REVIEW

Epigenetics application in the diagnosis and treatment of bladder cancer

Alfredo Harb-De la Rosa, MD, Matthew Acker, MD, Raj. A. Kumar, MD, Murugesan Manoharan, MD

Department of Urology, University of Miami Miller School of Medicine, Miami, Florida, USA

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Introduction: *Bladder cancer is the sixth most common cancer in the Western world. Patients with bladder cancer require close monitoring, which may include frequent cystoscopy and urine cytology. Such monitoring results in significant health care cost. The application of epigenetics may allow for a risk adapted approach and more cost-effective method of monitoring. A number of epigenetic changes have been described for many cancer sites, including the urinary bladder. In this review, we discuss the use of epigenetics in bladder cancer and the potential diagnostic and therapeutic applications.*

Materials and methods: *A comprehensive search of the English medical literature was conducted in PubMed using the terms microRNA regulation, DNA methylation, histone modification and bladder cancer.*

Results: *The most important epigenetic changes include DNA methylation, histone modification and microRNA regulation. Both DNA hypomethylation and hypermethylation have been associated with higher rate of cancer. The association of epigenetic changes with bladder cancer has led to the research of its diagnostic and prognostic implications as well as to the development of novel drugs to target these changes with the aim of achieving a survival benefit.*

Conclusions: *Recently, epigenetics has been shown to play a much greater role than previously anticipated in the initiation and propagation of many tumors. The use of epigenetics for the diagnosis and treatment of bladder cancer is an evolving and promising field. The possibility of reversing epigenetic changes may facilitate additional cancer treatment options in the future.*

Key Words: histone acetylation, bladder cancer, DNA methylation, microRNA regulation

Introduction

Bladder cancer is the sixth most common cancer in the Western world.[1] In the United States alone, it is estimated that 74,690 new cases were diagnosed in 2014 with 15,580 projected disease-specific deaths.[2,3]

The 5-year survival rate of bladder cancer has remained relatively low despite the development of various surgical and chemotherapeutic methods, mostly due to high rates of recurrence and metastatic spread.[4] The development of novel therapeutic strategies through the identification of key genes involved in the pathogenesis of bladder cancer has therefore become paramount.[5] In addition, bladder cancer patients typically require frequent surveillance with cystoscopies, imaging and urine cytologies, which results in a high annual expenditure.[6] Epigenetics may offer a potentially more cost-effective method of monitoring such patients. Epigenetics refers to inherited modifications that are not encoded in the DNA structure itself.[7] Recently, it has been shown that epigenetics plays a larger than anticipated role in cancer biology among other cell processes. A number of epigenetic changes have been found in several cancers including colon, lung, breast, prostate, and urinary bladder.[8] These changes to the genome present a possible therapeutic target due to their potential reversibility.[9] In this review, we discuss the use of epigenetics in the diagnosis of bladder cancer and potential prognostic and therapeutic applications.

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Address correspondence to Dr. Murugesan Manoharan, Department of Urology, P.O Box 016960 (M814), University of Miami Miller School of Medicine, Miami, FL 33101 USA

Epigenetics

Epigenetics is the study of the changes in gene activity that are not caused by changes in the DNA structure. Epigenetics includes, but is not limited to DNA methylation, microRNA (*miRNA*) regulation and histone modification.[10] DNA methylation involves the covalent addition of a methyl group from S-adenosyl-methionine to a nucleotide, mostly cytosine within *CpG* dinucleotides in the DNA.[11] This process is facilitated by DNA methyltransferases (*DNMT*). Post-transcriptional regulation of gene expression occurs through an epigenetic mechanism called RNA interference, which is mediated by a class of small noncoding RNAs called *miRNA*. De-regulation of miRNA expression resulting from epigenetic modifications occurring in transformed cells may lead to tumorigenesis.[12] Histone modification includes methylation, acetylation, and phosphorylation of the histone proteins in the chromatin material.

