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DNA Methylation in Promoter Region as Biomarkers in Prostate Cancer

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Abstract

The prostate gland is the most common site of cancer and the second leading cause of cancer death in American men. Recent emerging molecular biological technologies help us to know that epigenetic alterations such as DNA methylation within the regulatory (promoter) regions of genes are associated with transcriptional silencing in cancer. Promoter hypermethylation of critical pathway genes could be potential biomarkers and therapeutic targets for prostate cancer. In this chapter, we updated current information on methylated genes associated with the development and progression of prostate cancer. Over 40 genes have been investigated for methylation in promoter region in prostate cancer. These methylated genes are involved in critical pathways, such as DNA repair, metabolism, and invasion/metastasis. The role of hypermethylated genes in regulation of critical pathways in prostate cancer is discussed. These findings may provide new information of the pathogenesis, the exciting potential to be predictive and to provide personalized treatment of prostate cancer. Indeed, some epigenetic alterations in prostate tumors are being translated into clinical practice for therapeutic use.

Keywords

Prostate cancer; DNA methylation; Epigenetic variation; Biomarker

1. Introduction

Prostate cancer is the most common type of cancer and the second leading cause of cancer mortality in the American men. One man in six will develop prostate cancer during his lifetime, and one man in 34 will die of the disease (1). In 2010, it is estimated that 217,730 new cases will be diagnosed in the United States, and 32,050 men will die from the disease (2). The low mortality rate and gradual decrease of incidence rates, from 2000 to 2006, suggest that public awareness of early detection and advanced treatments of prostate cancer has begun to affect prostate cancer outcomes. However, the probability of developing prostate cancer sharply increases with age, e.g., ~30-fold increase among men over 40 years of age, compared to men under 40 years old. The aging of the current population means that the disease will become an even greater public health problem in the future.

There are substantial individual differences in the risk or progression of prostate cancer. In some patients with prostate cancer, the disease progresses relatively slow. In these cases, patients often die with prostate cancer rather than from prostate cancer. However, some cases grow aggressively and metastasize through the bloodstream and lymphatic system to other parts of the body. Currently, there are two important clinical challenges. The first challenge is the early detection of prostate cancer. Digital rectal examination (DRE) and serum prostate-specific antigen (PSA) are two main diagnostic tools. There is a considerable overlap in PSA levels between patients with prostate cancer and patients with benign prostatic hyperplasia (BPH). Approximately 25% of patients with prostate cancer show no

elevation of serum PSA and must be diagnosed by other methods (3). Therefore, the identification of biomarkers that can facilitate the diagnosis of prostate cancer at the early stages could improve the current standard of treatments. The second challenge is to determine which of prostate cancer's clinical forms a patient is presenting with, i.e., aggressive vs. indolent. This is critically important information given the significant morbidity associated with treatment interventions and could eventually help distinguish men who need intensive treatment from those who may be better served by watchful waiting. Currently, the level of PSA, clinical stage, and the grade of tumor (Gleason score) are used to estimate prognosis and determine treatment modalities. To overcome limitations of PSA and DRE, new biomarkers are demanded to improve the outcome of prostate cancer.

2. Role of DNA Methylation in the Promoter Regions in Prostate Cancer

Development and progression of prostate cancer are results of the accumulation of genetic and epigenetic alterations. Although genetic changes are involved in the inactivation of genes with important anticancer functions (e.g., tumor suppressor and DNA repair genes), DNA methylation in a promoter region is an important epigenetic mechanism for the downregulation (silencing) of expression of these genes. DNA methylation in the promoter region of tumor suppressor genes appears to occur at early stages of carcinogenesis and occurs with various frequencies. Therefore, epigenetic changes have the potential to be a new generation of biomarkers. Several types of epigenetic changes have been reported for prostate cancer including DNA hypermethylation, loss of imprinting, and altered histone modification patterns.

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 (4), including the prostate. A common molecular feature associated with tumorigenesis is hypermethylation of cytosines 5′ to guanosines (CpG) within the regulatory (promoter) region of suppressor gene genomic DNA (5–8). 5-methyl cytosine is unstable and mutates to thymine and methylated CpG sites degrade to TpG/CpA. In tumors, many CpG islands exhibit aberrant hypermethylation, resulting in gene silencing (Fig. 1). Many tumor suppressor genes have been found to be silenced by promoter hypermethylation in tumors.

It is firmly established that an increase of methylation across the promoter region affects transcription of genes. However, how methylated genes are downregulated is not completely known. Furthermore, the extent of methylation in the CpG islands required for gene silencing is not clear except for a short list of genes (9–18). Yet, regardless of mechanism, the observation of methylated promoter regions in silenced tumor suppressor genes in prostate tumor tissues suggests that DNA methylation may indicate a significant association with carcinogenesis and progression of prostate cancer.

3. Hypermethylated Genes in Prostate Tumor

The majority of previous publications in epigenetic research in prostate cancer focused on DNA hypermethylation. Indeed, a gene silencing by DNA hypermethylation in the promoter region is a more common event than a gene silencing by DNA mutations in carcinogenesis. Numerous studies on various hypermethylated genes in different cancers suggest that this is a key part of the carcinogenesis and progression of cancer.

Currently, over 40 genes have been investigated for their frequencies of hypermethylation and for their potential role in prostate cancer (Table 1). Most data in Table 1 were obtained from prostate tumor tissues. The functions of tumor suppressor genes in prostate cancer fall into four major categories: tumor suppressor genes, tumor cell invasion/metastasis,

metabolism, and DNA repair. Defected function of these genes by promoter hypermethylation can contribute to carcinogenesis and progression of prostate cancer.

3.1. Tumor Suppressors Genes

3.1.1. Caveolin-1 (CAV1)—Caveolin-1 (*CAV1*) is known as a tumor suppressor gene and involved in the vesicular transport, cholesterol balance, transformation, and tumorigenesis. Recent studies reported the dual function of *CAV1* both as a tumor suppressor gene and metastasis-promoting gene (19, 20).

Cui et al. found that 91% (20/22) of cases showed differential hypermethylation in the prostate tumor tissues when compared with adjacent normal tissues (20). Increased DNA methylation of *CAV1* was correlated with biochemical recurrence. Therefore, *CAV1* plays a role as a tumor suppressor gene which is silenced by hypermethylation in carcinogenesis in prostate. A recent study supports that *CAV1* is downregulated in prostate tumor due to hypermethylation in the promoter region of *CAV1* (21). However, Woodson et al. did not observe *CAV1* methylation in prostate tumor tissues (22). Karam et al. reported overexpression of *CAV1* as an established feature of prostate cancer and aggressive PSA recurrence (23). Moreover, *CAV1* is reported to upregulate fatty acid synthase (FASN), a tumor promoter, in the progression of prostate cancer (24). These data suggest that the methylation status of *CAV1* may not be a reliable biomarker for prostate cancer.

3.1.2. Cyclin-Dependent Kinase Inhibitors—The tumor suppressor gene *CDKN2*(*p16*) is one of the cyclin-dependent kinase inhibitors (CDKIs). *CDKN2A*($p16^{INK4a}$) is a key protein in the signaling pathway, which can be damaged by a variety of genetic and epigenetic changes including hypermethylation in prostate tumors. Aberrant CDKI expression is observed in many tumor tissues including prostate (25–28). The reported frequencies of *CDKN2A* promoter methylation are inconsistent in prostate tumors, ranging from 0 to 77% (25–27, 29–36). Perhaps these inconsistent results are due to different detection methods and/or different targets of methylated loci. For example, Gu et al. identified DNA methylation at the *Smal* site for 21 of 30 samples and found only one sample had an altered methylation pattern at the *Smal* site downstream of exon 1 of the *CDKN2A* (32). Since Herman et al. first reported inactivation of *CDKN*/*p16* by DNA methylation in prostate tumors (33), other researchers have investigated the role of hypermethylated *CDKN2A* in carcinogenesis and progression of prostate cancer (25–27, 29–35). Nguyen et al. observed methylation of $p16^{INK4a}$ only in exon 2. Although methylation at exon 2 may not be functional, this exon 2 methylation may be a potential biomarker for prostate tumor because of a high prevalence of methylation in tumor tissues (27). These results were confirmed by other groups, who reported that methylation occurred in the promoter region in 9%, 15% of tumors in exon 1 (26, 37), and 66% in exon 2 (26). Jeronimo et al. found that the $p16^{INK4a}$ gene was frequently methylated in tumor tissues (77%). However, the high frequency of methylation was also found in BPH (25). These data suggested that $p16^{INK4a}$ methylation may be a potential biomarker for an early detection of prostate cancer.

