# The Expanding Role of Epigenetics in the Development, Diagnosis and Treatment of Prostate Cancer and Benign Prostatic Hyperplasia

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**Purpose:** Prostate cancer research has focused significant attention on the mutation, deletion or amplification of the DNA base sequence that encodes critical growth or suppressor genes. However, these changes have left significant gaps in our understanding of the development and progression of disease. It has become clear that epigenetic changes or modifications that influence phenotype without altering the genotype present a new and entirely different mechanism for gene regulation. Several interrelated epigenetic modifications that are altered in abnormal growth states are DNA methylation changes, histone modifications and genomic imprinting. We discuss the status of epigenetic alterations in prostate cancer and benign prostatic hyperplasia progression. In addition, the rationale and status of ongoing clinical trials altering epigenetic processes in urological diseases are reviewed.

**Materials and Methods:** An online search of current and past peer reviewed literature on DNA methylation, histone acetylation and methylation, imprinting and epigenetics in prostate cancer and benign prostatic hyperplasia was performed. Relevant articles and reviews were examined and a synopsis of reproducible data was generated with the goal of informing the practicing urologist of these advances and their implications.

**Results:** Only 20 years ago the first study was published demonstrating global changes in DNA methylation patterns in tumors. Accumulating data have now identified specific genes that are commonly hypermethylated and inactivated during prostate cancer progression, including *GSTpi, APC, MDR1, GPX3* and *14-3-3σ*. Altered histone modifications, including acetylation and methylation, were also recently described that may modify gene function, including androgen receptor function. These epigenetic changes are now being used to assist in prostate cancer diagnosis and cancer outcome prediction. Epigenetic changes appear to have a role in benign prostatic hyperplasia development as well as in the susceptibility of the prostate to developing cancer. Treatments involving 5-*aza*-deoxycytosine and other, more selective DNA methyltransferase inhibitors remove methyl residues from silenced genes, generating re-expression, and are currently being used in therapeutic trials. Histone deacetylase inhibitors have shown promise, not only by directly reactivating silenced genes, but also as regulators of apoptosis and sensitizers to radiation therapy.

**Conclusions:** Evolving data support a significant role for epigenetic processes in the development of prostate cancer and benign prostatic hyperplasia. Epigenetic changes can predict tumor behavior and often distinguish between genetically identical tumors. Targeted drugs that alter epigenetic modifications hold promise as a tool for curing and preventing these diseases.

*Key Words: prostate, prostatic neoplasms, genetics, DNA methylation, chromatin*

## EPIGENETICS IN CANCER

The term epigenetic refers to mechanisms that permit the stable transmission of cellular traits without an alteration in DNA sequence or amount. Epigenetics encompasses many different phenomena, including DNA methylation, histone modifications, RNA interference and genomic imprinting, among others. Epigenetic changes modulate gene expression and can alter aspects of tumor phenotypes, including invasion, angiogenesis, motility and proliferation. The classic model of cancer progression, which was first described in 1971, describes the need for 2 discrete hits or inactivating events to silence the 2 alleles of a tumor suppressor gene involved in progression. Although this model originally focused on alterations to the DNA base sequence as inactivating events, it is now clear that epigenetic events may also function in this capacity. There is also developing evidence that epigenetic alterations have a role in other prostate growth disturbances, including BPH.

Epigenetics may explain other aspects of prostate tumor physiology. It is known that PCa occurs simultaneously at multiple foci throughout the peripheral prostate.[1] This suggests that a field effect process or processes occur in the entire peripheral prostate that directly triggers or passively allows the development of PCa. Epigenetic regulatory mechanisms appear uniquely sensitive to external influences, such as diet and oxidative stress, and they may modulate

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the effect of these entities on the prostate.[2] Epigenetic candidates for this field effect are global or specific changes in DNA methylation, histone modifications and alterations in genomic imprinting. We document what is known regarding epigenetic changes in prostate pathology and how they may be exploited diagnostically and therapeutically.

## ALTERATIONS IN DNA METHYLATION IN PCA

DNA methylation is the placement of a methyl(-CH3) group at the 5′-carbon on cytosine nucleotides adjacent to a guanine (CpG). Methylation is executed in eukaryotes by a series of conserved enzymes known as DNA MTases. CpGs are underrepresented in mammalian genomes and they occur in only 1% of the genome, lower than the expected statistical fraction of 6%, due to deamination, resulting in C-to-T conversion via uracil. The methyl donor for this reaction is supplied by SAM. This substrate is recycled through a folate and cobalamin dependent pathway. Hypomethylation or loss of methylation can be accelerated by altering this regenerative process through a deficiency of folate, vitamin B12 or other substrates (fig. 1).[3] CpG dinucleotides present in the genome are commonly clustered in groups or CpG islands located at or near the promoters and initial exons of many genes. When unmethylated, these promoter CpG islands are permissive for transcription. Approximately 60% of genes contain CpG islands in their promoters.

There are multiple roles for DNA methylation in mammals. Disruption of this process during early development by the inactivation of DNA MTases is lethal.[4] DNA methylation also has a putative role in genome defense.[5] Methylation is also involved with genome organization and silencing unneeded genes in differentiated cells. During this process DNA methylation acts as a stable tag on the promoter of a gene and which recruits methyl-binding proteins and other proteins, such as HDACs, to form large-scale heterochromatic structures that silence the associated genes.

