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PROSPECTS

Genetic and Epigenetic Aspects of Bladder Cancer

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Abstract Transitional cell carcinoma of the urinary bladder has a diverse collection of biologic and functional characteristics. This is reflected in differing clinical courses. The diagnosis of bladder cancer is based on the information provided by cystoscopy, the gold standard in combination with urinary cytology findings. Many tumor markers have been evaluated for detecting and monitoring the disease in serum, bladder washes, and urinary specimens. However, none of these biomarkers reported to date has shown sufficient sensitivity and specificity for the detection of the whole spectrum of bladder cancer diseases in routine clinical practice. The limited value of established prognostic markers requires the analysis of new molecular parameters of interest in predicting the prognosis of bladder cancer patients; in particular, the high-risk patient groups at risk of progression and recurrence. Over the past decade, there has been major progress elucidating of the molecular genetic and epigenetic changes leading to the development of transitional cell carcinoma. This review focuses on the recent advances of genetic and epigenetic aspects in bladder cancer, and emphasizes how molecular biology would be likely to affect the future therapies. J. Cell. Biochem. 95: 24–33, 2005.

Key words: bladder cancer; polymorphism; genetic alteration; methylation

Precise reason why specific individuals get bladder cancer remains unknown. Genetic polymorphism may be involved in tumorigenesis of urinary bladder, including xenobiotic metabolism, DNA repair, cell cycle control mechanisms, and many cytokine production [Kim et al., 2000, 2002, 2005; Wang et al., 2002; Jeong et al., 2004], which probably reflects not only different incidence rates in different countries [Morrison et al., 1984] but also the combined effects of environmental and hereditary factors. Recently, there has been major progress in both genetic polymorphism in relation to bladder cancer, and molecular genetic and epigenetic changes leading to the development of transitional cell carcinoma.

More than 90% of bladder cancers are transitional cell carcinomas and roughly 60% are low-grade superficial transitional cell carcinomas. The majority of these patients develop cancer recurrences after endoscopic resection, 16%–25% with high-grade cancers. Approximately 10% of patients with superficial bladder cancers subsequently develop invasive or metastatic disease. Almost 25% of patients with newly diagnosed bladder cancer have muscle-invasive disease, the vast majority being cancers of high histologic grade. Almost 50% of patients with muscle-invasive bladder cancer already have occult distant metastases [Koss, 1979; Cutler et al., 1982; Greenlee et al., 2001; Messing, 2002].

Transitional cell carcinoma is a mixture of heterogeneous cell populations having different metastatic potentials. Despite the apparent de novo clinical presentation of invasive bladder cancers, cytogenetic, and antigenic evidence supports the hypothesis that transitional cell carcinomas follow the general concept of multistep carcinogenesis and proceed through two distinct genetic pathways responsible for generating different cancer morphologies. These are the inactivation of cyclin-dependent kinase inhibitors in low-grade transitional cell carcinoma and early p53-mediated abnormalities in high-grade transitional cell carcinoma. The progression of transitional cell carcinoma correlates with genetic instability and accumulation

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of collaborative genetic lesions [Al-Sukhun and Hussain, 2003]. In addition, the advent of high-throughput is accelerating the identification process of the molecular events characteristic of bladder cancers' phenotype and subsequent behavior. The information provided by these analyses is resulting not only in the identification of novel therapeutic targets for bladder cancer, but also in the development of diagnostic tools.

CpG island methylation is rare in normal cells. It plays a role in X-chromosome inactivation in females and genomic imprinting, and increases with age and in vitro cell culture. Abnormal methylation of CpG islands can efficiently repress transcription of the associated gene in a manner akin to mutations and deletions, and act as one of the 'hits' in the Knudsen two-hit hypothesis for tumor generation [Jones and Laird, 1999; Baylin and Herman, 2000]. There are several examples of aberrant CpG island promoter hypermethylation of tumor suppressor genes, genes involved in cell–cell adhesion, and genes involved with DNA repair in relation to bladder cancer [Maruyama et al., 2001; Tada et al., 2002; Dulaimi et al., 2004]. Examples in which one copy of a tumor suppressor gene is either mutated or lost and the wild-type copy is transcriptionally silenced by hypermethylation have been found [Robertson, 2001]. It has recently been recognized that aberrant hypermethylation events can occur early in tumorigenesis and disrupt pathways that may predispose cells to malignant transformation. Aberrant methylation was detectable in preneoplastic lesions and the frequency of aberrations increased with disease progression [Belinsky et al., 1998; Nuovo et al., 1999; Wong et al., 1999]. Thus, ample evidence exists to support the notion that DNA hypermethylation events could act as a primary inactivating event contributing directly to tumorigenesis of the urinary bladder. This review focuses on the recent advances of genetic polymorphism, genetic alterations, and hypermethylation events in relation to bladder transitional cell carcinoma.