Another *CDKI*, the *CDKN2A*/$p14^{ARF}$, generated from an alternative splicing process that replaces the first exon of $p16^{INK4a}$, has been known as a growth suppressor. Therefore, epigenetic alterations of $p14^{ARF}$ may affect $p16^{INK4a}$/*RB1* pathways in the tumorigenesis and progression of prostate cancer. The $p14^{ARF}$ promoter has been methylated in various cancers, glioma (38), bladder (39), leukemia (40), head and neck (41), and prostate cancers (25–27, 30, 31, 36, 37, 42). Based upon eight independent studies, frequencies of $p14^{ARF}$ methylation in prostate cancer range from 0 to 37% (25–27, 30, 31, 36, 37, 42). With the exception of two studies (27, 31), most studies reported low methylation frequencies that

ranged from 0 to 6%. The *p16*^{INK4a} and *p14*^{ARF} are frequently comethylated, which may deregulate the *RB1* or p53 pathway (42). However, promoter methylation in *p14*^{ARF} is rare in prostate tumors. Therefore, methylation in *p16*^{INK4a} rather than *p14*^{ARF} may be the predominant event in the *INK4a*/*ARF* loci in tumor tissues.

3.1.3. Cyclin A1 (CCNA1) and Cyclin D2 (CCND2)—The cell cycle is controlled by a family of cyclin-dependent kinases (CDKs). Cyclin A1 (CCNA1) activates two different CDKs and functions in both S phase and G2 (43, 44), while cyclin D2 (CCND2) is involved in the regulation of transition from G1 to S (45). Abnormal expression of *CCND2* may disrupt the normal cell cycle, and therefore, it is considered as both an oncogene and tumor suppressor gene. Aaltomaa et al. reported that expressions of *CCNA1* and *CCND2* were interrelated in prostate cancer tissues (46, 47).

Shames et al. observed a higher frequency of hypermethylation of *CCNA1* in both prostate tumors and benign tissues (48). However, Wegiel et al. reported that levels of CCNA1 protein and mRNA expression were significantly higher in prostate tumors than in adjacent benign tissues (47).

Aberrant expression of *CCND2* by DNA methylation has been noted in prostate cancer (45, 49). The frequencies of methylation in *CCND2* were significantly higher in prostate tumors (32%) than in normal tissues (6%) (45). Studies observed a positive correlation between the methylation in *CCND2* and clinicopathological features such as Gleason score and preoperative serum PSA (45, 50). Moreover, methylation status of *CCND2* was significantly associated with the risk for recurrence among prostate cancer patients who underwent a prostatectomy treatment (51). Henrique et al. further reported that *CCND2* methylation levels were significantly higher in prostate tumors compared to tissues of high-grade prostatic intraepithelial neoplasia (HGPIN), BPH, or normal prostate, whereas mRNA expression levels followed the opposite trend (49). They found that high *CCND2* methylation levels correlate with clinicopathological parameters of tumor aggressiveness. Altogether, *CCND2* promoter methylation, but not cyclin A1 gene, may be a useful prostate cancer biomarker for the identification of the aggressive prostate cancer that may benefit from different therapeutic modalities.

3.1.4. Death-Associated Protein Kinase—Death-associated protein kinase (DAPK) is a serine/threonine kinase involved in apoptosis pathway (52). Overexpression of *DAPK* induces apoptosis, whereas loss of its function leads to protection against apoptosis (53). Therefore, *DAPK* may function as a suppressor of metastasis. A repressed expression of *DAPK* by hypermethylation in the promoter region has been shown for various human cancers (52, 54, 55). The methylation frequencies in prostate cancer range from 0% to 36% in four independent studies (29, 30, 36, 56). In addition, Mishra et al. observed that methylation level of DAPK in a prostate cancer cell line (LNCaP) is significantly higher than one in a normal cell line (RWPE1) through global methylation analysis (57). However, *DAPK* overexpression and repressed function in prostate tumors (58) suggest that *DAPK* activity may be damaged at a posttranslational level in cancer cells (59). Based on its unclear function and a persistently low frequency of methylation in both tumors and normal tissues, *DAPK* needs to be further tested for a potential biomarker for prostate cancer.

3.1.5. Fragile Histidine Triad—Fragile histidine triad (*FHIT*) is known as a tumor suppressor gene and frequently methylated in various cancers such as lung (60), leukemia (61), ovarian (62), skin (63), cervical (64), gastric (65), renal (66), and prostate cancers (29, 67). Previous studies indicate that FHIT is a proapoptotic factor (68). Guo et al. (69) reported that downregulation of FHIT protein in more than half of the prostate tumors is determined by immunohistochemistry. However, these results were not confirmed by

another study (70). Although there are indications for a potential role of *FHIT* methylation in prostate cancer, previous studies show its limited value due to a persistently low frequency of methylation in tumors and normal tissues (29, 57, 67).

3.1.6. Hypermethylated in Cancer 1—The tumor suppressor hypermethylated in cancer 1 (*HIC1*) is a transcriptional repressor, which is epigenetically silenced in solid tumors (71–73). Loss of heterozygosity (LOH) of the short arm of chromosome 17 (17p) is a frequent genetic alteration in human cancers. Moreover, frequent LOH or DNA methylation changes occur in a more telomeric region at 17p13.3. In the animal study, heterozygous $HIC1^{+/-}$ mice developed spontaneous malignant tumors of different types (74, 75). These results suggest that *HIC1* may be involved in tumorigenesis. Three studies investigated methylation in the promoter region of *HIC1* in prostate tumors. Results of three studies indicated that CpG island at the *HIC1* was methylated in 89–100% of prostate tumors (30, 56, 76). However, the methylation status of *HIC1* in prostate tumors parallels the respective normal tissue, although a high proportion of tumors are methylated. Therefore, DNA methylation sites in *HIC1* gene are not good candidates as prognostic markers for progression or early detection of prostate cancer (30, 76).

3.1.7. Lipoprotein Lipase—Lipoprotein lipase gene (*LPL*) is common locus of the somatic deletions in prostate tumors. Gallucci et al. reported *LPL* deletion in 76% of prostate tumor determined by fluorescence in situ hybridization (FISH) (77). *LPL* deletion was associated with higher stages, biochemical/clinical progression, and Gleason grade. Only one published study evaluated methylation status in *LPL* using 56 prostate tumors and matching normal tissue pairs. Kim et al. found that 21 samples out of 56 primary cancers (38%) were methylated in the *LPL* promoter region, while methylation was not detected in any normal tissues. In addition, the methylation status in *LPL* was positively associated with the preoperative PSA levels (67). These data suggest that biallelic inactivation of *LPL* by gene deletion and hypermethylation may affect progression of prostate cancer.

3.1.8. Paired-Like Homeodomain Transcription Factor 2 (PITX2)—Paired-like homeodomain transcription factor 2 gene (*PITX2*) encodes a member of the RIEG/PITX homeobox family, which is in the bicoid class of homeodomain proteins. The protein acts as a transcription factor, and it is involved in the development of several major organs. *PITX2* expression is induced by the Wnt pathway, and the protein mediates cell-type-specific proliferation by inducing growth-regulating genes (78). Methylation in *PITX2* was reported as one of the best validated methylated genes for predicting distant recurrence outcome of breast cancer by Maier et al. (79). These results were validated by an independent cohort and confirmed by two additional studies. Harbeck et al. reported that *PITX2* methylation can predict outcome in node-negative, tamoxifen-treated breast cancer (80). *PITX2* promoter methylation is also a biomarker for disease recurrence, early distant metastasis, and poor overall survival in breast cancer patients (81).

Recently, two cohort studies ($N = 605$ (82); $N = 476$ (83)) showed prostate cancer patients with high *PITX2* methylation had threefold higher chance of biochemical recurrence than patients with low *PITX2* methylation. They also showed the prognostic capability of *PITX2* methylation status in patient strata defined by the Gleason score. These results were supported by Vanaja et al. (84). Methylation profile of six genes including *PITX2* was significantly associated with prediction of biochemical, local, and systemic recurrence of prostate cancer. Together, the data show the ability of *PITX2* methylation status to provide prognostic information beyond the traditional Gleason score. Therefore, the prognostic potential of the *PITX2* methylation may help to determine a personalized treatment.