DNA methylation, hypermethylation and hypomethylation

Among epigenetic alterations, DNA methylation has been studied extensively.[10] DNA methylation is typically the formation of a covalent bond of a methyl group to the 5 carbon position of a cytosine ring, although it can occur to adenine or thymine rings as well.[8] DNA methylation often occurs in a *CpG* dinucleotide (*CpG* island), a dinucleotide in which a cytosine is directly followed by a guanine. Methylation of *CpG* islands has been associated with the silencing of tumor-suppression genes.[13] This silencing can be transferred through several generations of cells. In methylation of *CpG* islands, both strands are methylated at the cytosine base. After replication, one strand is methylated due to semi-conservation. This state is known as hemimethylation. DNA methyltransferases have a high affinity for hemimethylated substrates, which methylate the remaining cytosine. In this way, the silencing persists through several cell divisions.

The hypermethylation of DNA has been established as an epigenetic abnormality observed in several malignant tumors, particularly in *CpG* islands.[13] Therefore, analysis of hypermethylated *CpG* islands is a promising method of detection and classification of cancers.[14] Hypermethylation involves silencing genes that perform tumor suppression, DNA repair and other key processes. Hypermethylation requires a number of enzymes called DNA methyltransferases (*DNMT*),[15] Figure 1. Four members of the *DNMT* family have been indentified in mammals: *DNMT1*, *DNMT3A*, *DNMT3B* and *DNMT3L*. *DNMT1* is the most essential for cancer cell growth.[11]

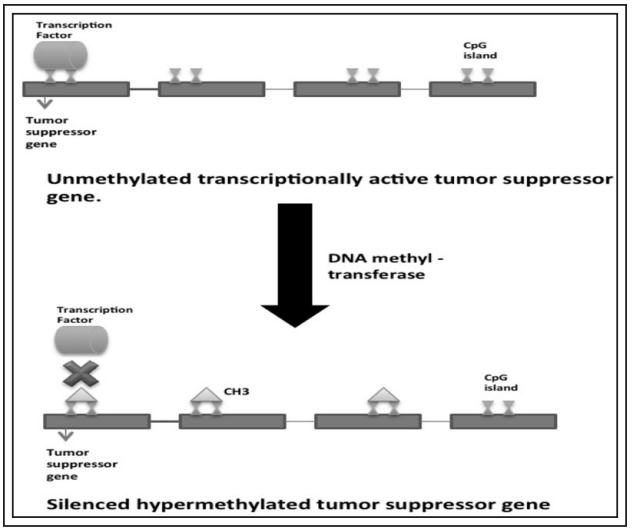

Figure 1. Epigenetic silencing of a tumor suppressor gene carried out by DNA methyl-transferase through hypermethylation of CpG dinucleotides.

DNA hypomethylation involves the removal of methyl groups from methylated DNA strands. It is facilitated by a group of enzymes known as demethylases.[15] Similar to hypermethylation, DNA hypomethylation has also been associated with a higher risk of cancer.[16] DNA hypomethylation is also a potential biomarker for bladder cancer. The gene *LINE-1* is one such marker.[17] However, the relationship between this epigenetic alteration and bladder cancer is still unclear, and DNA hypomethylation has predominantly shown to be useful as a post-diagnosis marker.

MicroRNA regulation

Regulation of post-transcriptional gene expression takes place through the epigenetic mechanism of RNA interference. *MiRNAs* are a class of small noncoding RNAs that regulate this process. Within tumor cells, dysregulation of *miRNA* expression can occur, affecting its differentiation, proliferation and apoptosis. The overexpression of *miRNA* in tumor cells has been termed oncogenic whereas *miRNA* that shows reduced expression is referred to as a tumor suppressor.[18] In a recent paper that assessed the expression of *miR-200c*, *miR-30b* and *miR-141* in tissue samples of patients with bladder cancer and healthy adjacent tissue, it was reported that *miR-141* was up-regulated in 91% of malignant tissue samples whereas *miR-200c* and *miR-30b* were over-expressed in 79% and 64% respectively.[19] Yu et al recently reported the anti-metastatic properties of *miR-34a* by inhibiting epithelial mesenchymal transition (EMT) related proteins in bladder cancer. EMT is a transient process in which epithelial cells acquire a mesenchymal phenotype by

losing intercellular adhesions normally maintained by a group of proteins called *E-cadherins* with the subsequent increase in cell motility. Proteins related to EMT include *N-cadherin*, *Beta-catenin* and *Vimentin*. *MiR-34a* consistently promoted *E-cadherin* protein expression and inhibited *N-cadherin*, *Beta-catenin* and *Vimentin* protein expression. This data indicated that *miR-34a* functions as a tumor suppressor in bladder cancer.[20]

