate cancer, RASSF1 promoter methylation is a common event, occurring in 54%-96% of samples (55, 58, 68, 78, 98, 99). RASSF1 promoter methylation was detected in some non-malignant prostate tissue samples (78, 100) but not in many others (55, 58, 68). A large percentage (64%) of PIN samples exhibit RASSF1 promoter hypermethylation (55). Increased RASSFI promoter methylation is also associated with advanced tumors (i.e., those with high Gleason scores) (55, 78, 98). These findings indicate that RASSF1 promoter methylation occurs in the early stages of prostate cancer development, increases as the cancer progresses, and is a potential tumor biomarker for prostate cancer diagnosis and risk assessment.

There are possibly additional genes with putative tumor suppressor function undergoing epigenetically inactivation in prostate cancer including KAI1 (a prostate-specific tumor metastasis suppressor gene) (101), inhibin-alpha (a member of the TGF-beta family of growth and differentiation factors)(102) and DAB2IP, a novel GTPase-activating protein for modulating the Ras-mediated signal pathway (103). However, the hypermethylation reported in these studies is not significant enough to suggest a causal role in prostate carcinogenesis or a role as a biomarker for prostate cancer diagnosis.

3.1.6. Hypermethylation occurs early in prostate carcinogenesis

Transformation of benign epithelial glands to premalignant lesions and then to invasive carcinoma represents a multi-step process of prostate cancer development. Several morphological alterations have been proposed as potential precursor lesions such as high grade PIN (104) and proliferative inflammatory atrophy (PIA) (105). High grade PIN consists of architecturally benign prostatic acini and ducts, lined by cytological atypical cells and is the most likely precursor of prostate cancer because of its morphological and genetic similarities to invasive prostate cancer (106) (Figure 1). PIA consists of discrete foci of proliferative glandular epithelium with the morphological appearance of simple atrophy or postatrophic hyperplasia, occurring in association with inflammation (105, 107).

GSTP1 gene methylation has been frequently found in PIN (21, 87, 108), along with other genes such as RARβ2 (21), APC, MGMT, and RASSF1 (55).

Normally, PIA expresses elevated GSTP1 in response to increased oxidative stress (107), however, GSTP1 methylation also occurs in PIA lesions (108), but to a much less extent compared to that in PIN.

The potential contribution of GSTP1 inactivation through hypermethylation in the progression of premaliganant prostate lesions to prostate carcinogenesis has not been established. Recent studies show evidence that epigenetic lesions can lead to genetic lesions in cancer and specific epigenetic lesions such as hypermethylation of MGMT and GSTP1 may lead to specific genetic lesions: G to A transitions and steroid-related adducts in DNA respectively (109). Nelson *et al* (110) proposed a model in which GSTPI acts as a 'caretaker' gene during the pathogenesis of prostate cancer. Loss of GSTP1 activity

renders prostate cells vulnerable to genome damage associated with chronic prostatic inflammation and repeated exposure to carcinogens, which in turn promote transformation to high grade PIN and thus prostate cancer.

3.2. DNA hypomethylation

DNA methylation in mammalian genomes is a defense mechanism by which repetitive DNA (which accounts for at least 50% of genome's content) is transcriptionally silent to prevent it from propagating (111). Demethylation of normally methylated DNA, also known as hypomethylation, can disrupt this defense mechanism leading to structural and functional alterations of the genome. There are two types of hypomethylation: global or genomic hypomethylation, which refers to an overall decrease of 5-methylcytosine content in the genome, and localized or gene-specific hypomethylation, which refers to a decrease in cytosine methylation relative to the "normal" methylation level. This latter process affects specific regions of the genome, such as the promoter regions of protooncogenes or normally highly methylated sequences such as repetitive sequences and oncogenes (112). Both global and gene-specific hypomethylation have been implicated in human cancer.