3.1.9. Prostaglandin-Endoperoxide Synthase 2—Prostaglandin-endoperoxide synthase 2 (*PTGS2*) is a key regulator of inflammation and may play a role in prostate carcinogenesis. The two PTGS isoforms, *PTGS1* and *PTGS2*, differ in their expression patterns. While *PTGS1* is constitutively expressed in most tissues, *PTGS2* is usually not expressed and is induced by inflammation, hypoxia, and *Wnt* signaling (85). An elevated expression of *PTGS2* is frequently reported in different human cancer sites including prostate. *PTGS2* over expression and enzymatic activation can enhance the level of antiapoptotic protein B-cell CLL/lymphoma 2 (*BCL2*) and matrix metalloproteinase (*MMP*) family. Antiapoptotic and proproliferative and inflammatory functions of *PTGS2* support its role in tumorigenesis. However, other studies show that *PTGS2* may not be expressed or downregulated in prostate tumor. Bastian et al. observed *PTGS2* gene is silenced in prostate cancer by hypermethylation (86, 87). Range of methylation in *PTGS2* promoter was 0–88% of prostate tumor (30, 86, 88–90).

Methylation at the *PTGS2* gene was significantly different in prostate tumor and in BPH. These data indicated that methylation in *PTGS2* could be a reliable biomarker which can distinguish tumor from nontumor tissues (88). Moreover, the CpG island hypermethylation at *PTGS2* correlated with seminal vesicle infiltration, capsular penetration, pathologic T-stage, and recurrence (89). However, there was no PTSG2 methylation in hormone-refractory metastatic prostate cancer (87).

3.1.10. RAS Association Domain Family Protein 1 Isoform A—The RAS family of proto-oncogenes plays a key role in signal transduction pathways involved in cellular proliferation and survival, interacting with other regulatory circuits of cell growth and death. Overexpression of RAS may cause reduction of growth factor dependency, resistance to apoptosis, or other features of the tumor phenotype. However, RAS association domain family protein 1 isoform A (*RASSF1A*), a tumor suppressor gene, was known to be associated with the DNA repair proteins and with the apoptotic effect (91). Inactivation by methylation of *RASSF1A* may deregulate the DNA repair pathway and cell-cycle control in the tumor. Methylation in *RASSF1A* promoter gene was found in a large fraction of various tumors including prostate (92). In prostate tumors, *RASSF1A* promoter methylation is a common event, occurring in 21–99% of tumor tissues (25, 29– 31, 35, 36, 90– 96). *RASSF1A* promoter methylation is also positively associated with aggressiveness of prostate cancer (29, 92, 93). In addition, Aitchison et al. reported that there was over 50% of methylation in normal epithelial cells and benign prostatic tissues as well as prostatic intraepithelial neoplasms (96). These findings indicate that *RASSF1A* promoter methylation may be associated with early event of carcinogenesis and progression.

3.1.11. Solute Carrier Family 5A8 (SLC5A8)—Solute carrier family 5 (iodide transporter) (*SLC5*) is a solute-linked carrier gene family that contains 12 sodium-coupled transporters for several chemicals (97). *SLC5A8* is downregulated by methylation, obesity, or chronic hypoxia, while it is up regulated by lactate, butylate, TNF (tumor necrosis factor)-α, or nitric oxide (NO) (98). The potential function of *SLC5A8* protein in normal prostate tissues is likely to mediate concentrative uptake of butyrate and propionate, all of which are inhibitors of histone deacetylases (HDACs). SLC5A8 can also transport a variety of pharmacologically relevant monocarboxylates, e.g., various nonsteroidal anti-inflammatory drugs such as ibuprofen and ketoprofen (99) especially transport pyruvate into epithelial cells, and may explain a potential tumor suppressive role (100). *SLC5A8* was identified as a differentially methylated gene by restriction landmark genome scanning which provides a global analysis of methylation events in colon cancer cell lines and lung tumor (101, 102). Since then, increasing evidence suggests that gene silencing of *SLC5A8* may contribute to the carcinogenesis and progression of tumors. *SLC5A8* promoter methylation and gene

silencing were detected in lung, brain, thyroid, gastric, pancreatic, breast, and prostate tumors (100, 102– 112).

We previously reported hypermethylation of *SLC5A8* in prostate (111) and pancreatic tumors (110), and its expression was restored by treatment with either 5-azacytidine or TSA in cancer cell lines (111). Although these results hint a potential role of HDACs on *SLC5A8* expression, aberrant methylation represents the principal mechanism for inactivating *SLC5A8* in prostate tumor.

3.1.12. Solute Carrier Family 18 (Vesicular Monoamine) Transporter 2—Solute carrier family 18 (vesicular monoamine) transporter 2 (*SLC18A2*) transports monoamines, such as dopamine, serotonin, and histamine, from the cytosol into vesicles for storage and/or exocytotic release during neurotransmission or autocrine/paracrine factor release (113). Although *SLC18A2* is expressed in prostate tumors, biological function in normal and tumor prostate tissues is unknown. However, several of the monoamines that are substrates for SLC18A2-mediated transport have been shown to influence growth, proliferation, migration, or apoptosis of prostate cancer cells in vitro and in vivo. Kristiansen et al. reported that 50% of tumor tissues had silenced *SLC18A2* expression, by performing microarray analyses (114). A recent study confirmed that *SLC18A2* is frequently downregulated in tumor tissues by methylation, as compared with nonmalignant prostate tissue samples. Level of expression of *SLC18A2* is also negatively associated with risk for biochemical recurrence after radical prostatectomy (115).

3.1.13. Tumor Necrosis Factor Receptor Superfamily, Member 10C and 10D (TNFRSF10C and 10D)—The TNF receptor superfamily member 10C is one of several TNF-related apoptosis-inducing ligand (TRAIL)-like decoy receptors. *TNFRSF10C* is located on 8p21.3, which is a common prostate cancer susceptibility region (116, 117). *TNFRSF10C* encodes for DCR1 and is involved in the inhibition of the apoptosis pathway. TNFRSF10C lacks the intracellular death domain and appears unable to induce apoptosis. The extracellular domains of TNFRSF10C compete with those of DR4 or DR5 for TRAIL binding. Thus, TNFRSF10C inhibits apoptosis induction through DR4 and DR5 (118). Previous studies reported that frequent loss of expression of *TNFRSF10C* by aberrant methylation of promoter regions in human tumor tissues (118, 119) and low expression of *TNFRSF10C* was associated with tumor recurrence (120). Hypermethylation of *TNFRSF10C* promoter region had been reported in prostate tumor tissues, with a range from 0 to 78% (117, 118, 121, 122). A recent German study reported that *TNFRSF10D*, which codes for DCR2, was also downregulated by methylation in tumors (120).

3.1.14. NK3 Homeobox 1 (NKX3.1) and NK2 Transcription Factor Related, Locus 5 (NKX2.5)—The *NKX3.1* is located on 8p21, which is a common prostate cancer susceptibility region (123). This gene is an NK family homeodomain protein and a tumor suppressor gene that is downregulated in the early phases of prostate cancer. Like its cardiac homolog, NKX2.5, *NKX3.1* acts synergistically with serum response factor (SRF) (124).

Loss of function of the *NKX3.1* homeobox gene in the mouse prostate leads to deregulated expression of oxidative damage response genes and increased levels of 8-oxy-dG, correlated with the onset of PIN (125, 126). Downregulation of *NKX3.1* was observed throughout prostate cancer progression (125, 127, 128). In addition, downregulation of *NKX3.1* is frequently observed with overexpression of *MYC*, an oncogene, at the early stage of prostate cancer (125). Asatiani et al. found hypermethylation at CpG sites −921, −903, and −47 of *NKX3.1* in tumors, as compared with adjacent normal cells (129). However, these data were not supported by another study. Lind et al. reported that downregulation of *NKX3.1* expression might not be caused by DNA methylation, but other epigenetic mechanisms

(130). Chung et al. reported that *NKX2.5* promoter was significantly highly methylated in prostate tumor, as compared to normal tissues (131). These results were confirmed by another group (132). We expect that further methylation information at their promoters will be available.