GENETIC POLYMORPHISM

Genetic variation in the human genome is an emerging resource for studying cancer, a complex set of diseases characterized by both environmental and genetic contributions. It is becoming increasingly apparent that most of population-attributable cancer heritability is related not to the rare deleterious gene defects but to polymorphic variations in the DNA sequence [Ponder, 2001]. This concept has been substantiated in a variety of cancer settings, particularly by the genetic epidemiology of bladder cancer. N-Acetylation of aromatic amine procarcinogens by N-acetyltransferase 2 (NAT2) is generally considered to be a detoxifying mechanism [Cartwright et al., 1982]. Slow and rapid acetylators were shown to have variable susceptibility to bladder cancer. Several comparative studies among different ethnic groups observed less than 20% and more than 55% of slow acetylators among Asians and Whites [Grant et al., 1983; Sinues et al., 1992]. Hein [1988] combined data from 12 studies and noted a statistically significant relative risk for bladder cancer of 1.5 in slow versus rapid acetylators. Among Korean subjects in our study, 7.1% of bladder cancer patients and 11.0% of controls had slow acetylator genotypes [Kim et al., 2000]. Yu et al. [1994] observed that a difference in prevalence of slow acetylator (the high-risk phenotype) among the three ethnic groups (Whites, Blacks, and Asians) closely paralleled their varying incidence of bladder cancer. It might be inferred that the lower frequencies of slow acetylator genotypes in Asians relative to the Westerners could result in less incidence of bladder cancer.

Glutathione (GSH) and GSH-dependent enzymes are involved in the cellular metabolism and detoxification of cytotoxic and carcinogenic products. Accordingly, much interest is concentrated on the significance of polymorphism in the glutathione *S*-transferase (GST) supergene families, as these enzymes metabolize exogenous and endogenous molecules involved in cell-specific functions, such as proliferation and apoptosis [Nebert, 1994]. The absence of GST-μ (GSTM1) activity is due to homozygous deletion of the gene. A similar polymorphism of the GSTT1 encoding the θ class enzymes were discovered [Pemble et al., 1994]. GSTM1 null genotype has attracted much attention due to its possible association with increased susceptibility to certain malignancies such as lung cancer [Seidegard et al., 1986] and bladder cancer [Bell et al., 1993; Katoh et al., 1998], although the high risk of bladder cancer in individuals with the GSTM1 null genotype is still controversial. Kim et al. [2000] demon-

strated an association between the GSTM1 null genotype and increased risk of bladder cancer. On the other hand, few studies showed GSTT1 being a possible risk factor in bladder cancer. Kempkes et al. [1996] reported that the frequency of GSTT1 null genotype was similar in patients with urothelial cancer and controls. Brockmoller et al. [1996] reported that there was no significant difference in the GSTT1 genotype between urothelial cancer cases and controls in all subjects including smokers and non-smokers, although a significantly high frequency of GSTT1 null genotype in the cancer cases was observed in the non-smoker group. To identify the effects of GSTM1-null and GSTT1-null types and smoking status on the bladder cancer incidence with ethnicity as determined by the previous 29 reports, Kim et al. [2002] compared the frequencies of GSTM1-null and GSTT1-null genotypes and estimated smoking prevalence with the age-standardized bladder cancer incidence. In the univariate and multivariate analyses with the ecological data of various countries and ethnic groups, cigarette smoking positively, but the frequency of the GSTT1-null type negatively, correlated with the age-standardized bladder cancer incidence. These results suggest that the GSTT1-null genotype might not be a risk factor but a protective factor of bladder cancer.

Genes encoding a number of cytokines are polymorphic. Tumor necrosis factor-α (TNF-α) is a multifunctional cytokine. Genotype changes at position −308 of the TNF-α promoter are frequently observed in several cancers [Warzocha et al., 1998; Sasaki et al., 2000]. Vascular endothelial growth factor (VEGF) is a major angiogenic factor. VEGF mRNA is markedly upregulated in human tumors, such as kidney and bladder cancer [Brown et al., 1993]. Several studies reported that the cancer stage and grade were significantly associated with the TNF-α and VEGF genotypes [Jeong et al., 2004; Kim et al., 2005]. These cytokines have angiogenic function, possibly critical for cancer invasion and metastasis. High-grade and stage bladder cancers are more prone to invasion and metastasis, which suggests that the aggressiveness of primary bladder cancer might be associated with the TNF-α and VEGF genotypes.

The mutagenic base, 8-oxoguanine, is removed from damaged DNA by base excision repair. The human 8-oxoguanine DNA glycosylase 1 (hOGG1) gene encodes DNA glycosylase that catalyzes the excision of the mutagenic lesion 8-oxoguanine from oxidatively damaged DNA [Tchou et al., 1991; Lee et al., 1993]. A Ser326Cys polymorphism in hOGG1 has been identified in bladder cancers [Kim et al., 2005]. Distribution of the hOGG1 codon 326 genotypes (Cys326Cys versus Ser326Ser and Ser326Cys) of controls was significantly different from the bladder cancer patients in that Cys326Cys genotype displayed a protective effect against bladder cancer development, compared to Ser326Ser and Ser326Cys genotypes. Currently, there is no convincing explanation that Cys326Cys is a highly protective genotype. These reflect that hOGG1 may not be the only gene associated with oxidative damage. An alternative DNA oxidative damage repair pathway to minimize the effects of 8-oxoguanine in genomes was reported [Klungland et al., 1999].