3.2.1. Global hypomethylation

Net decreases in the content of methylcytosines in cancer often exceed the localized increases in DNA methylation associated with carcinogenesis (113, 114). For example, in colon adenocarcinomas, the genomic 5-methylcytosine content is reduced by an average of 10% (115). Global DNA hypomethylation has also been found in the premalignant or early stages of some neoplasms (115, 116) and is implicated as an important factor for tumor progression (84, 117). It is unclear whether this epigenetic alteration is a cause or consequence of tumorigenesis. To add to the complexity, hypomethylation induced by disrupting DNMT1 has been found to either inhibit (118) or promote (119) tumor growth. In a well studied model of mouse intestinal neoplasia, mice carrying a germ-line mutation in the APC gene ($APC^{Min/+}$) crossed with mice heterozygous for a DNMT1 mutation showed a dramatic reduction in tumor number compared with Min mice crossed with wild-type DNMT1 (118, 120). In contrast, genomic hypomethylation has been associated with the induction of T cell lymphomas in mice carrying a hypomorphic DNMT1 allele, which reduces DNMT1 expression to 10% of wild-type levels and results in substantial genome-wide hypomethylation in all tissues. Whether hypomethylation promotes or inhibits tumor progression might be related to differences in model systems or tissue specificity (121).

The initial findings regarding DNA methylation in the prostate came from studies by Bedford and Helden (122) more than a decade ago. They observed that the overall 5-methylcytosine content in DNA from prostates with benign prostatic hyperplasia and metastatic tumors was significantly lower than that in DNA from nonmetastatic prostate tumors. Further studies found that global hypomethylation is associated with the clinical stage (84, 123) and metastatic state (124) of prostate cancer. By examining the global 5-methylcytosine levels in 30 prostate cancer samples using an antibody that reacts with 5-methylcytosine, a recent study found that prostate cancer cells have a pronounced decrease in global methylation compared with benign and normal tissue (125). A known consequence of hypomethylation is genetic instability, thus, chromosomal aberrations are often associated with hypomethylation in prostate cancer (124).

3.2.2. Gene-specific hypomethylation

Gene-specific hypomethylation was first noted more than 20 years ago. In 1983, Feinberg and Vogelstein (126) showed that genes from cancer cells were substantially hypomethylated when compared to normal cells. They further reported that, compared with adjacent normal tissues, cancer tissues contained two hypomethylated ras oncogenes, c-Ha-ras and c-Ki-ras (127).

Hypomethylation of loci transcriptionally controlled by methylation may enhance transcription of associated genes (128). In the prostate, the PLAU gene is highly expressed in most prostate cancer tissues (129) and invasive prostate cancer cell lines (130). The PLAU gene encodes urokinase plasminogen activator, a multifunctional protein that can promote tumor invasion and metastasis in several malignancies including prostate cancer. Although gene amplification has been attributed to PLAU overexpression (130), recent evidence suggests that DNA methylation may also play a role in the regulation of PLAU gene in prostate cancer (131). Hypomethylation of the PLAU promoter is associated with its increased expression in hormone-independent prostate cancer cells, higher invasive capacity *in vitro*, and increased tumorigenesis *in vivo* (131). However, in normal prostate epithelial cells and in hormone-dependent LNCaP cells, the PLAU promoter is methylated resulting in lower expression of the gene (131).

Other hypomethylated genes in prostate cancer include CAGE, a novel cancer/testis antigen gene (132), heparanase (HPSE) (133), CYP1B1(134) and XIST (135). Hypomethylation of CAGE, which occurs at a frequency of approximately 40% in prostate cancers, is responsible for its exclusive expression in cancer tissues (136). Data from our laboratory shows that HPSE, an endo-beta-D-glucuronidase, and CYP1B1 are overexpressed and substantially hypomethylated in prostate cancer compared with benign prostatic hyperplasia samples (133, 134).

There is little information regarding the paradoxical coexistence of global and regional hypo- and hypermethylation in cancer in which DNA methyltransferase activity is generally high (35, 137, 138). DNA methylation has been considered as a mechanism by which tissue-specific expression of genes are regulated (139). Therefore, the observed gene specific hypomethylation in cancer may result from disrupted transcriptional inhibition of normally silenced tumor promoting genes. Additionally, gene specific hypomethylation may be associated with global hypomethylation (140) but not with gene specific

hypermethylation (141). Thus, hypo- and hypermethylation may contribute individually to the process of carcinogenesis (141).

3.3. Factors that affect DNA methylation

3.3.1. Age

In the United States, prostate cancer is primarily a disease of the elderly. Among males under 40 years of age prostate cancer is extremely rare affecting only 1 in 12,000. In contrast, prostate cancer affects 1 in 44 males aged 40 to 59, 1 in 7 males aged 60 to 79 and over half of males over 80 years of age (142). These statistics clearly demonstrate that prostate cancer susceptibility is highly influenced by age.