3.1.15. Stratifin (SFN/14-3-3σ)—The p53-regulated gene *14-3-3σ* is a putative tumor suppressor gene involved in cell-cycle regulation and apoptosis following DNA damage. In response to DNA damage, *14-3-3σ* enforces a G2/M arrest by inhibiting the cyclin B1–cdc2 complex from entering the nucleus. This allows DNA repair before cell-cycle progression (133). *14-3-3σ* undergoes frequent epigenetic silencing in several types of cancer, including prostate cancer, suggesting that the loss of 14-3-3σ expression may be causally involved in tumor progression (134). However, there were similar high frequency of *14-3-3σ*methylation in both of prostate cancer and BPH (133, 135). Thus, promoter methylation at *14-3-3σ* may not be a specific biomarker for prostate cancer.

3.2. Genes Involved in Metabolism

The specific causes of prostate cancer are not known, but multiple etiological factors, including genetics, hormones, diet, infection, and environmental exposures, are thought to play significant roles. Although the precise role of androgens and their receptors in carcinogenesis and progression of prostate cancer has not been fully studied, previous studies suggest that these processes are important (136, 137). The production of estrogens from androgens is mediated by the aromatase enzyme, the aberrant expression of which plays a critical role in the development of malignancy in a number of tissues (138). Differences in the activities of these enzymes are determined to a large extent by genetic and epigenetic changes in the genes encoding them.

3.2.1. Androgen Receptor—It had been known that androgens stimulate the growth of prostate cells through the androgen receptor (*AR*) (139). There are two well-known AR target genes, *PSA* and *TMPRSS2–ETS* fusion genes. The exact roles of *PSA* and *TMPRSS2–ETS* in prostate cancer are not fully defined yet. While silencing of *AR* expression leads to decrease growth and induce apoptosis in vitro (140–142), overexpression of *AR* also induces growth inhibition and apoptosis (143). In addition to prostatectomy and radiation therapy, androgen deprivation is one of the most effective treatments for prostate cancer. However, many advanced prostate cancers turn into a castrate-resistant cases. Prostate tumor cells in this stage grow aggressively without stimulation of androgens. Androgen receptor is one of the most frequently overexpressed proteins in the castrate-resistant cases (144). Jarrard et al. (145) reported a significant association between *AR* promoter methylation and its expression in vitro using prostate cancer cell lines.

Several groups found *AR* promoter methylation in 8–39% of the prostate tumor tissues (56, 133, 146–149). Frequencies of *AR* promoter methylation are higher in castrate-resistant cases than ones in primary prostate tumor tissues (146, 148). Until now, the biological significance of *AR* silencing by promoter methylation in castrate-resistant prostate cancer is not clear. Recently, Wang et al. reported that *AR* selectively upregulates M-phase cell-cycle genes in castrate-resistant cells, including ubiquitin-conjugating enzyme E2C (*UBE2C*), a gene that inactivates the M-phase checkpoint. They also found that epigenetic marks at the *UBE2C* enhancer are present in castrate-resistant cells and direct *AR*-enhancer binding and *UBE2C* activation (139). On the other hand, Schayek et al. found that progression to metastatic stage in a cellular model of prostate cancer is associated with methylation of *AR*, and *AR* suppresses the insulin-like growth factor-I receptor (*IGF*), therefore suggesting roles of *IGF* for stimulating *AR* signal in castrate-resistant prostate cancer (149).

3.2.2. Estrogen Receptors—Estrogens are effective against androgen-dependent prostate cancer, but paradoxically, estrogens might also be involved in the causation of this malignancy (150). The biological actions of estrogens are meditated by the estrogen receptor (*ER*) (151). There are two ERs which are highly homologous DNA-binding domains but different N-terminus and ligand-binding domains. Stimulation of *ER a*(*Esr1*) leads to aberrant proliferation, inflammation, and premalignant pathology, whereas activation of *ERβ*(*Esr2*) appears to have beneficial effects regarding cellular proliferation and a putative protective role against carcinogenesis (138).

Both *ER*s, *Esr1* and *Esr2*, are downregulated in prostate tumor tissues (152, 153). Promoter methylation is the primary mechanism responsible for low expression of *ER*s (147, 154, 155). *Esr1* expression is associated with a poor prognosis for hormonal therapy (156), and its hypermethylation is correlated with cancer progression (157). The range of *Esr1* methylation in prostate cancer is diverse from 19 to 95% (31, 147, 157, 158). *Esr2* may serve as a tumor suppressor gene because it protects against uncontrolled cell proliferation in normal prostate cells (155). However, high expression of *Esr2* in prostate tumors is associated with increased risk for recurrence and distant metastasis (153, 159). Therefore, *Esr2* may have multiple roles in carcinogenesis and progression. The frequency of *Esr2* promoter methylation ranges from 65 to 83% in prostate tumors (147, 160, 161). The extent of ERs promoter methylation is significantly higher in prostate tumors than in the BPH samples (158, 161). In addition, the percentage of methylated CpG sites in *Esr2* promoter increased progressively from 0.29% (normal) to 35% (grade 4/5 prostate cancer) (154).

3.2.3. Retinoic Acid Receptor β (RARβ)—Retinoic acid receptor β (*RARβ*) is known as a tumor suppressor gene by interacting with retinoic acid. Expression of retinoic acid receptor B (*RARβ*) is reported to be absent or downregulated in tumor tissues (162). The *RARβ2* promoter is aberrantly methylated in many cancers, including prostate cancer (163). Several groups reported that frequencies of methylation of the *RARβ2* promoter range from 40% to 84% of primary prostate cancers but rarely in normal prostate tissues or BPH samples (29, 35, 56, 95, 121, 163–166). Moderate or high frequencies of *RARβ* promoter methylation were also observed in urine or blood samples, respectively (31, 36, 87). Therefore, *RARβ2* gene methylation may be an ideal biomarker candidate for early detection of prostate cancer (56, 163).

3.2.4. Glutathione S Transferase P1—Glutathione S transferase P1 (GSTP1) is involved in the detoxifying process and elimination of potentially genotoxic foreign compounds by conjugating glutathione into toxic chemicals. These processes protect prostate cells from DNA adducts and carcinogenesis (167). Thus, defective *GSTP1* activity may increase DNA mutations and, therefore, may increase the prostate cancer risk (168). Because of its consistently frequent hypermethylation in the promoter region in prostate cancer, *GSTP1* is perhaps one of the most studied genes in prostate cancer.

Lee et al. first reported a high frequency of *GSTP1* hypermethylation in prostate tumor tissues (169). Since then, numerous studies confirmed similar results consistently. Methylation of the *GSTP1* promoter region occurs in 26–100% of tumor tissues (25, 29–31, 35, 42, 56, 88, 90, 93, 95, 169–180). However, this methylation is rarely detected in normal prostate or BPH tissues. *GSTP1* methylation was also detected consistently with high frequency in urine, blood, and ejaculates of prostate cancer patients, while either low or no methylation was detected in the samples from healthy controls (31, 36, 87, 181–183). Different frequencies of *GSTP1* promoter hypermethylation between tumor and normal prostate tissues make an ideal biomarker for prostate cancer. To increase the accuracy of detection, some investigators used multiple gene panel approaches, had commonly chosen

GSTP1, and studied its promoter hypermethylation as a biomarker for prostate cancer incidence, progress, and recurrence or survival (31, 36, 165, 184).

3.2.5. Cellular Retinol-Binding Protein 1—Effects of retinoids on prostate gland or prostate cell lines implicate retinoids in the regulation of prostate growth and suppression of prostate cancer development (185). Retinoids exert their effects through a variety of binding proteins including cellular retinol-binding protein (CRBP), retinol-binding proteins (RBP), cellular retinoic-acid-binding protein (CRABP), and two classes of nuclear proteins, i.e., retinoic acid receptors (RARs) and retinoic acid X receptors (RXRs) (186). *CRBP1* is postulated to promote apoptosis via its upregulation of all *trans*-retinoic acid (ATRA) synthesis. Therefore, loss of *CRBP1* could disrupt a retinoic-acid-mediated apoptosis pathway and hence support prostatic tumor progression (187). Low expression of *CRBP1* by promoter methylation has been associated with the malignant tumor tissues including prostate (188, 189). *CRBP1* promoter hypermethylation was selectively found in prostate cancer tissue, rare in BPHs or normal prostate tissues (25, 189, 190). Low expression and hypermethylation in *CRBP1* occur frequently in prostate tumors. However, data indicated that *CRBP1* hypermethylation is not an early event in tumorigenesis (189).