Numerous single nucleotide polymorphism (SNP) studies have been currently reported in relation to the bladder cancer (Table I). The existence of low-penetrance cancer predisposing polymorphisms is undisputable; unfortunately, very few consistent gene-disease associations have been identified so far. It is hoped, that the ongoing worldwide efforts in obtaining large and informative DNA collections, combined with the rapid development of high-throughput genotyping technologies, will provide useful prognostic markers for clinicians applicable in clinical setting.

GENETIC ALTERATIONS

Cytogenetic studies have identified many structural and numerical chromosomal changes in bladder transitional cell carcinomas. Chromosomal abnormalities, including increased or decreased number of chromosomes (hyperdiploidy and aneuploidy), marker chromosomes, and the chromosomes of abnormal size or configuration, have also been shown to correlate with an increased risk of tumor recurrence and cancer progression [Sandberg et al., 1986; Falor and Ward-Skinner, 1988]. Molecular genetic studies on bladder cancer have demonstrated deletion of several chromosomal arms, including 3p, 6q, 9q, 11p, 17p, and 18q [Sandberg and Berger, 1994]. Loss of 9q were demonstrated in low as well as high-grade bladder cancer suggesting that the loss of 9q may be a primary event in the genesis of bladder cancer [Tsai et al., 1990]. Among the early

TABLE I. Genetic Polymorphisms and the Risk of Bladder Cancer Development

Gene	Polymorphisms	Odds ratio (95% CI*)	References
p53	Arg72Pro	4.7 (2.1–10.4)	Soulitzis et al. [2002]
CCND1 (CyclinD1)	241 A/G	1.8 (1.1–2.8)	Wang et al. [2002]
NQO1 (NAD(P)H dehydrogenase, quinone 1)	609 C/T	1.6 (1.0–2.7)	Choi et al. [2003]
CYP2E1 (Cytochrome P450, family 2, subfamily E, polypeptide 1)	C1019 (c1/c1)	1.8 (1.1–2.9)	Choi et al. [2003]
RGS6 (Regulator of G-protein signalling 6)	rs2074677 C → T	0.7 (0.5–1.0)	Berman et al. [2004]
XRCC1 (X-ray repair complementing defective repair in Chinese hamster cells 1)	Arg399Gln	0.6 (0.4–1.0)	Kelsey et al. [2004]
XRCC3 (X-ray repair complementing defective repair in Chinese hamster cells 3)	Thr241Met	1.3 (0.9–1.9)	Stern et al. [2002b]
		0.6 (0.4–0.9)	Shen et al. [2003]
GPX1 (Glutathione peroxidase 1)	Pro198Leu	2.6 (1.5–4.8)	Ichimura et al. [2004]
CDH1 (E-cadherin)	−160 C/A	3.5 (1.3–9.6)	Zhang et al. [2003]
GSTM1 (Glutathione *S*-transferase μ)	Null-genotype	1.6 (1.2–2.2)	Brockmoller et al. [1996]
		1.7 (1.2–2.5)	Bell et al. [1993]
		1.7 (1.1–2.8)	Katoh et al. [1998]
		1.7 (1.1–2.7)	Kim et al. [2005]
		1.8 (1.1–3.0)	Kempkes et al. [1996]
		1.8 (1.1–2.9)	Kim et al. [2000]
		2.3 (1.1–5.5)	Jeong et al. [2003]
		2.8 (1.1–6.4)	Toruner et al. [2001]
GSTT1 (Glutathione *S*-transferase θ)	Null-genotype	2.6 (1.1–6.0)	Brockmoller et al. [1996]
		0.6 (0.4–1.0)	Kim et al. [2005]
GSTP1 (Glutathione *S*-transferase π)	313 A/G	2.4 (1.1–4.9)	Toruner et al. [2001]
		2.4 (1.1–4.9)	Habdous et al. [2004]
hOGG1 (8-oxoguanine DNA glycosylase 1)	Ser326Cys	2.0 (1.2–3.5)	Kim et al. [2005]
XPD (Xeroderma pigmentosum D)	Gln751Lys	0.8 (0.4–1.3)	Stern et al. [2002a]
NAT2 (N-acetyltransferase 2)	NAT2*5B	2.7 (1.0–7.4)	Brockmoller et al. [1996]

*CI, confidence interval.

changes appearing in superficial bladder cancers are deletions of 11p and 8p, and gains of 8q and 1q [Fadl-Elmula et al., 2001]. In contrast, deletions of 17p were not identified in superficial noninvasive papillary lesions, while 60% of invasive cancers exhibited loss of heterozygosity (LOH) of 17p, suggesting involvement in the progression of bladder cancer [Olumi et al., 1990; Presti et al., 1991].