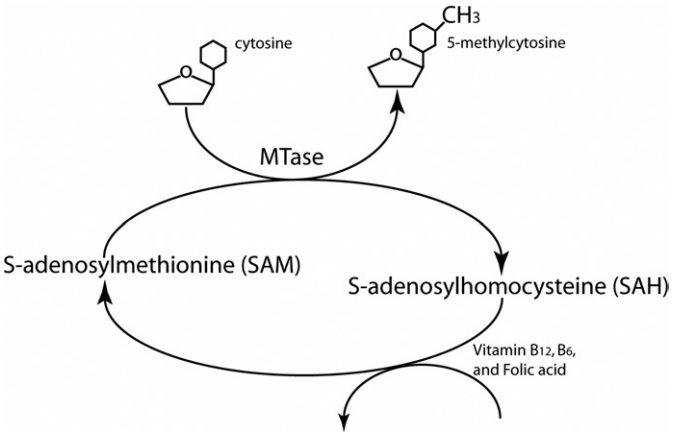


FIG. 1. DNA methylation and diet. MTase transfers methyl group from SAM to cytosine base. SAM is converted into S-adenosylhomocysteine. Regeneration of S-adenosylhomocysteine into SAM requires vitamins $B_{12}$ and $B_6$ in addition to folate, through several intermediate steps (not shown). Deficiencies or excesses in any of these components may alter methylation status. Methylation only occurs on cytosine nucleotides adjacent to guanine.

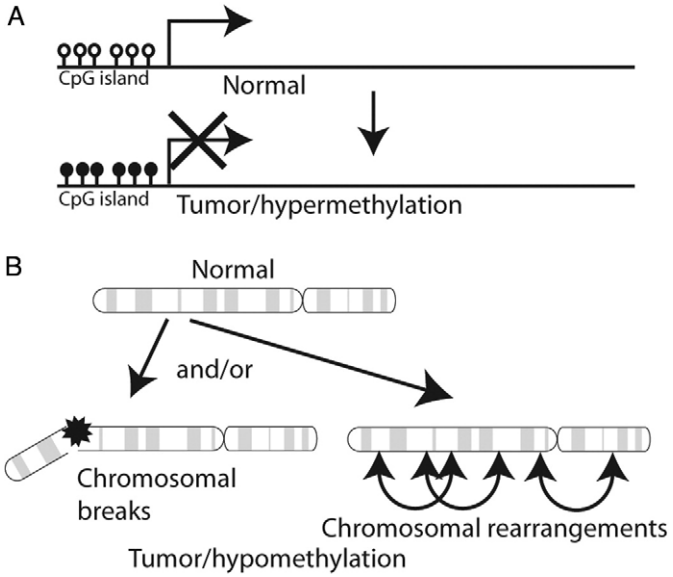


FIG. 2. DNA methylation alterations in cancer. *A*, promoter and transcription start site (arrow) of hypothetical gene is shown. Clustered CG dinucleotides, called CpG island, are unmethylated (open circles) when gene is transcriptionally active in "normal" state. Hypermethylation at CG dinucleotides (closed circles) silences associated gene in *cis* and may occur in cancer and other premalignant states. *B*, hypomethylation, or loss of methylation, occurs throughout genome in tumor cells and can result in increased frequency of chromosomal breakage or rearrangements.

Changes in DNA methylation during cancer formation have seemingly divergent effects on the cell. A common alteration is the focal addition of methyl groups to CpG islands, so-called hypermethylation, which occurs in the promoters of tumor suppressor and other genes. This results in silencing the associated gene in the absence of a mutation or deletion (fig. 2, *A*). A synchronous finding is the global loss of DNA methylation, a hallmark of cancer, which occurs throughout the genome. This change predisposes to chromosomal alterations, such as chromosomal breaks, large translocations of chromosomal segments and aneuploidy (fig. 2, *B*).[6] Methylated and typically silenced proto-oncogenes or cancer promoting genes, including *HRAS*, can also lose their methylation and become active.[7]

DNA MTases are traditionally split into 2 categories, that is de novo enzymes, which establish new methylation patterns, and maintenance enzymes, which replicate existing patterns.[8] In humans DNMT1 is a maintenance MTase, and DNMT3a and 3b perform de novo functions. The enzymatic activity of DNMT1 is 2 to 3-fold higher in PCa cell lines and cancer tissues compared with that in BPH lines and tissues.[9] DNMT2 and DNMT3a are up-regulated in the hormone refractory PCa variant LnCaP-r[10] compared to the hormonally sensitive parent line, suggesting that alterations in methylation may also be important for the development of androgen resistant cancer. In humans a polymorphism (a DNA sequence alteration among individuals) was recently identified in the de novo MTase DNMT3b that increases the risk of PCa development 2.8-fold.[11] It is associated with an increased frequency of gene promoter hypermethylation.

Table 1 lists many of the genes that are hypermethylated and silenced in PCa. Notably many of these genes are often also mutated or deleted in PCa, demonstrating that direct