3.2.6. Multidrug Resistance 1 (MDR1/ABCB1)—Multidrug resistance 1 (MDR1) is a transmembrane calcium-dependent efflux pump to detoxify xenobiotics or induce multidrug resistance with GSTs. It is reported to be inactivated in prostate cancer, and some reports showed significantly high hypermethylation at *MDR1* promoter compared to BPH (30, 87, 90, 122, 191). A recent global methylation study showed 6.2- and 13.7-fold higher methylation at *MDR1* in AR-positive (LNCaP) and AR-negative prostate cancer cells (DU145 and PC3), respectively, compared to normal prostate epithelial cell lines (RWPE1) (57). However, Cho et al. showed no significant differences in frequency of *MDR1* methylation among normal ($N = 20$), PIN ($N = 25$), and prostate cancer tissues ($N = 35$), while the prevalence of *MDR1* methylation was as high as 100% (121). Recent multigene methylation analyses showed that the frequency of methylation in *MDR1* gene in prostate cancer samples was 55.3 and 11.6% in BPH. Multigene methylation models, which contain *MDR1* and GSTs, may serve as a good biomarker for prostate cancer (192).

3.2.7. Endothelin B Receptor Gene (EDNRB)—Endothelin B receptor interacts with endothelins to regulate several critical biological processes and may induce cell death by apoptosis and inhibit tumor progression (193). Several studies reported that the *EDNRB* promoter is hypermethylated in a high proportion of prostate tumors and that much less frequency of methylation was found in normal tissues (30, 87, 194, 195). However, other studies found that *EDNRB* methylation frequencies in prostate tumors and paired normal were same, although a high proportion of tumors are methylated (88, 95, 196). Because a high methylation is present in normal and tumor tissues, methylation in *EDNRB* cannot be considered as a specific biomarker for prostate cancer.

3.2.8. EPH Receptor A7 (EPHA7)—Ephrins and EPHS are involved in embryonic development and play a key role for the differentiation of the nervous and vascular systems (197, 198). Their signaling pathway networks with the Wnt signaling pathway during embryogenesis, tissue regeneration, and carcinogenesis (199). Recent expression microarray data, which were profiling androgen-dependent and castrate-resistant cells, revealed that EPHA7 is downregulated in castrate-resistant cells (200). Silencing of *EPHA7* is reactivated by 5-aza treatment (198). These data are supported by a significant correlation between methylation and loss of expression of *EPHA7* (201). A recent report showed higher frequency of methylation of *EPHA7* promoter region in prostate tumor tissues than hyperplasias (42% vs. 19%) (198). A role of *EPHA7* methylation in progression of prostate

cancer was confirmed by a positive association between hypermethylation and Gleason scores (198).

3.2.9. Tazarotene-Induced Gene 1—Tazarotene-induced gene 1 (*TIG1*) is frequently silenced in prostate tumors (202). This gene, also known as retinoid-acid-receptor-responsive 1 gene, was first identified as an RA-responsive gene (203). Several researchers reported that *TIG1* was methylated frequently in prostate tumors, but was not or barely low methylated in normal tissues or BPH (88, 122, 164, 183, 204, 205). Zhang et al. further found that the methylation of *TIG1* and *RARβ* was positively correlated. Therefore, it is possible that the methylation of the retinoid response gene *TIG1* occurred in response to the methylation and inactivation of *RARβ*. In addition, concordant hypermethylation of retinoid signaling genes, e.g., *RARβ* or *TIG1* (164), was observed.

Ellinger et al. analyzed the diagnostic and prognostic possibilities of methylation analysis in serum DNA of prostate cancer patients. They found hypermethylation in *TIG1* was more frequent in prostate cancer patients (10%) than in BPH (0%) and healthy individuals (0%) (88). Although the levels of hypermethylation frequency for specific genes are usually lower in serum or urine DNA than those in prostate tissues (Table 1), use of non-invasive biosamples may be worth it for the specific diagnosis of prostate cancer (87).

3.2.10. Aldehyde Dehydrogenase 1A2 and 1A3—Aldehyde dehydrogenases (*ALDH*s) are a group of $NAD(P)^+$-dependent enzymes involved in metabolism of wide variety of aliphatic and aromatic aldehydes (206). *ALDH1A2*, known as retinaldehyde dehydrogenases (RALDHs), and 1A3 are embryonically lethal in gene knockout mice and involved in retinaldehyde oxidation into retinoic acid (RA), a compound with prodifferentiation properties. Most prostate cancer patients show a decreased prostatic RA concentration, and altered retinoid metabolism has been noted in prostate cancer (207, 208). Kim et al. reported *ALDH1A2* promoter region was hypermethylated in primary prostate tumors, as compared with normal prostate specimens (209). Their results are supported by Touma et al., who observed a lower expression of *ALDH1A2* in all prostate tumor FFPE sections relative to normal prostate tissue on the same sections. Therefore, *ALDH1A2* is suggested as a tentative tumor suppressor gene in prostate cancer, and its alteration is suspected as an early event in prostate cancer. *ALDH1A3* was reported to be androgen responsive (210), and upregulation of *ALDH1A3* can increase the oxidation of retinal to RA. Shames et al. reported hypermethylation in the promoter region of *ALDH1A3* in prostate tumor (48). Recently, disulfiram, an inhibitor of *ALDH*s and demethylation agent, showed inhibition of prostate cancer cell growth (211). Thus, promoter methylation at *ALDH1A2* or *1A3* is a suspected biomarker for prostate cancer diagnosis or prevention.

3.3. Tumor Cell Invasion/Metastasis

Metastasis is an extremely complicated process, which occurs through a series of sequential steps that include the invasion, transport, adhesion at a distant site, and outgrowth into a secondary organ. Although metastases are the cause of 90% of human cancer mortality, little is known about the genetic and biochemical determinants of metastasis.

3.3.1. Adenomatous Polyposis Coli—The methylated adenomatous polyposis coli (*APC*) gene causes familial adenomatous polyposis, which is an inherited disorder characterized by extensive colon polyps and the development of colorectal cancer in early adulthood. The APC is involved in the Wnt signal transduction pathway (212). The APC complex is known to function as a gatekeeper in the cell, preventing the transcription of gene products that promote cell proliferation and survival rather than differentiation and apoptosis (213). Hypermethylation of *APC* implies silencing of this gatekeeper, making the

cell vulnerable to further epigenetic and genetic changes and, thus, progression toward the development of invasive cancer.

APC promoter methylation is common in various human tumors, especially colon (214). Most studies found a prevalence of 14–100% in prostate cancer tissues but only 5–6% in noncancerous tissues (25, 29–31, 35, 36, 50, 51, 86, 89, 90, 93, 121, 122, 166, 184, 192, 204, 215, 216). Recent studies found that methylation in *APC* is associated with progression of prostate cancer (50, 51, 217). In two small cohorts of prostate cancer patients, a threefold statistically significantly increased HR for promoter methylation in *APC* has been reported among the patients who experienced *PSA* recurrence, metastasis, or death (50, 51). Richiardi et al. found that hypermethylation in the promoter of the *APC* gene is involved in prostate cancer progression using large survival analysis of two independent series of unselected prostate cancer patients (217). Rogers et al. reported somewhat low methylation frequency of *APC* in urine collected after DRE; however, overall, 100% of patients with biopsy-proven prostate cancer had at least one gene methylation among *APC*, *GSTP1*, and *EDNRB* in urine vs. 60% of those without evidence of prostate cancer on biopsy (195). A recent multiplex urine assay study for prostate cancer diagnosis (184) showed that the sensitivities of *APC* (52%) in the urine sediments were similar to those seen by other investigators, who demonstrated a similar sensitivity for *APC* (36).