New high-throughput microarray technologies make it possible to gain a comprehensive insight into the molecular basis of human diseases [Bubendorf, 2001]. Search for the genes that are differentially expressed in human tumors has been greatly facilitated by the DNA microarray technology. In this technology, the RNA expression level of hundreds or thousands of genes in a tumor can be surveyed simultaneously. Recently, the tissue microarray technology has also been developed to facilitate large-scale molecular analyses in hundreds of tumors simultaneously [Kononen et al., 1998]. These microarray technologies have widened applications in the urological field. However, only few studies dealt with molecular classification of bladder cancer expression profiling using DNA microarrays. Sanchez-Carbayo et al. [2003] introduced cDNA microarrays containing 17,842 known genes to allow hierarchical clustering of early-stage and invasive transitional carcinomas. It is possible to separate carcinoma in situ from papillary superficial lesions and subgroups within early-stage and invasive cancers displaying different overall survival. The most extensive expression profiling study of bladder cancers reported to date has dealt with the development of a predictive classifier of Ta, T1, and T2 + bladder carcinoma subclasses. Dyrskjot and coworkers [Dyrskjot, 2003; Dyrskjot et al., 2003] reported the identification of clinically relevant subclasses of bladder carcinoma using expression microarray analysis of bladder cancers. Cluster analysis identified three major stages, Ta, T1 and T2-4, with the Ta tumors further classified into subgroups. A 32-gene molecular classifier was able to classify benign and muscle-invasive cancers with close correlation to pathological staging. This also provided new predictive information on disease progression in Ta tumors compared with conventional staging. Furthermore, gene-expression profiles characterizing each stage and subtype identified their biological properties, producing new potential targets for therapy. Dyrskjot et al. [2004] recently reported microarray expression profiling to examine the gene expression patterns in superficial transitional cell carcinoma with surrounding carcinoma in situ, without surrounding carcinoma in situ lesions, and in muscle inva-

sive carcinomas. They identified a few gene clusters that contained genes with similar expression levels in transitional cell carcinoma with surrounding carcinoma in situ and invasive carcinomas. A 16-gene molecular classifier for identification of the carcinoma in situ gene expression signature was constructed and suggested to be useful in the follow-up of bladder cancer patients.

The application of tissue microarrays represents a high-throughput approach for validation of potential novel markers for bladder cancer by immunohistochemistry or in situ hybridization in paraffin blocks [Kononen et al., 1998; Schraml et al., 1999; Richter et al., 2000; Nocito et al., 2001]. Several reports describe the rapid evaluation of targets of interest, such as cytoskeletal actin associated gelsolin or E-cadherin [Rao et al., 2002], Na, K-ATPase or cell-cycle-related markers [Espineda et al., 2003]. The most extensive tissue microarray in bladder cancer, analyzing over 2000 bladder carcinomas, revealed the prognostic utility of cyclin E [Richter et al., 2000]. Focus is intensified in this field to automate the construction of tissue microarrays.

The usefulness of the microarrays has not yet been evaluated by enough clinical trials. Identification of tumor subtypes within the superficial disease and patients more likely to develop positive lymph nodes or distant metastases is critical subclassification issues to be investigated. In the near future, gene profiling will provide an effective means of predicting the response to specific therapeutic regimes based on the molecular signatures of the tumors associated with their chemosensitivity or resistance to anticancer drugs. Moreover, the discovery of molecular pathways altered in cancer progression, as well as the identification of molecule-susceptible targets, would lead to the development of novel alternative therapies. The classical tumor marker concept of an individual biological determinant will be substituted by the use of cluster of genes as predictive classifiers.

DNA replication errors (RER) have been characterized by the addition or deletion of bases within the simple mononucleotide or dinucleotide repeat sequences in the DNA of tumors compared with the matching DNA of normal tissue specimens [Thibodeau et al., 1993]. Microsatellite instability (MSI) is the alteration of the length of simple repetitive sequences (microsatellites) throughout the genome. It is due to dysfunction of the DNA mismatch repair genes (hMSH2, hMLH1, hMSH6, PMS1, PMS2) leading to the accumulation of DNA replication errors. These alterations mainly affect microsatellite sequences in some cases residing in the coding sequence of important growth regulatory genes, hence contributing to the development of cancer [Peltomaki and de la Chapelle, 1997]. That is to say, RER is judged based on the degree of microsatellite instability, which is believed to result in a rapid accumulation of somatic mutations once carcinogenesis is initiated. The status of RER may thus be important for determining both the prognosis and the optimal therapeutic strategy of carcinomas in the future [Lothe et al., 1993]. Microsatellite alterations in bladder cancer have been reported to be associated with invasive phenotype and young age [Sardi et al., 1999; Thykjaer et al., 2001]. Regardless of the underlying mechanism, many groups are testing the possibility of using this microsatellite alteration phenotype for the early diagnosis and follow up of bladder cancer by detecting these shifted DNA bands in exfoliated cell in urine, or bladder washings [Uchida et al., 1996; Larsson et al., 2001; Seripa et al., 2001]. However, microsatellite alterations in urine are indicators not only of malignancy but also of inflammatory conditions [Christensen et al., 2000]. Thus, their role will be additive to the current diagnostic methods.

