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The role of deoxyribonucleic acid methylation in development, diagnosis, and prognosis of bladder cancer☆

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Abstract

Alterations in global levels and regional patterns of deoxyribonucleic acid methylation are among the earliest and most common events known to occur in human cancer. The mutational and epigenetic effects of this covalent deoxyribonucleic acid modification to the development of bladder cancer are well recognized. The contribution of aberrant methylation to mutational hot spots located within genes, transcriptional silencing, and chromosomal instability is reviewed in the context of its relevance to bladder carcinogenesis. Understanding how such processes evolve during the progression of bladder cancer is essential for using these molecular changes in the clinical setting. The recent development of sensitive and specific techniques for quantifying methylation changes in urine specimens and bodily fluids underscores the potential use of this molecular marker for early detection and surveillance of bladder cancer. Further refinement of these molecular biological techniques holds much promise for the use of methylation markers for bladder cancer diagnosis, risk stratification, and disease prognostication.

Keywords: DNA methylation; Bladder cancer; Diagnosis; Prognosis

1. Introduction

It is estimated that 51,420 new cases of bladder cancer will be diagnosed, and 13,060 people will die of the disease in the United States in 2006 [1]. Carcinoma of the bladder is currently the second leading cause of genitourinary cancer mortality after prostate cancer. The most common form of bladder cancer accounting for 90% of clinical presentations in the United States is urothelial carcinoma (also known as transitional cell carcinoma). This phenotype most commonly occurs as papillary, low-grade disease for which transurethral resection and selected use of intravesical, immunotherapy, or chemotherapy is the standard of care. Tumor recurrence with superficial disease is quite common and can be as high as 70% after local resection, with approximately 10% to 30% of recurrent cancers progressing to invasive disease. Although the vast majority of urothelial carcinomas present as superficial disease and are amenable to transurethral resection, nearly 30% of urothelial carcinomas are muscle invasive at their initial clinical presentation [2].

Invasive or high-grade bladder cancers typically appear as flat urothelial carcinomas that are frequently associated with dysplastic changes and carcinoma in situ (CIS). Squamous cell carcinoma represents another histologic variant of bladder cancer that is uncommon in the United States (5% to 7% of reported cases). The highest incidence of squamous cell carcinoma of the bladder occurs in regions such as Northeast Africa and the Middle East, where schistosomiasis is endemic. Squamous cell carcinoma has been reported in as many as 60% of patients with bladder cancer with a history of neurogenic voiding dysfunction, and is presumably the result of long-standing irritation from chronic infections or indwelling catheters [3].

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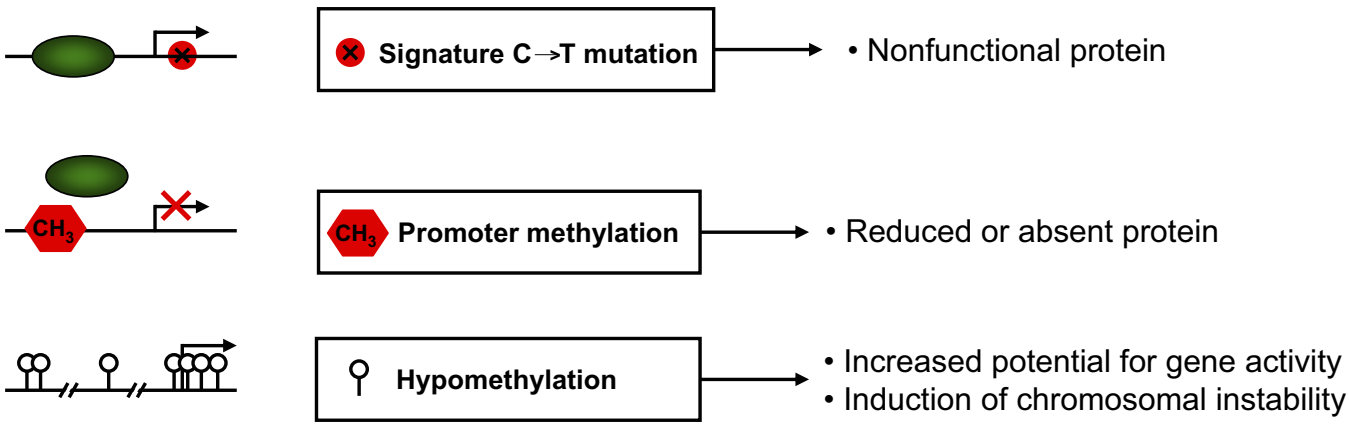