3.3.2. CD44 (CD44)—*CD44* is a transmembrane glycoprotein that is involved in signal transduction and cell–cell and cell–matrix interactions by serving as a receptor. It codes a lipid raft protein like *CAV1* or E-cadherin. Lipid rafts are also involved in angiogenesis and local invasion (19). The *CD44* expression in prostate tumor tissues is lower than ones in adjacent normal tissues. This low expression is correlated with *CD44* promoter methylation (22, 178). Gao et al. reported that decreased *CD44* expression is associated with Gleason score and the distant metastatic progression of prostate cancer (218). Therefore, *CD44* is considered as a metastasis suppressor gene. Furthermore, *CD44* expression and its promoter methylation may correlate with not only tumorigenesis but also progression of prostate cancer (219). However, there are inconsistent results for *CD44* promoter methylation in many reports (22, 28, 87, 95, 122, 178, 219, 220).

3.3.3. E-Cadherin (CDH1)—The E and one of the key proteins in the maintenance of cell differentiation and the normal architecture of epithelial tissues (221). DNA methylation-induced *CDH1* silencing was observed in prostate tumor and was associated with tumorigenesis, metastasis, and poor patient outcome (29). Treatment with the demethylating agent 5-aza restored E-cadherin expression in the E-cadherin-negative prostate cancer cell lines (222). The prevalence of methylation varies from 0 to 77% (22, 28–31, 35, 36, 45, 95, 122, 160, 178, 222, 223). The reason for the discrepancy among these studies may come from technical issues, e.g., different CpG targets, detection methods, and samples, but also tumor status issues. Li et al. reported that the overall methylation frequencies of E-cadherin promoter were high in advanced stage samples (70%) and low in early stage (33%) prostate tumors (222). In addition, a recent study reported that methylated and unmethylated E-cadherin gene expression is dominant in primary prostate cancer and bone metastasis, respectively (223). These data suggested that *CDH1* methylation might be a useful biomarker to assess progression of prostate cancer (222).

3.3.4. H-Cadherin (CDH13)—H-cadherin (*CDH13*) belongs to the cadherin family of cell surface glycoproteins responsible for selective cell recognition and adhesion (224). Like *CDH1*, previous reports suggested a role for *CDH13* in cancer invasion and metastasis in human cancers (29, 225, 226). Low expression by *CDH13* methylation has frequently been observed in various cancers (225), including prostate cancer (29, 45, 226). *CDH13* was known as a tumor suppressor gene because low expression of *CDH13* resulted in significant

inhibition of tumor growth (227). However, data from animal study suggested that *CDH13* is not involved in the metastasis (228). Although the molecular and biological mechanisms underlying the functions of *CDH13* are unknown, several groups reported *CDH13* promoter methylation in prostate tumors (29, 226). However, Cho et al. reported that the frequency of *CDH13* promoter methylation in prostate cancer was not different from that in BPH tissues (53.6 and 53.3%, respectively) (122).

3.3.5. S100 Calcium-Binding Protein A2 (S100A2) and A6 (S100A6)—Although most S100 proteins are commonly upregulated in tumors and this is often associated with tumor progression, *S100A2* has been documented as a tumor suppressor in some cancers but as an oncogene in others (229). In the case of prostate cancer, Rehman et al. reported that *S100A2* is downregulated (230). *S100A2* methylation was seen in 94% of prostate tumor and 100% of cases of metastatic cancer. However, *S100A2* methylation was also seen in 75% of cases of nonmalignant tissues and in 100% of cases of BPH (25). One interesting fact was age-related increase in *S100A2* methylation levels. This age-related methylation of *S100A2* might be zone dependent because it was observed in a transition zone lesion, but not in a lesion from the peripheral zone (25).

S100A6 is coexpressed with *S100A2* in prostate tissue. *S100A6* methylation was absent in nonmalignant tissues and 100% in BPH tissues, whereas methylation was seen in 52% of prostate tumors. Loss of *S100A6* proteins is frequent in prostatic tumors (230).

3.3.6. Tissue Inhibitor of Metalloproteinase-2 and -3—MMPs are proteolytic enzymes that degrade the extracellular matrix and the basement membrane. High expressions of this enzyme have been associated with tumor growth, invasion, and tumor-induced angiogenesis (231). These pathways are controlled by the balance between the levels of the MMPs and tissue inhibitors of metalloproteinases (TIMPs) (232). Thus, TIMPs are called angiogenesis inhibitors.

TIMP-2 is one of the frequently investigated members of this family because of its involvement in cancer progression and metastasis in a variety of human cancers (233, 234). Pulukuri et al. observed that 25 (60%) of 42 prostate tumors were methylated in comparison with 5 (16%) of 32 normal prostate samples (235). These findings further supported that majority of the prostate cancer tissues have weak or no expression of *TIMP-2* when compared with BPH or normal prostate tissues (235). However, these results were not confirmed by a previous study (236). Ross et al. found that *TIMP-2* was expressed in a majority of prostate tumors and correlated with clinical stages and recurrence. *TIMP-2* expression appears to have a tumor-promoting role in prostate cancer and warrants further investigation (236). This was in contrary to the Pulukuri's study which indicated antitumor effects.

The roles of *TIMP-3* in cancer progression were investigated by several groups. High expression of *TIMP-3* reduces metastasis, induces apoptosis, increases drug sensitivity, and inhibits tumor growth (237–239). A low expression by promoter methylation of *TIMP-3* has been reported to be associated with poor outcomes (240). A recent global methylation study showed 12.08- and 22.3fold higher methylation at *TIMP-3* in AR-positive (LNCaP) and AR-negative cells (DU145 or PC3), respectively, compared to normal prostate epithelial cell lines (RWPE1) (57). The promoter region of *TIMP-3* was found to be methylated in 97% of prostate tumors (25). However, other studies reported low (0%) and 6% frequencies of *TIMP-3* methylation (30, 56), while additional two studies found *TIMP-3* promoter methylation in 37 and 41% of urine sediments from prostate cancer patients (31, 36). As a diagnostic biomarker in urine DNA, value of *TIMP-3* may be limited due to low frequency of methylation in normal samples.

3.3.7. SRC Family Tyrosine Kinase (FYN)—The SRC family of kinases (SFKs) is the largest family of nonreceptor protein tyrosine kinases and is responsible for signal transduction during differentiation, adhesion, and migration. Aberrant SRC/SFK activity has been widely implicated in cancer development. Several lines of evidence indicate a role for SFKs in the development of prostate cancer, e.g., SFK overexpression in prostate cancer cell lines and tissues (241).

Posadas et al. reported overexpression of *FYN*, a member of SFK, in prostate cancer cell lines and tissues than in normal tissues (242). Sorensen et al. reported frequent aberrant methylation in the *FYN* promoter region in both prostate cancer cell lines and primary prostate tumors. In addition, methylation-induced silencing was confirmed by Western blot and RT-PCR results (243). Methylation at *FYN* promoter should be further investigated to be evaluated as a biomarker of prostate cancer.

3.3.8. Neutral Endopeptidase 24.11—Neutral endopeptidase 24.11 (*NEP*), one of cell surface peptidases, is expressed in prostate. This protein inactivates growth factors needed in the growth of castrate-resistant prostate cancer (244). Therefore, loss of *NEP* activates protein kinase B (Akt), which may accelerate prostate tumor growth (245). Several investigators reported hypermethylation in *NEP* promoter in prostate tumor tissues (87, 244, 246). Usmani et al. observed that methylation of the *NEP* promoter was present only in castrate-resistant prostate cancer cell lines not in androgen-dependent prostate cancer cell lines. Reactivation of *NEP* by demethylating agent 5-aza-2′-deoxycytidine shows that hypermethylation of *NEP* is associated with a loss of *NEP* expression in prostate tumor (244). Further studies are needed to elucidate the impact of *NEP* promoter methylation on the progression to castrate-resistant prostate cancer.

3.4. DNA Repair Genes

Although the specific causes of prostate cancer are not known, androgens, estrogens abnormalities, inflammation, and DNA repair capacity have been implicated. DNA is constantly damaged by endogenous oxygen free radicals and exogenous chemicals. DNA mutations are estimated to spontaneously occur 20,000–40,000 times everyday (247, 248). The DNA repair process is important to the survival of cell; therefore, different repair pathways are available to reverse the different types of DNA damage. In fact, over 250 DNA repair enzymes participate in this process (249, 250). Defects in these DNA repair pathways may increase persistent mutations in daughter cell generations, genomic instability, and ultimately prostate cancer risk.