EPIGENETIC ALTERATIONS

The hereditability of newly acquired traits is a hallmark of clonal expansion in cancers. The inheritance of information on the basis of gene expression levels is known as epigenetics, as opposed to genetics, which refers to information inherited on the basis of gene sequence. Enzymatic methylation of the C-5 position of cytosine residues can affect epigenetic inheritance by altering the expression of genes and by transmission of DNA methylation patterns through cell division. Thus, in addition to its well-known role in deamination mutational hotspots in human DNA, DNA methylation may contribute to gene inactivation in cancer. DNA methylation is a powerful mechanism for the suppression of gene activity. It should be emphasized that this inverse correlation has been demonstrated conclusively only for methylation in the

promoter regions and not in transcribed part of genes. The methyl groups do not affect base pairing but can influence protein–DNA interactions by protruding into the major groove [Razin and Riggs, 1980].

Several tumor suppressor genes related to the bladder cancer contain CpG islands in their promoters, prompting many studies to investigate the role of methylation in silencing of these genes (Table II). Hypermethylation in the 5′ promoter region of genes is associated with transcriptional silencing and is an alternative mechanism for down-regulating gene expression rather than gene deletion or mutation. Hypermethylation of the promoter region of the p16INK4A gene in a subset of patients with superficial transitional cell carcinomas has been described and may be an early event in transitional cell carcinoma pathogenesis [Orlow et al., 1999]. Other genes also have been found to undergo aberrant promoter methylation in transitional cell carcinoma but are not found in the normal bladder mucosa associated with these tumors, including DBCCR1 (deleted-in bladder cancer 1 gene), and the 5′ end of transmembrane protein containing epidermal growth factor and follistatin domains [Salem et al., 2000]. Maruyama et al. [2001] determined the methylation status of 10 genes in 98 surgically resected bladder cancers, and calculated the median methylation index (MI), a reflection of the methylated fraction of the genes tested. All of the tumors were transitional cell carcinomas except for one squamous cell carcinoma. Their results indicated that multiple genes are methylated during the process of bladder cancer development. They found frequent methylation of four genes (two members of the cadherin family-CDH1 and CDH13-; a recently identified putative tumor suppressor gene, RASSF1A; and APC). Methylation of these genes and the MI correlated with one or more parameters

TABLE II. Promoter Methylation of Genes in Relation to Human Bladder Cancer

Gene	Chromosomal locus	Methylated rate (%)	References
p16INK4A (cyclin-dependent kinase inhibitor 2A)	9p21	14.9	Orlow et al. [1999]
		7	Maruyama et al. [2001]
		26.5	Chan et al. [2002]
		11	Tada et al. [2002]
		26	Valenzuela et al. [2002]
		60	Chang et al. [2003]
		7	Dulaimi et al. [2004]
p14ARF (cyclin-dependent kinase inhibitor 2A)	9p21	0	Chang et al. [2003]
		35	Dulaimi et al. [2004]
APC (adenomatous polyposis coli)	5q21	35	Maruyama et al. [2001]
		69	Dulaimi et al. [2004]
RASSF1A (RAS association domain family protein 1A)	3p21	56.4	Lee et al. [2001]
		35	Maruyama et al. [2001]
		47.5	Chan et al. [2003]
		51	Dulaimi et al. [2004]
CDH1 (E-cadehrin)	16q22	43	Bornman et al. [2001]
		63.3	Chan et al. [2002]
		47	Horikawa et al. [2003]
		36	Maruyama et al. [2001]
		84	Ribeiro-Filho et al. [2002]
		48	Tada et al. [2002]
CDH13 (H-cadehrin)	16q24.2-q24.3	29	Maruyama et al. [2001]
FHIT (fragile histidine triad gene)	3p14.2	16	Maruyama et al. [2001]
RARβ (retinoic acid receptor β)	3p24	15	Maruyama et al. [2001]
		87.8	Chan et al. [2002]
GSTP1 (glutathione *S*-transferase π)	11q13	11	Maruyama et al. [2001]
		5.1	Chan et al. [2002]
		13	Tada et al. [2002]
DAPK (death-associated protein kinase 1)	9q34.1	4	Maruyama et al. [2001]
		58.2	Chan et al. [2002]
		29	Tada et al. [2002]
MGMT (O-6-methylguanine-DNA methyltransferase)	10q26	2	Maruyama et al. [2001]
		5.1	Chan et al. [2002]
		17	Tada et al. [2002]
LNMA3 (laminin, α 3)	18q11.2	45	Sathyanarayana et al. [2004]
LNMB3 (laminin, β 3)	1q32	21	Sathyanarayana et al. [2004]
LNMC2 (laminin, γ 2)	1q25-q31	23	Sathyanarayana et al. [2004]
hMLH1 (MutL homolog 1)	3p21.3	13	Tada et al. [2002]
VHL (Von Hippel-Lindau tumor suppressor)	3p26-p25	4	Tada et al. [2002]
DBCCR1 (deleted in bladder cancer 1)	9q32-q33	52	Habuchi et al. [2001]