It is also clear that aberrant promoter hypermethylation occurs due to aging in normal human tissues (143-145). In colorectal mucosa, methylation of a CpG island on the ERα gene increases linearly with age (146). A recent study from our laboratory shows that the methylation prevalence of ERα increased dramatically with age from 50.0% in patients aged 60 years and under to 89.7% for patients aged 70 years and over (147). It is possible that age-related gene methylation/inactivation increases the susceptibility of cells to neoplastic transformation. Thus, age-dependent gene methylation may be an important early event leading to cancer initiation and may represent a mechanism linking aging and prostate cancer.

3.3.2. Race

Compared with all other cancers, prostate cancer has the highest racial disparity in incidence and mortality (142). In the United States the incidence of prostate cancer is highest in African-Americans, followed by Caucasians, Hispanics, Asian/Pacific Islanders, and American Indians (148-151). African-Americans are also more likely to have a family history of prostate cancer and are younger at the time of diagnosis (152). These racial differences have been hypothesized to result from differences in genetics, diet, socioeconomic status, lifestyle, etc. However, epigenetic mechanisms, through interactions with genetic, environmental and dietary factors, may also play roles in these differences.

Two studies by Woodson et al (68, 70) examined racial differences in the methylation status of the GSTP1, CD44, E-cadherin, RASSF1, RARβ2, EDNRB, Annexin-2, and CAV1 genes in prostate tumors and observed only a slightly higher frequency of CD44 methylation among African-Americans relative to Caucasians. By comparing GSTP1 methylation in prostate cancer samples with their clinical and pathological outcomes, a study from our laboratory showed that among African-Americans, cases with GSTP1 methylation are 13.3 times more likely to have PC whereas in Caucasians this ratio is only 3.8. These results suggest that GSTP1 hypermethylation is a very sensitive diagnostic marker for African-Americans (153). In another study, we observed a significant higher methylation frequency for ERα and ERβ in prostate tumors isolated from Western men than that from Asian men (Li, LC, unpublished data). Future work in this area may aid in our understanding of the molecular basis of race-related disease, as well as identify biomarkers that can better detect and assess prostate cancer in a particular ethnic group.

3.3.3. Environmental and dietary factors

Environmental and dietary factors are believed to contribute to differences in cancer incidence among populations with different dietary habits and life styles. DNA methylation may mediate some of the effects of environmental exposures and lifestyle factors on disease risk with early studies showing an effect of methyl-deficient diets on global methylation (154). In liver cancer, chronic alcohol exposure and associated hepatitis and cirrhosis are accompanied by high levels of methylation of CDKN2A and other genes (155). In the colon, a small study suggested that high-fiber diets are associated with reduced levels of ER methylation, whereas reduced estrogenic hormone levels (via premature menopause) were associated with higher levels of methylation (156). Results from a case-control study of 1,294 prostate cancer patients and 1,451 controls also supports a favorable role of dietary folate on prostate cancer risk (157).

The rates of clinically significant prostate cancer have been shown to be 15-fold higher in men from the United States than in men from Asian countries (158). The high levels of soy consumed in Asian countries are thought to be one factor that may be responsible for this discrepancy. A traditional Asian diet contains soy products such as tofu, soy flour and soy milk (159). The average intake of soy isoflavone in Asian diets is estimated to be 50 mg/day where the average American eats, only a few milligrams of isoflavones per day (160). In an animal study, the effect of an isoflavone compound on DNA methylation was examined in male mice fed a diet containing genistein. Consumption of genistein was found to correlate positively with changes in prostate DNA methylation at CpG islands of a few mouse genes (161).

Further, Fong et al (162) found that the DNA hypermethylation of RARB can be reversed by genistein, resulting in reactivation of RARB expression in LNCaP and PC3 prostate cancer cells. The same group earlier showed that the tea polyphenol (-)-epigallocatechin-3-gallate (EGCG), the major polyphenol from green tea, can inhibit DNMT activity and reactivate the methylation-silenced RARB gene in prostate cancer cells (163).

Smoking may also affect DNA methylation. Enokida et al demonstrated significant correlation of methylation status of multiple genes with smoking status in prostate cancer (164).