3.4.1. Methylguanine-Methyltransferase—DNA repair genes can be classified into several distinct pathways, including the direct reversal (DR) pathway. The only known enzyme in the DR pathway is methylguanine-methyltransferase (*MGMT*). *MGMT* transfers the alkyl group at the O^6 position of guanine to a cysteine residue within its active site, leading to the direct restoration of the natural chemical composition of DNA without the need for genomic reconstruction. Therefore, defective *MGMT* activity is associated with an increased mutation rate (251). Reports regarding *MGMT* methylation in prostate tumor tissues have been inconsistent.

While three studies reported a low frequency of *MGMT* promoter hypermethylation (0–2%) in prostate tumor tissues (29, 30, 56), others observed higher prevalence of hypermethylation (19–76%) (25, 31, 36, 37, 42, 93, 252). Two other groups reported 15 and 19% *MGMT* hypermethylation frequencies in urine sediment samples from prostate cancer patients, respectively (31, 36). These data suggest that *MGMT* promoter methylation can be

a potential biomarker for early detection and surveillance of prostate cancer. However, larger studies will be necessary to resolve these inconsistent results.

4. Conclusions

Although a few large-scale genome-wide analyses of epigenetic variations are currently ongoing, most published studies are small scale with a retrospective design. Therefore, meta-analysis or large studies should be performed to obtain the complete extent and pattern of differential DNA methylation in the promoter region in the critical genes. Since epigenetic changes are involved in carcinogenesis and progression of prostate cancer, information of these epigenetic changes may provide clues for better diagnostic, prognostic, and predictive modalities than existing ones. The ultimate goals of these epigenetic studies are to improve patients' outcomes and enhance quality of life.

A number of clinical trials and therapies are targeting methylated genes. Unlike DNA somatic mutations, DNA methylations are reversible. Thus, hypermethylated tumor suppressor genes can be reactivated with drugs. Several demethylating agents such as 5-azacytidine (Vidaza) and 5-aza-2′-deoxycytidine (decitabine) have been approved as treatments for the myelodysplastic syndrome (MDS) and leukemia (253–255). Some MDS patients treated with 5-azacytidine showed a significant survival benefit (256). However, a major limitation of these therapies is their nonspecific target approach, which may induce unintended side effects. Therefore, not only tumor suppressor genes but also silenced oncogenes can be reactivated. Future studies should focus on developing drugs that can target specific genes.

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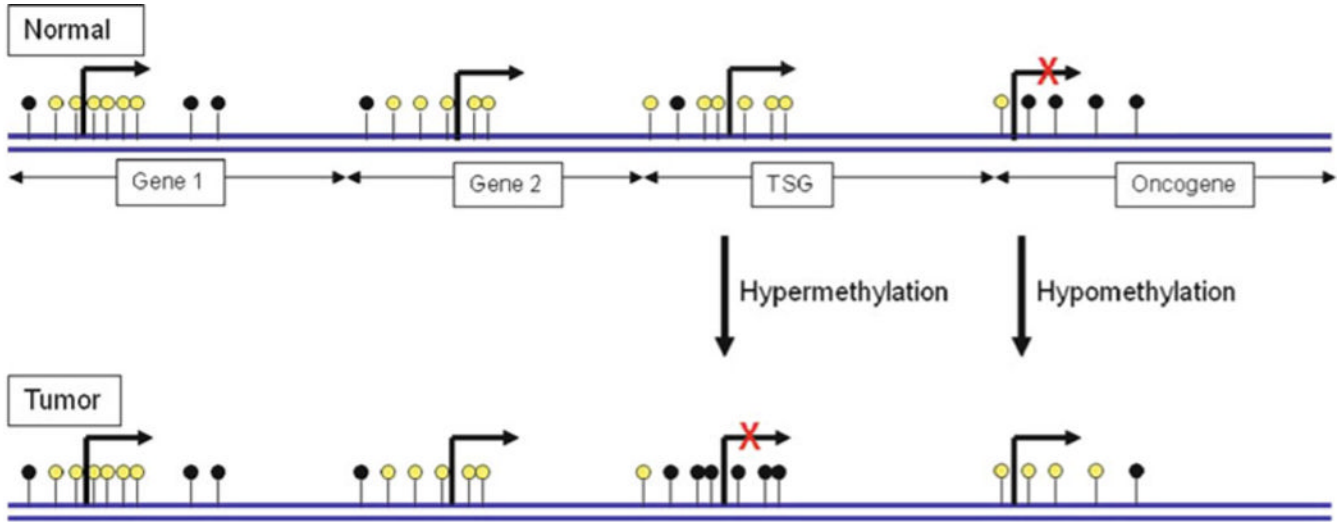

Fig. 1.

Role of DNA methylation in cancer: unmethylated and methylated CpG sites are indicated by *white* and *black circles*, respectively. This figure shows a representative region of genomic DNA in normal and tumor cell. The promoter regions in gene1, gene2, and tumor suppressor gene are rarely methylated in normal cells and, therefore, expressed. Cytosines 5′ to guanosines (CpG) islands in promoter region of tumor suppressor gene are methylated, and it results in gene silencing. Conversely, hypomethylation in the promoter region of oncogene in tumor reactivates transcription.

Table 1

Frequencies of methylated genes in prostate tumor and biosamples

Gene	Common name	Function	Frequency	References
ALDHla2	Aldehyde dehydrogenase 1 family, member A2	Tumor suppressor (synthesis of RA)	100% (7/7)[a]	(208)
ALDHla3	Aldehyde dehydrogenase 1 family, member A3	Tumor suppressor (synthesis of RA)	21% (5/24)	(48)
AFC	Adenomatous polyposis coli	Tumor suppressor	12% (2/17)[b]	(194)
			90% (66/73)	(30)
			14% (11/76)[c]	(89)
			92% (36/39)[c]	(165)
			57% (21/37)	(92)
			27% (27/101)	(29)
			100% (118/118)	(25)
			41% (182/447)	(216)
			79% (48/61)	(203)
			65% (117/179)	(121)
			3.0[d]	(51)
			83% (44/53)[c]	(85)
			73% (131/179)	(121)
			27% (21/79)	(50)
			82% (59/72)	(203)
			64% (109/170)	(191)
			83% (65/78)	(88)
			51% (48/95)[b]	(36)
			51% (58/113)[b]	(183)
			51% (18/35)	(120)
			48% (25/52)[b]	(31)
			78% (88/113)	(35)
AR	Androgen receptor	Steroid hormonal response	29% (2/7)[a]	(148)
			13% (2/15)	(145)
			8% (3/38)	(146)
			25% (6/24)	(147)
			15% (16/109)	(56)
			39% (30/76)[c]	(132)
CAV1	Caveolin-1	Tumor suppressor	91% (20/22)	(20)
			100% (4/4)	(21)
			0% (0/8)	(22)
CCNA1	Cyclin A1	Tumor suppressor	79% (19/24)	(48)

Gene	Common name	Function	Frequency	References
CCND2	Cyclin D2	Tumor suppressor	25% (21/83)	(50)
			32% (32/101)	(45)
			99% (117/118)	(49)
			1.78[d]	(51)
CD44	CD44 molecule	Tumor invasion/metastasis (lipid-raft-associated)	78% (31/40)	(219)
			33% (30/90)	(22)
			3% (1/30)[c,e]	(28)
			68% (27/40)	(218)
			32% (36/111)	(177)
			72% (58/81)	(94)
			0% (0/18)[c]	(86)
			20% (2/8)	(256)
			22% (39/179)	(121)
CDH1	E-cadherin	Tumor invasion/metastasis (lipid-raft-associated)	31% (29/95)[b]	(36)
			0% (0/30)[c,e]	(28)
			54% (19/35)	(221)
			70% (14/20)[a]	(222)
			27% (27/101)	(29)
			0% (0/111)	(177)
			0% (0/73)	(30)
			4% (5/114)	(35)
			61% (49/81)	(94)
			30% (6/20)	(159)
			77% (40/52)[b]	(31)
			24% (22/90)	(22)
			69% (70/101)	(45)
			21% (38/179)	(121)
CDH13	H-cadherin	Tumor invasion/metastasis (lipid-raft-associated)	45% (68/151)	(225)
			31% (31/101)	(29)
			54% (96/179)	(121)
CDKN2A (p16^{INK4a})	Cyclin-dependent kinase inhibitor 2A	Tumor suppressor	73% (8/11)	(27)
			3% (3/101)	(29)
			6% (4/73)	(30)
			77% (91/118)	(25)
			66% (21/32)	(26)
			13% (3/24)	(34)
			70% (21/30)	(32)
			4% (5/113)	(35)