of worse prognosis, specifically high tumor stage and grade, muscle invasion, nonpapillary growth pattern, and shortened survival. In multivariate analyses, CDH1 methylation-positive status was independently associated with poor survival. Sathyanarayana et al. [2004] analyzed the methylation pattern of LAMA3, LAMB3, and LAMC2, which encoded laminin-5, in 128 bladder cancers and 71 urine samples. Methylation of LAMA3 and LAMB3, and MI were correlated with several parameters of poor prognosis (grade, stage, growth pattern, invasion, and ploidy pattern), whereas methylation of LAMC2 and MI were associated with shortened patient survival. Tada et al. [2002] determined the frequency of aberrant promoter hypermethylation of 7 genes, hMLH1, O6-methylguanine-DNA-methyltransferase (MGMT), p16, Von Hippel-Lindau (VHL), death-associated protein kinase (DAP-kinase), glutathione *S*-transferase P1 (GST-P1) and E-cadherin in superficial bladder cancers. Simultaneous hypermethylation of three genes or more among the seven genes was a significant concordance between the number of methylated genes and the development of recurrence. In particular, the recurrence rate for 24 months was 88% for hypermethylation of DAPkinase and 28% for nonmethylation of DAP-kinase. The methylation profile may represent a new potential biomarker of risk prediction in transitional cell carcinoma.

SUMMARY

The origin of bladder cancer is not fully explained by a single risk factor. Numerous factors may be involved in carcinogenesis, progression and patient's survival, including genetic polymorphisms, genetic and epigenetic alterations such as chromosomal alterations, and loss of heterozygous (LOH) of specific allele and aberrant DNA methylation, and factors controlling the cell cycle, cell proliferation and apoptosis. In spite of numerous SNP studies in relation to bladder cancer, only a few genetic polymorphisms give us marginal information for patient's prognosis. It is expected that the increasing capacities of available DNA collections, coupled with the rapid development of high-throughput genotyping technologies, will vastly accelerate the research on bladder cancer susceptibility. Though the usefulness of the microarrays has not yet been evaluated by enough clinical trials, this technology will allow a rapid molecular analysis of thousands of tumors within a few hours, with the premise of a better tool for biological classification of bladder cancers. The epigenetic silencing of tumor suppressor genes is interesting from a clinical standpoint because it is possible to reverse epigenetic changes and restore gene function onto a cell. Treatment with DNA methylation inhibitors can restore the activities of dormant genes such as CDKN2A and decrease the growth rate of cancer cells in a heritable fashion. DNA repair capacity can be restored by activation of MLH1. It should therefore be possible to partially reverse the cancer phenotype by the use of methylation inhibitors. Aforementioned genetic and epigenetic signatures will allow a better chance of cure opting for the most appropriate treatment, while maintaining quality of life.

REFERENCES

Al-Sukhun S, Hussain M. 2003. Molecular biology of transitional cell carcinoma. Crit Rev Oncol Hematol 47:181–193.

Baylin SB, Herman JG. 2000. DNA hypermethylation in tumorigenesis: Epigenetics joins genetics. Trends Genet 16:168–174.

Belinsky SA, Nikula KJ, Palmisano WA, Michels R, Saccomanno G, Gabrielson E, Baylin SB, Herman JG. 1998. Aberrant methylation of p16(INK4a) is an early event in lung cancer and a potential biomarker for early diagnosis. Proc Natl Acad Sci USA 95:11891–11896.

Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW. 1993. Genetic risk and carcinogen exposure: A common inherited defect of the carcinogen-metabolism gene glutathione *S*-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J Natl Cancer Inst 85:1159–1164.

Berman DM, Wang Y, Liu Z, Dong Q, Burke LA, Liotta LA, Fisher R, Wu X. 2004. A functional polymorphism in RGS6 modulates the risk of bladder cancer. Cancer Res 64:6820–6826.

Bornman DM, Mathew S, Alsruhe J, Herman JG, Gabrielson E. 2001. Methylation of the E-cadherin gene in bladder neoplasia and in normal urothelial epithelium from elderly individuals. Am J Pathol 159:831–835.

Brockmoller J, Cascorbi I, Kerb R, Roots I. 1996. Combined analysis of inherited polymorphisms in arylamine *N*-acetyltransferase 2, glutathione *S*-transferases M1 and T1, microsomal epoxide hydrolase, and cytochrome P450 enzymes as modulators of bladder cancer risk. Cancer Res 56:3915–3925.

Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Dvorak HF, Senger DR. 1993. Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. Am J Pathol 143:1255–1262.