All these studies indicate that environmental and dietary factors may influence the risk of prostate cancer via epigenetic pathways and some factors may possess preventive and therapeutic potential in prostate cancer.

4. ABBERATIONS OF HISTONE MODIFICATIONS IN PROSTATE CANCER

Accumulating evidence indicates that histone modifications play important roles in regulating gene expression during prostate cancer initiation, progression and metastasis. Notably, the enhancer of the zeste homolog

2, Drosophila (EZH2) gene is involved in multiple epigenetic abnormalities. EZH2 encodes a polycomb protein which contains a SET domain and thus has histone methyltransferase activity and can catalyze the addition of a methyl group to histone at K27 (165). Varambally *et al* were the first to link the EZH2 gene to prostate cancer by observing that EZH2 is overexpressed in hormone-refractory and metastatic diseases (166). Following this initial study, a number of important discoveries have been made indicating that EZH2 may play a causal role through several epigenetic pathways in cancer progression. For example, EZH2 has been shown to silence the expression of DAB2IP, a putative tumor suppressor, in prostate cancer cells by adding repressive methyl groups to H3-K27 on the DAB2IP promoter and by inducing histone deacetylation (167). Further, EZH2 was recently found to control DNA methylation through direct physical contact with DNA methyltransferase (168). Thus, overexpression of EZH2 in cancer could cause multiple epigenetic aberrations affecting a number of genes. In support of the early study in prostate cancer, overexpression of EZH2 was recently found to be associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma, cancers of the endometrium, breast, and prostate (169). This strongly suggests that EZH2 is a promising prognostic marker for prostate cancer (166).

Another gene that appears to be tightly controlled by histone methylation is the prostate-specific antigen (PSA) gene which is an AR responsive gene and contains the AR response element (ARE) in its 5' regulatory region. Methylation of H3-K4 is associated with transcriptional inactivation of the PSA gene in the prostate cancer cell line LNCaP, and AR-mediated transcription of the PSA gene was accompanied by rapid decreases in di- and tri-methylated H3 at lysine 4 (170). In addition, a lysine specific demethylase (LSD1) has been found to interact with AR to stimulate the AR-dependent transcription of PSA in LNCaP cells by removing the methyl group at H3-K9 (10). These findings may suggest that LSD1 is a potential therapeutic target for prostate cancer. Furthermore Metzger *et al* have identified an inhibitor of LSD1, pargyline which can block AR-dependent transcription via blocking histone demethylation (10).

Another important aspect of aberrant histone modification in prostate cancer is the loss of acetylation of H3 and H4 resulting from increased HDAC activity. One study has directly demonstrated changes in histone acetylation associated with a particular gene locus by using the chromatin immunoprecipitation (ChIP) assay (103). Other indirect studies reported that treatment of prostate cancer cells with HDAC inhibitors increased expression of specific genes such as insulin-like growth factor binding protein 3 (171) and carboxypeptidase A3 (CPA3) (172), thereby inferring a role for histone acetylation in gene regulation.

The 1,25-(OH)2 vitamin D3 acts through the vitamin D receptor to exert cell cycle regulatory antiproliferative effects in a variety of tumor cells, including prostate (173-176). However, prostate cancer cells display a range of sensitivities to the antiproliferative effects of 1,25-(OH)2 vitamin D3 (177, 178), although the reasons for this range of sensitivity are unclear. Prostate cancer cells that are insensitive to 1,25-(OH)2 vitamin D3 have increased levels of nuclear receptor co-repressor SMRT (silencing mediator of retinod and thyroid), which could result in increased deacetylase activity and decreased transcriptional activity of the vitamin D receptor (179). In addition, combined treatment of prostate cancer cell lines with the HDAC inhibitor trichostatin A (TSA) and 1,25-(OH)2 vitamin D3 synergistically inhibits cell proliferation (179). This finding may be useful in the clinical setting in which use of 1,25-(OH)2 vitamin D3 and its analogues in combination with HDAC inhibitors could activate the vitamin D receptor while minimizing unwanted side effects associated with 1,25-(OH)2 vitamin D3, such as hypocalcaemia.