Histone modification

Histones are proteins around which the double stranded DNA is coiled forming a structure known as nucleosome. Each nucleosome contains eight histone molecules (octamer): one pair each of *H2A*, *H2B*, *H3*, and *H4*. Both the histones and the DNA can be modified to silence or activate genes. Consequently, the proteins translated (or not translated) can cause the silencing or activation of other genes, creating a chain effect. Histone modification includes methylation, acetylation, and/or phosphorylation. Histone structure consists of two domains, a globular domain and a "tail" that contains the NH_2 terminal. Modification typically occurs on the "tail" domain at different amino acid positions. Nucleosomes are considered the basic unit of eukaryotic chromatin. There are essentially two types of chromatin: heterochromatin and euchromatin. Heterochromatin is highly condensed and is thus harder to transcribe, effectively silencing most genes within it. Euchromatin is more loosely packed and the genes are more easily transcribed. Like DNA methylation, identifying histone modifications has the potential for diagnosis or prognostication of bladder cancer. A well-studied histone modification is the *trimethylation (m3) of histone 3 lysine 27 (H3K27m3*). One study was able to correctly estimate gene expression from this marker in 65% of the subjects studied.[21] A list of genes involved in bladder cancer with its corresponding frequency is shown in Table 1.

TABLE 1. **Mutated genes and involvement frequency in bladder cancer[37]**

Gene	Frequency
KDM6A	20%-25% of bladder cancers
MLL2	27% of bladder cancers
ARID1A	25% of bladder cancers
EP300	15% of bladder cancers
MLL	> 10% of bladder cancers
MLL3	> 10% of bladder cancers
CREBBP	> 10% of bladder cancers
CHD7	> 10% of bladder cancers
SRCAP	> 10% of bladder cancers

Marker potential

Diagnosis

The use of epigenetics for the diagnosis of bladder cancer has great potential. It could reduce the need of invasive tests for tumor detection, including cystoscopy, which can miss some small or in-situ tumors despite good technique. DNA tests can be done easily using the polymerase chain reaction (PCR), which rapidly and accurately amplifies the subject's DNA, allowing it to be screened for genetic abnormalities.[22] One of the most validated techniques to establish the presence or absence of methylation in a gene within a tumor sample is methylation-specific PCR (MSP). This technique requires only small quantities of DNA and allows for a rapid analysis of multiple markers.[23] DNA tests can be done by simply using voided urine from bladder cancer patients. A number of recent studies have found several potential diagnostic markers. One study found the genes *IRF8*, *p14* or *sFRP1* predicted the presence of bladder cancer with a sensitivity of 86.7% and a specificity of 94.7%. Methylation of *SFRP1*, *IRF8*, *APC* and *RASSF1A* were also indicative of a higher tumor grade.[24] Chung et al reported two sets of parallel genes, the first of which contained: *MYO3A, CA10, NKX6-2* and *DBC1* or *SOX11, which had* 81% sensitivity and 97% specificity for the detection of bladder cancer. The second set contained: *MYO3A, CA10, NKX6-2, DBC1* and *SOX11* or *PENK, which had* 85% sensitivity and 95% specificity. Thus, a total of six methylation markers were identified.[25] Yu et al studied an 11-gene set which confirmed bladder cancer with a sensitivity of 91.7% and an accuracy of 87%.[26] There are a number of genes that could be used as potential DNA markers including *APC*, *RASSF1A*, *SFRP1*, *GDF15*, *HSPA2*, *TMEFF2*, *HOXB2*, *KRT13*, and FRZB.[6,14,27] The functions of these genes have been listed in Table 2.