Fig. 1. DNA methylation in the development of bladder cancer. (1) Mutational effects: deamination of 5-methylcytosine results in the signature cytosine (C) to thymine (T) transition mutations observed in mutational hot spots for genes, such as p53. (2) Epigenetic effects: promoter methylation may lead to reduced or absent protein as a result of disruption of local chromatin structure, inhibition of transcription factor binding, or exclusion of transcriptional machinery from methylated promoter sequences. (3) Hypomethylation may lead to increased potential for gene activity or genomic instability via alterations in chromatin structure, recombination between repetitive elements, or re-expression of retrotransposons. (Color version of figure is available online.)

The striking differences in the biologic potential of superficial and invasive urothelial carcinomas are reflected in the genetic alterations underlying each histologic variant. Two molecular pathways to the development of urothelial carcinoma were first described in a series of 216 bladder tumors, in which chromosome 9 loss of heterozygosity (LOH) and mutations of the p53 tumor suppressor gene were examined [4]. A high frequency of chromosome 9 LOH (34%), but a low frequency of p53 mutations (3%), were observed in papillary tumors. In contrast, CIS and dysplastic urothelium contained a low frequency of chromosome 9 LOH (12%), but a high number of p53 gene mutations (65%). Mutations of the p53 gene are believed to occur early during development of CIS and dysplastic lesions, while LOH of chromosome 9 occurs as a later event that may be required for tumor progression and invasiveness. Early acquisition of p53 mutations may lead to genome-wide instability, thus increasing the likelihood of disease progression in this tumor phenotype. The presence of both chromosome 9 defects and p53 alterations may be required for the development of invasive tumors. There is strong evidence based on chromosomal mapping studies that one or more bladder tumor suppressor genes are located on 9q [5,6]. Loss of such key cellular growth regulators may play a role in the development of noninvasive papillary bladder tumors. Accumulation of molecular alterations such as p53 gene mutations may facilitate progression to invasive disease.

Significant changes in the global levels and regional patterns of deoxyribonucleic acid (DNA) methylation are among the earliest and most frequent events known to occur in human cancer [7]. Alterations in DNA methylation have a direct impact on the mutational and epigenetic components of neoplastic transformation. Application of novel molecular biologic techniques has increased our appreciation of the widespread changes in methylation patterns that occur during bladder carcinogenesis. There are 3 important effects of DNA methylation on the genome that must be considered in the development of this disease: (1) mutational burden of 5-methylcytosine, (2) epigenetic effects of promoter methylation on gene transcription, and (3) potential gene activation and induction of chromosomal instability by DNA hypomethylation (Fig. 1) [8,9]. The mechanisms by which normal regulatory processes are affected by DNA methylation will be discussed in the framework of recent discoveries.

2. Mutational burden of DNA methylation

The covalent modification of cytosine by DNA (cytosine-5) methyltransferase results in the formation of 5-methylcytosine. This enzymatic modification occurs primarily at the CpG palindrome in vertebrate DNA and is a ubiquitous but regulated phenomenon essential for normal mammalian development [10]. Methylation of cytosine residues was first shown to be mutagenic in bacteria and accounts for the phenomenon of hot spots for spontaneous base substitutions in DNA [11]. Methylation of cytosines at CpG dinucleotides has increased the probability of C→T transition mutations between 12 and 42-fold, and has resulted in approximately 5-fold depletion of this sequence throughout the genome [12,13]. The coding regions of important genes, including those that influence cell cycle activities, are frequently methylated at CpG dinucleotides and are sites of mutational hot spots [14]. The presence of C→T transitions located at CpG dinucleotides are signature mutations attributable to the deamination of 5-methylcytosine to thymine and can result in production of nonfunctional protein (Fig. 1).