Gene	Common name	Function	Frequency	References
			37% (19/52)[b]	(31)
			15% (8/53)	(37)
			12% (11/95)[b]	(36)
			10% (3/30)[c]	(28)
			60% (3/5)[a]	(33)
CRBP1	Cellular retinol-binding protein 1	Steroid hormonal response (control of retinoids)	81% (96/118)	(25)
			47% (17/36)	(188)
			34% (34/101)	(189)
P14^{ARF}	Cyclin-dependent kinase inhibitor 2A	Tumor suppressor	4% (2/53)	(37)
			6% (6/95)[b]	(36)
			37% (19/52)[b]	(31)
			4% (5/118)	(25)
			0% (0/73)	(30)
			3% (1/32)	(42)
			6% (1/16)	(26)
			22% (2/9)	(27)
DAPK	Death-associated protein kinase	Tumor suppressor	36% (39/109)	(56)
			1% (1/101)	(29)
			0% (0/73)	(30)
			28% (27/95)[b]	(36)
			10.9–18.7[f]	(57)
EDNRB	Endothelin receptor type B	Steroid hormonal response (cell adhesion)	49% (36/73)	(30)
			72% (58/81)	(94)
			70% (23/35)	(193)
			100% (80/80)	(87)
			50% (9/18)[b]	(86)
			83% (40/48)	(195)
			66% (8/12)[b]	(194)
EPHA7	EPH receptor A7	Steroid hormonal response (cell differentiation, apoptosis)	42% (20/48)	(197)
Esr1	Estrogen receptor alpha	Steroid hormonal response	90% (28/31)	(157)
			19% (14/73)	(30)
			95% (36/38)	(146)
			41% (64/156)	(156)
Esr2	Estrogen receptor beta	Steroid hormonal response	83% (19/23)	(160)
			65% (13/20)	(159)

Gene	Common name	Function	Frequency	References
			79% (30/38)	(146)
FHIT	Fragile histidine triad gene	Tumor suppressor	15% (15/101)	(29)
			65% (15/23)	(67)
			$> 10^6$	(57)
FyN	SRC family tyrosine kinase	Tumor invasion/metastasis (cell differentiation)	67% (12/18)	(242)
GSTP1	Glutathione S transferase P1	Steroid hormonal response (metabolism)	58% (7/12)	(175)
			81% (68/84)[c]	(179)
			39% (31/80)[b]	(179)
			26% (20/76)	(89)
			86% (37/43)	(164)
			85% (89/105)	(176)
			36% (36/101)	(29)
			88% (96/109)	(56)
			84% (99/118)	(177)
			100% (18/18)	(178)
			95% (69/73)	(30)
			87% (32/37)	(92)
			79% (22/28)[b]	(170)
			71% (43/61)	(169)
			95% (112/118)	(25)
			75% (24/32)	(42)
			72% (58/81)	(94)
			79% (89/113)	(35)
			48% (25/52)[b]	(31)
			83% (79/95)[b]	(36)
			42% (71/168)[c]	(87)
			28% (5/18)[c]	(86)
			93% (74/80)	(87)
			100% (20/20)	(168)
			91% (52/57)	(171)
			75% (24/32)	(172)
			44% (4/9)[g]	(180)
			90% (18/20)	(181)
			94% (16/17)	(173)
			42% (71/168)[c]	(182)
			91% (63/69)	(174)
HIC1	Hypermethylated in cancer 1	Tumor suppressor	99% (108/109)	(56)
			67% (52/78)	(75)

Gene	Common name	Function	Frequency	References
			100% (73/73)	(30)
LPL	Lipoprotein lipase	Tumor suppressor (metabolism of lipids)	38% (21/56)	(67)
MDR1/ABCB1	Multidrug resistance 1, ATP-binding cassette, subfamily B (MDR/TAP), member 1	Steroid hormonal response	48% (36/76)	(89)
			83% (15/18)[c]	(86)
			55% (97/177)	(190)
			88% (64/73)	(30)
			100% (35/35)	(120)
			51% (91/179)	(121)
MGMT	O^6-methylguanine DNA methyltransferase	DNA repair	26% (14/53)	(37)
			34% (21/62)	(251)
			2% (2/109)	(56)
			19% (22/118)	(25)
			25% (8/32)	(42)
			76% (28/37)	(92)
			0% (0/101)	(29)
			l% (1/73)	(30)
			19% (10/52)[b]	(31)
			15% (14/95)[b]	(36)
NEP	Neuroepithelial tyrosine kinase	Tumor cell invasion/metastasis	17% (3/18)[c]	(86)
			14% (3/21)	(243)
			73% (16/22)	(245)
NKX3.1		Tumor suppress (defense for oxidative damage)	83% (33/40)	(128)
NKX2.5		Tumor suppress (defense for oxidative damage)	30% (6/20)	(130)
PITX2	Paired-like homeodomain 2	Tumor suppress	3.4[d]	(81)
			2.99[d]	(82)
			100% (17/17)	(83)
PTGS2	Prostaglandin-endoperoxide synthase 2	Tumor suppressor	88% (64/73)	(30)
			11% (8/76)	(89)
			71% (38/53)	(85)
			68% (54/80)	(87)
			65% (51/78)	(88)
			0% (0/18)[c]	(86)
RARβ	Retinoic acid receptor beta	Steroid hormonal response	*79%* (11/14)	(162)
			71% (25/35)	(120)
			91% (39/43)	(164)

Gene	Common name	Function	Frequency	References
			79% (33/42)[c]	(165)
			53% (54/101)	(29)
			78% (85/109)	(56)
			84% (42/50)	(163)
			70% (79/113)	(35)
			35% (18/52)[b]	(31)
			40% (32/81)	(94)
			39% (7/18)[c]	(86)
			62% (59/95)[b]	(36)
RASSF1A	Ras association domain family 1	Tumor suppressor	21% (16/76)	(89)
			71% (37/52)	(91)
			99% (117/118)	(25)
			53% (54/101)	(29)
			96% (70/73)	(30)
			84% (31/37)	(92)
			74% (97/131)	(93)
			73% (38/52)[b]	(31)
			49% (40/81)	(94)
			78% (88/113)	(35)
			78% (74/95)[b]	(36)
			17% (3/18)[c]	(86)
			50% (7/14)	(95)
S100A2	S100 calcium-binding protein A2	Tumor cell invasion/metastasis	94% (32/34)	(229)
			99% (117/118)	(25)
S100A6	S100 calcium-binding protein A6		52% (14/27)	(229)
SFN	14-3-3σ	Tumor suppressor	87% (45/52)[c]	(132)
			99% (121/122)	(134)
SLC5A8	Solute carrier family 5, member 8	Tumor suppressor	70% (7/10)	(110)
SLC18A2	Vesicular monoamine transporter 2	Tumor suppressor	88% (15/17)	(114)
TIG1	Tazarotene-induced gene 1	Steroid hormonal response (chloroplast trigger factor)	53% (26/50)	(163)
			55% (17/31)	(204)
			70% (43/61)	(203)
			10% (16/168)[c]	(182)
			96% (77/80)	(87)

Gene	Common name	Function	Frequency	References
			42% (75/179)	(121)
TIMP-2	Tissue inhibitor of metalloproteinase-2	Tumor cell invasion/metastasis	60% (25/42)	(234)
TIMP-3	Tissue inhibitor of metalloproteinase-3	Tumor cell invasion/metastasis	41% (37/91)[b]	(36)
			37% (19/52)[b]	(31)
			6% (7/109)	(56)
			97% (114/118)	(25)
			0% (0/73)	(30)
TNFRSFlOC/DcR1	TNF receptor superfamily, member 10c	Tumor suppressor	65% (117/180)	(121)
			50% (25/50)	(117)
			78% (46/59)	(116)
			0% (0/35)	(120)
TNFRSF1OD/DcR2	TNF receptor superfamily, member 10D	Tumor suppressor	38% (5/8)	(119)

[a] Cell culture

[b] Urine samples

[c] Serum DNA

[d] Hazard ratio

[e] Bone marrow

[f] Methylation fold compared to normal cells

[g] Ejaculates