Prognosis

A recent study has shown that epigenetic screening can be helpful in determining the prognosis of patients with bladder cancer. Methylation of the metastasis suppressor gene, *KISS1*, was shown to be associated with higher grade and higher stage tumors.[28] The *KISS1 gene* has been identified as suppressing metastasis in melanoma and breast cancer cells. This gene encodes for a 145-amonoacid protein that is processed into kisspeptins, which play a role at inhibiting cancer metastasis of different tumor types. In their report, Cebrian et al evaluated the effect of *KISS1* methylation on its expression and the clinical relevance in bladder cancer. Their results revealed that

TABLE 2. **Genes and functions**[38-45]

Gene	Function
APC	Cell proliferation, differentiation, migration, polarity
RASSF1A	Cell cycle regulation, apoptosis, microtubule stability
SFRP1	Cellular senescence
GDF15	Proliferation regulation and apoptosis
HSPA2	Sperm maturity, function and fertility
TMEFF2	Believed to be a tumor suppressor
HOXB2	Decreases tumor proliferation
KRT13	Encodes keratin
FRZB	Expressed in developing and mature chondrocytes

KISS1 was hypermethylated in bladder cancer and that the methylation of the gene and its transcript expression could be used as a biomarker and a predictor of outcome for bladder cancer patients.[28]

Therapeutic targets

Tumor suppressor gene silencing through an epigenetic mechanism is carried out through systematic enzyme catalyzation processes. Reactivation of these genes has become an attractive target for cancer therapy. There are several drugs that are being evaluated. These are broadly grouped into two categories: DNA methyl transferases (*DNMT*) inhibitors and histone deacetylase (*HDAC*) inhibitors.[15]

DNMT inhibitors

Nucleoside analogs are a large part of *DNMT* inhibitors. As mentioned previously, methylation typically occurs in *CpG* islands at the two cytosine bases (one on either strand).[8] The understanding of the mechanism of methylation by *DNMT* suggests that inhibition of methylation can occur at a number of steps including recognition of the target sequence, flipping of the target base, formation of the initial covalent bond, methyl transfer to carbon 5 of cytosine and release of the substrate. Nucleoside analogs substitute the nitrogenous bases in DNA with analog structures to prevent methylation. These nucleosides are converted to deoxyribonucleoside triphosphates and subsequently incorporated into newly synthesized DNA in place of cytosine residues. *DNMT* is inactivated through binding to these newly synthesized residues that replace cytosine residues next to guanine, resulting in global hypomethylation.[29,30] Zebularine is one such drug, which is an analog of cytosine. Studies have shown that synthetic oligonucleotides containing Zebularine form tight complexes with bacterial methyltransferases leading to a potent inhibition of DNA methylation. One study tested whether Zebularine was an effective inhibitor of mammalian DNA methylation in human bladder carcinoma cells lines. It was noted that Zebularine could induce expression of a human tumor suppressor gene that had been silenced by methylation after just 48 hours of treatment.[31-33]

HDAC inhibitors

There are a number of drugs that show *HDAC* inhibitory capacity. Due to their diversity, they are broadly categorized into five groups based on chemical composition and structure: short-chain fatty acids, hydroxamic acids, cyclic tertrapeptides, benzamides and aliphatic acids. The difficulty in using them lies in minimizing the toxicity of the drug, while maximizing the effect. Several *HDAC* inhibitory drugs have been tested for treatment of urological cancers including: Romidepsin, Vorinostat, and Panobinostat, however, trials have shown poor response and high toxicity levels.[34] Some reviews have suggested *HDAC* inhibitors show promise due to good bioavailability and, used in combination with cytotoxic chemotherapy, have acceptable toxicity. However their tumor inhibitory effect could not be independently assessed.[35] A combination of *HDAC* inhibitors and *DNMT* inhibitors can have a synergistic effect on apoptosis, differentiation and/or cell growth arrest in cancer cells.[36]

Conclusions

Epigenetics has been shown to play a much greater role than previously thought in the initiation and propagation of tumors. The use of epigenetics for the diagnosis and treatment of bladder cancer appears to be a promising field. Drugs using epigenetic targets are undergoing tests for their toxicity, safety, and potential synergistic effects with other drugs. Epigenetic profiling also has the potential to identify patients at high risk of bladder cancer through non-invasive urine tests. Such testing could help identify patients at an earlier stage of the disease. The possibility of epigenetic changes being "reversed" makes such epigenetic modification therapy a powerful potential option for cancer treatment in the future. □

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