Bubendorf L. 2001. High-throughput microarray technologies: From genomics to clinics. Eur Urol 40:231–238.

Cartwright RA, Glashan RW, Rogers HJ, Ahmad RA, Barham-Hall D, Higgins E, Kahn MA. 1982. Role of *N*-acetyltransferase phenotypes in bladder carcinogenesis: A pharmacogenetic epidemiological approach to bladder cancer. Lancet 2:842–845.

Chan MW, Chan LW, Tang NL, Tong JH, Lo KW, Lee TL, Cheung HY, Wong WS, Chan PS, Lai FM, To KF. 2002. Hypermethylation of multiple genes in tumor tissues and voided urine in urinary bladder cancer patients. Clin Cancer Res 8:464–470.

Chan MW, Chan LW, Tang NL, Lo KW, Tong JH, Chan AW, Cheung HY, Wong WS, Chan PS, Lai FM, To KF. 2003. Frequent hypermethylation of promoter region of RASSF1A in tumor tissues and voided urine of urinary bladder cancer patients. Int J Cancer 104 :611–616.

Chang LL, Yeh WT, Yang SY, Wu WJ, Huang CH. 2003. Genetic alterations of p16INK4a and p14ARF genes in human bladder cancer. J Urol 170:595–600.

Choi JY, Lee KM, Cho SH, Kim SW, Choi HY, Lee SY, Im HJ, Yoon KJ, Choi H, Choi I, Hirvonen A, Hayes RB, Kang D. 2003. CYP2E1 and NQO1 genotypes, smoking and bladder cancer. Pharmacogenetics 13:349–535.

Christensen M, Wolf H, Orntoft TF. 2000. Microsatellite alterations in urinary sediments from patients with cystitis and bladder cancer. Int J Cancer 85:614–617.

Cutler SJ, Heney NM, Friedell GH. 1982. Longitudinal study of patients with bladder cancer: Factors associated with disease recurrence and progression. In: Bonney WW, Prout GR, Jr., editors. Bladder cancer. Baltimore, MD: Williams & Wilkins. pp 35–46.

Dulaimi E, Uzzo RG, Greenberg RE, Al-Saleem T, Cairns P. 2004. Detection of bladder cancer in urine by a tumor suppressor gene hypermethylation panel. Clin Cancer Res 10:1887–1893.

Dyrskjot L. 2003. Classification of bladder cancer by microarray expression profiling: Towards a general clinical use of microarrays in cancer diagnostics. Expert Rev Mol Diagn 3:635–647.

Dyrskjot L, Thykjaer T, Kruhoffer M, Jensen JL, Marcussen N, Hamilton-Dutoit S, Wolf H, Orntoft TF. 2003. Identifying distinct classes of bladder carcinoma using microarrays. Nat Genet 33:90–96.

Dyrskjot L, Kruhoffer M, Thykjaer T, Marcussen N, Jensen JL, Moller K, Orntoft TF. 2004. Gene expression in the urinary bladder: A common carcinoma in situ gene expression signature exists disregarding histopathological classification. Cancer Res 64:4040–4048.

Espineda C, Seligson DB, James Ball W, Jr., Rao J, Palotie A, Horvath S, Huang Y, Shi T, Rajasekaran AK. 2003. Analysis of the Na, K-ATPase alpha- and beta-subunit expression profiles of bladder cancer using tissue microarrays. Cancer 97:1859–1868.

Fadl-Elmula I, Kytola S, Pan Y, Lui WO, Derienzo G, Forsberg L, Mandahl N, Gorunova L, Bergerheim US, Heim S, Larsson C. 2001. Characterization of chromosomal abnormalities in uroepithelial carcinomas by G-banding, spectral karyotyping and FISH analysis. Int J Cancer 92:824–831.

Falor WH, Ward-Skinner RM. 1988. The importance of marker chromosomes in superficial transitional cell carcinoma of the bladder: 50 patients followed up to 17 years. J Urol 139:929–932.

Grant DM, Tang BK, Kalow W. 1983. Polymorphic *N*-acetylation of a caffeine metabolite. Clin Pharmacol Ther 33:355–359.

Greenlee RT, Hill-Harmon MB, Murray T, Thun M. 2001. Cancer statistics, 2001. CA Cancer J Clin 51:15–36.

Habdous M, Siest G, Herbeth B, Vincent-Viry M, Visvikis S. 2004. Glutathione *S*-transferases genetic polymorphisms and human diseases: Overview of epidemiological studies. Ann Biol Clin (Paris) 62:15–24.

Habuchi T, Takahashi T, Kakinuma H, Wang L, Tsuchiya N, Satoh S, Akao T, Sato K, Ogawa O, Knowles MA, Kato T. 2001. Hypermethylation at 9q32-33 tumour suppressor region is age-related in normal urothelium and an early and frequent alteration in bladder cancer. Oncogene 20:531–537.

Hein DW. 1988. Acetylator genotype and arylamine-induced carcinogenesis. Biochim Biophys Acta 948:37–66.