Apart from changes of histone modifications of individual genes in prostate cancer, global changes have recently been studied in prostate cancer (180). By staining prostate cancer tissue samples with antibodies against five modified histones, including acetylated H3K18, H3K9, H3K12, and di-methylated of H3K4, H4 at arginine (R) 3, the authors found that changes in these modifications are predicative of clinical outcome. However the mechanistic basis of such changes and the genes affected are currently unclear.

5. INTERACTION BETWEEN DNA METHYLATION AND HISTONE MODIFICATION

Although DNA methylation and histone acetylation can each separately modulate gene expression, they also interact to form a transcriptionally inactive chromatin state by binding methylated DNA binding proteins such as MeCP2. This interaction then recruits histone deacetylase activity to methylated promoters, resulting in gene silencing (181). In addition, DNMTs can directly recruit HDAC activity to silence gene expression (182, 183). In vitro studies have demonstrated that DNA methylation and histone acetylation cooperate in regulating expression of several genes in prostate-derived cell lines. For example, TSA and the demethylating agent 5-aza-deoxycytindine cooperatively activate DAB2IP mRNA expression in PC-3 cells, which have low basal levels of acetylated H3 (103). Another gene that is regulated by both DNA methylation and histone acetylation is the RARB gene (184). All RARB-negative cells (LNCaP, PC3, and DU145) are hypoacetylated at both H3 and H4 of its promoter. Combined TSA and all-trans retinoic acid treatment after 5-azacytidine treatment increased the accumulation of acetylated histone levels, which led to the activation of the methylated RARB promoter and expression of RARB (184). These studies provide evidence that promoter hypermethylation and histone deacetylation cooperate in maintaining inactive chromatin and provide a rationale for a combined treatment with DNA methylation and HDAC inhibitors in reversing epigenetic silencing of key tumor suppressor genes.

Another line of evidence suggests that DNA methylation also interacts with histone methylation to modulate chromatin structure and regulate gene transcription (185, 186). For example, MeCP2 binds to methylated CpG dinucleotides, recruits HDAC activity and facilitates methylation of H3K9 thereby reinforcing an inactive chromatin state (186). In Neurospora, histone methylation directs DNA methylation mediated by the adaptor protein, heterochromatin protein HP1 (187). This mechanism could also be operational in mammals in which a direct interaction between DNMTs and histone methyltransferase has been observed (185, 188). Although it is unclear which methylation event occurs first, available data supports both possibilities (189, 190). However, in LNCaP prostate cancer cells, transcriptional shutdown of the GSTP1 gene requires several sequential changes involving DNA methylation as the initial event, followed by histone deacetylation, and then histone methylation (191).

6. EPIGENETIC CHANGES AS PROSTATE CANCER BIOMARKERS

To be clinically applicable, an ideal tumor biomarker must be readily detectable in clinical specimens obtained through minimally invasive procedures. DNA hypermethylation seems to fulfill this requirement and has been considered a promising biomarker for several reasons (192, 193). First, unlike mutations, methylation always occurs in defined regions (i.e., CpG islands) and can be detected using techniques with high sensitivity such as methylation-specific PCR (194) and high resolution such as bisulfite genomic sequencing (195). Second, hypermethylated DNA is associated with virtually every type of tumor (196), with each type apparently having its own signature of methylated genes. Examples are the prevalence of methylation of GSTP1 in prostate cancer (84, 85), von Hippel–Lindau gene in renal cancer (95, 197), MLH1 in colon cancer (198), and APC in esophageal cancer (199). Third, some methylation occurs early in cancer development (193).

Because GSTP1 is the most frequently methylated gene in prostate cancer, attempts have been made to detect prostate cancer by identifying methylated GSTP1 CpG islands in clinical samples such as plasma and serum (90, 200), prostate secretions (201, 202), voided urine (203-207), and prostate biopsy specimens (208, 209). Goessl *et al.* (205) examined DNA methylation of the GSTP1 gene in urine after prostatic massage and detected prostate cancer with a specificity of 98% and an overall sensitivity of 73%. In a similar study using post-biopsy urine samples (206), the specificity was 67% and the sensitivity was only 58%. These numbers are even lower if simple voided urine is used as the DNA source (204). GSTP1 methylation was detected in 36%-72% of plasma samples from patients with prostate cancer (90, 200). Harden *et al.* (209) compared the results of a blinded histologic review of sextant biopsy samples from 72 excised prostates with the relative methylation levels of GSTP1 (defined as the ratio of methylation level of GSTP1 to that of a reference gene, MYOD1). They found that histology alone detected prostate carcinoma with a sensitivity of 64% and a specificity of 100%, whereas the combination of histology and GSTP1 methylation at an assay threshold of greater than 10 (a cut-off level for GSTP1/MYOD1 ratio) detected prostate carcinoma with a sensitivity of 75% and a specificity of 100%. This increase represents an 11% improvement in sensitivity compared with histology alone. GSTP1 methylation can also be used to detect occult prostate cancer cells in lymph nodes. Kollermann *et al.* (210) found evidence of GSTP1 hypermethylation in 90% of lymph nodes from prostate cancer patients, but in only 11.1% of lymph nodes from a non-cancer cohort, suggesting a role of GSTP1 methylation detection in molecular staging of prostate cancer. Another gene assessed for feasibility as a prostate cancer biomarker is CD44. Although hypermethylation of CD44 can be readily detected in the serum of prostate cancer patients, there is lack of specificity for the disease because CD44 is also found in normal epithelial specimens (74).

Using the methylation status of a single gene as a biomarker for prostate cancer has certain limitations such as insufficient sensitivity, lack of specificity in differentiating prostate cancer from non-malignant diseases and from cancers originating from other organs, and poor risk assessment. An examination of the methylation pattern of multiple genes may overcome such limitations and offer better diagnostic and prognostic possibilities than that of a single gene. By profiling the methylation pattern of multiple genes in prostate tissue, several recent studies (55, 58) have shown improved sensitivity and specificity in detecting prostate cancer. For example, examining the methylation of GSTP1, APC, RASSF1, and MDR1 can distinguish primary prostate cancer from benign prostate tissues, with sensitivities of 97%-100% and specificities of 92%-100% (58). Similar methylation patterns were observed in studies conducted with Korean prostate cancer patients (55) and Western patients (58). Three of four genes (GSTP1, RASSF1A, APC, and MGMT) studied in both populations have similar high prevalence (55, 58), indicating the existence of a unique prostate cancer-specific methylation fingerprint that is not defined by race/ethnicity.

These studies however, have only examined primary or metastastic tissues. For methylation profiling of multiple genes to be a clinically practical tumor marker, these results need to be validated in clinical specimens of prostate cancer patients. In this regard, a recent study evaluated the methylation status of a panel of nine genes and found that the methylation of four genes including CDKN2A, $p14^{ARF}$, MGMT, and GSTP1 detected 87% of prostate cancers with 100% specificity (207).

Although most current studies focus on candidate gene approaches, there is an urgent need to perform genome-wide screening of unknown methylated loci in prostate cancer and add these loci to the pool of known methylated genes for methylation profiling. We can envision a scenario in the future in which microchips spotted with five to 10 genes representing the best prostate cancer methylation fingerprints will be available for rapid, accurate diagnosis and risk assessment of patients with prostate cancer.

Another potentially useful application of methylation profiles is in the molecular classification of prostate cancer. The current prostate cancer classification system is dependent largely on histopathologic observations that are unable to predict whether a latent tumor will progress to a clinically relevant tumor, and unable to predict the response of tumors to androgen ablation treatment. Classification based on methylation profiles alone or in combination with pathologic diagnosis could be useful in predicting the behavior of a tumor. Attempts have been made to use both quantitative methylation analysis of the GSTP1 gene and a histologic review in the diagnosis of prostatic sextant biopsies. Using these techniques, improved sensitivity and specificity were noted (209).

Lastly, DNA methylation detection may also aid in early tumor diagnosis. Histopathological examination and immunostaining of basal cell specific cytokeratin are commonly used to distinguish various forms of precursor lesions of the prostate from benign glands and prostate cancer. However neither of them has a prognostic value in predicting which premalignant lesion will be stable and which will progress to invasive cancer, thus methylation may be a surrogate marker for PIN and PIA. In addition, PIN lesions have been found to pre-date the onset of cancer by at least 5 to more than 10 years (211), thus identifying PIN lesions through DNA methylation may dramatically improve the early diagnosis prostate cancer.

7. EPIGENETIC MODULATORS FOR PROSTATE CANCER TREATMENT

7.1. Reversal of hypermethylation by DNA methyltransferase inhibitors

Unlike genetic alterations of the genome, epigenetic changes in DNA methylation are potentially reversible ones. The reversible nature of epigenetic gene silencing makes this process an attractive target for cancer therapy (113). 5-azacytidine and 5-aza-deoxycytidine (decitabine) are nucleoside analogue inhibitors of DNMTs and have been widely used in *in vitro* cell culture systems to reverse abnormal DNA hypermethylation and restore gene expression. However, only limited success has been achieved in clinical trials with these drugs (212, 213). In a phase II study conducted by Thibault *et al.* (213), 14 men with progressive, metastatic prostate cancer that recurred after total androgen blockade and flutamide withdrawal, were treated with decitabine. However only two of 12 patients evaluated for response had stable disease, with delayed time to progression. The authors concluded that decitabine is a well tolerated regimen with modest clinical activity against hormone-independent prostate cancer.

Because nucleoside analogue inhibitors of DNMTs have many potential side effects such as myelotoxicity (214), mutagenesis (215), and tumorigenesis (216), nonnucleoside analogue DNA methyltransferase inhibitors might be an alternative for clinical use. Lin *et al.* (217) reported that procainamide, a widely used anti-arrhythmia drug, reversed GSTP1 CpG island hypermethylation and restored GSTP1 expression in LNCaP cells grown *in vitro* or *in vivo* as xenograft tumors in athymic nude mice. Procainamide can also restore expression of several other genes silenced by promoter methylation (218, 219). The demethylating effect of procainamide is thought to occur through inhibition of DNMT-catalyzed transfer of methyl groups from S-adenosylmethionine to DNA (220). In addition, compounds with a weak demethylating effect such as those existing in various dietary plants have been identified (162, 163) and may be useful for cancer chemoprevention and chemotherapy.

Although demethylating agents may protect against some cancers (118), they may also promote genomic instability and increase the risk of cancer in other tissues (119). Indeed, hypomethylation can have hazardous effects such as promoting carcinogenesis as demonstrated in certain model systems (119). Therefore, caution should be used in selecting the type of cancer patient for clinical trials involving DNA methyltransferase inhibitors. It is also important to note that the favorable effects of DNMT inhibitors and even the effects of knocking down DNMT1 per se may be independent of the mechanisms of epigenetic reactivation (221).

7.2. Reversal of hypomethylation for the suppression of overexpressed genes

Early studies found that gene transcription from a transfected plasmid DNA can be suppressed by *in vitro* DNA methylation of the upstream promoter by using SssI methylase (222, 223). Recently, a new class of oligonucleotides termed methylated oligonucleotide (MON) have been used to manipulate sequence-specific DNA methylation *in vitro* and *in vivo* (38, 224). This technique uses a synthetic oligonucleotide in which the cytosine residues are replaced by 5'-methylcytosine. Binding of the MON to one strand of the DNA forms a hemimethylated DNA intermediate, which has a "replication fork"-like structure and is a preferred substrate of DNMTs. The latter methylates the second strand and spreads the methylation around the targeted site (224). Introduction of a MON into PC-3 prostate cancer cells that targets the ESR2 gene promoter results in sequence-specific methylation and the suppression of ESR2 gene expression (38). In an *in vivo* study of mice with implanted hepatocellular carcinoma, injection of a MON targeting the IGF2 gene led to improved survival (224). However, before this technique can be used as a therapy, its efficacy in achieving sustained inhibition of gene expression needs to be compared with other gene silencing approaches such as RNA interference (RNAi).

RNAi was initially discovered as a posttranscriptional mechanism of gene silencing through targeted cleavage of homologous mRNA using short interfering RNA (siRNA). Later it was found to also function at the transcriptional level through targeted *de novo* methylation of DNA and histone at the gene promoter region. Two groups have used siRNAs to target the CpG island regions of the E-cadherin, HER2 and elongation factor 1alpha (EF1A) gene promoter and achieved expression inhibition of the targeted gene (225, 226). This approach could be potentially useful in suppressing

hypomethylation and thus overexpressed tumor promoting genes.

7.3. HDAC inhibitors for activating transcriptionally silenced genes

HDAC complexes enzymatically remove the acetyl group from lysine residues of the amino-terminal tails of histones maintaining chromatin in a transcriptionally inactive state (227). This transcriptional blockade can be overcome by agents that inhibit HDACs (8). A variety of agents, many of which are natural products, exhibit HDAC inhibitory activity and therefore may have antitumor activity. Commonly used HDAC inhibitors include TSA (228), sodium butyrate (228), depsipeptide (FR901228, FK228) (229), valproic acid (230), MS-275 (231), suberoylanilide hydroxamic acid (SAHA) (232), pyroxamide (232), and phenylbutyrate (233). Some of these agents such as depsipeptide are in clinical trials**.** A comprehensive review of various HDAC inhibitors in cancer treatment has been published recently (234).

A number of *in vitro* studies have used the antiproliferative effects of various HDAC inhibitors in cultured human prostate cancer cells. All the agents tested inhibit prostate cancer cell growth (232, 233), but the underlying mechanism varies widely. For example, sodium butyrate and TSA synergize with 1,25(OH)2 vitamin D3 to inhibit the growth of LNCaP, PC-3 and DU-145 prostate cancer cells by inducing apoptosis (13). Valproic acid induces prostate cancer cell apoptosis by increasing the expression of several pro-apoptotic genes (230). Although the study did not address whether there were locus-specific histone acetylation changes, a marked global decrease in nuclear HDAC activity was noticed in valproic acid-treated cells (230). Other growth inhibitory mechanisms have also been identified such as increasing expression of the cell cycle regulator CDKN1A (232, 235, 236), decreasing telomerase activity (228), and suppressing angiogenic factors, such as VEGF and bFGF (229). It is widely believed that the observed decrease in histone deacetylating activity induced by HDAC inhibitors causes the induction of gene transcription. In several instances however, HDAC inhibitors may actually decrease expression of hyper-acetylated genes (228, 229). Notably, depsipeptide inhibits PC-3 cell growth by suppressing the expression of VEGF mRNA despite the fact that it induces accumulation of acetylated histones in chromatin associated with the VEGF gene promoter (229). It is unclear why accumulation of acetylated histones in the VEGF gene promoter causes transcriptional inhibition of the associated gene.

Several HDAC inhibitors have also been tested in animal models of prostate cancer and exhibited promising antitumor activity (232, 235, 237, 238). SAHA, at a dose without detectable toxicity, reduced tumor growth by 97% in mice transplanted with CWR222 human prostate tumors (237). Similarly, depsipeptide, sodium butyrate, and tributyrin slow prostate cancer tumor growth by 50%-98% depending on the cell line used for establishing xenografts in mice (235, 238).

7.4. Combined action of DNA methyltransferase inhibitors and HDAC inhibitors

It is unlikely that a single agent has the potential to reverse epigenetic silencing of genes without inducing adverse effects related to cytotoxicity or undesired epigenetic effects. Emerging evidence supports the concept that epigenetic silencing of genes requires the sequential action of multiple mechanisms including DNA methylation, histone methylation and acetylation, and chromatin remodeling (191). Several studies have shown that the combination of HDAC and DNMT inhibitors can generate additive or synergistic effects on apoptosis, differentiation and/or cell growth arrest in cancer cells (239-241). HDAC inhibitors such as TSA can potentiate the induction of tumor suppressor genes by DNA demethylating agents in cancer cells (241). To maximally achieve gene reactivation, it may be necessary to simultaneously block the processes essential to both the formation and maintenance of the transcriptionally repressive chromatin associated with such genes (103).

8. CONCLUSIONS AND FUTURE DIRECTIONS

Despite the accumulating evidence that a large number of genes are dysregulated in prostate cancer due to aberrant epigenetic control, some fundamental questions remain: What drives epigenetic changes in prostate cancer? Are they the cause or consequence of tumor transformation? Only after we have found answers to these questions, will epigenetic-based diagnosis and treatment of prostate cancer become a reality.

Before we fully understand the cause and consequence of global hypomethylation, therapeutics targeting of DNMTs in cancer should be used with caution. Ideal treatments are those that can selectively activate a group of methylated genes without inducing undesired demethylation of the rest of the genome. Given the close relationship between DNA methylation and histone deacetylation in epigenetic inactivation, a combination of DNMT and histone deacetylation inhibitors may be an attractive strategy for the treatment of prostate cancer patients.

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