Horikawa Y, Sugano K, Shigyo M, Yamamoto H, Nakazono M, Fujimoto H, Kanai Y, Hirohashi S, Kakizoe T, Habuchi T, Kato T. 2003. Hypermethylation of an E-cadherin (CDH1) promoter region in high grade transitional cell carcinoma of the bladder comprising carcinoma in situ. J Urol 169:1541–1545.

Ichimura Y, Habuchi T, Tsuchiya N, Wang L, Oyama C, Sato K, Nishiyama H, Ogawa O, Kato T. 2004. Increased risk of bladder cancer associated with a glutathione peroxidase 1 codon 198 variant. J Urol 172:728–732.

Jeong HJ, Kim HJ, Seo IY, Kim HJ, Oh GJ, Chae SC, Lim JS, Chung HT, Kim JJ. 2003. Association between glutathione *S*-transferase M1 and T1 polymorphisms and increased risk for bladder cancer in Korean smokers. Cancer Lett 202:193–199.

Jeong P, Kim EJ, Kim EG, Byun SS, Kim CS, Kim WJ. 2004. Association of bladder tumors and GA genotype of -308 nucleotide in tumor necrosis factor-alpha promoter with greater tumor necrosis factor-alpha expression. Urology 64:1052–1056.

Jones PA, Laird PW. 1999. Cancer epigenetics comes of age. Nat Genet 21:163–167.

Katoh T, Inatomi H, Kim H, Yang M, Matsumoto T, Kawamoto T. 1998. Effects of glutathione *S*-transferase (GST) M1 and GSTT1 genotypes on urothelial cancer risk. Cancer Lett 132:147–152.

Kelsey KT, Park S, Nelson HH, Karagas MR. 2004. A population-based case-control study of the XRCC1 Arg399Gln polymorphism and susceptibility to bladder cancer. Cancer Epidemiol Biomarkers Prev 13:1337–1341.

Kempkes M, Golka K, Reich S, Reckwitz T, Bolt HM. 1996. Glutathione *S*-transferase GSTM1 and GSTT1 null genotypes as potential risk factors for urothelial cancer of the bladder. Arch Toxicol 71:123–126.

Kim WJ, Lee HL, Lee SC, Kim YT, Kim H. 2000. Polymorphisms of *N*-acetyltransferase 2, glutathione *S*-transferase mu and theta genes as risk factors of bladder cancer in relation to asthma and tuberculosis. J Urol 164:209–213.

Kim WJ, Kim H, Kim CH, Lee MS, Oh BR, Lee HM, Katoh T. 2002. GSTT1-null genotype is a protective factor against bladder cancer. Urology 60:913–918.

Kim EJ, Jeong P, Quan C, Kim J, Bae SC, Yoon SJ, Kang JW, Lee SC, Wee JJ, Kim WJ. 2005. Genotypes of TNF-α,

VEGF, hOGG1, GSTM1 and GSTT1: Useful determinants for the Clinical Outcome of Bladder Cancer. Urology 68:70–75.

Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, Seeberg E, Lindahl T, Barnes DE. 1999. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc Natl Acad Sci USA 96:13300–13305.

Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP. 1998. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 4:844–847.

Koss LG. 1979. Mapping of the urinary bladder: Its impact on the concepts of bladder cancer. Hum Pathol 10:533–548.

Larsson PC, Beheshti B, Sampson HA, Jewett MA, Shipman R. 2001. Allelic deletion fingerprinting of urine cell sediments in bladder cancer. Mol Diagn 6:181–188.

Lee YS, Lee HS, Park MK, Hwang ES, Park EM, Kasai H, Chung MH. 1993. Identification of 8-hydroxyguanine glycosylase activity in mammalian tissues using 8-hydroxyguanine specific monoclonal antibody. Biochem Biophys Res Commun 196:1545–1551.

Lee MG, Kim HY, Byun DS, Lee SJ, Lee CH, Kim JI, Chang SG, Chi SG. 2001. Frequent epigenetic inactivation of RASSF1A in human bladder carcinoma. Cancer Res 61: 6688–6692.

Lothe RA, Peltomaki P, Meling GI, Aaltonen LA, Nystrom-Lahti M, Pylkkanen L, Heimdal K, Andersen TI, Moller P, Rognum TO, Fossa SD, Haldorsen T, Langmark F, Brogger A, de la Chapelle A, Borresen AL. 1993. Genomic instability in colorectal cancer: Relationship to clinicopathological variables and family history. Cancer Res 53: 5849–5852.

Maruyama R, Toyooka S, Toyooka KO, Harada K, Virmani AK, Zochbauer-Muller S, Farinas AJ, Vakar-Lopez F, Minna JD, Sagalowsky A, Czerniak B, Gazdar AF. 2001. Aberrant promoter methylation profile of bladder cancer and its relationship to clinicopathological features. Cancer Res 61:8659–8663.

Messing EM. 2002. Urothelial tumors of the urinary tract. In: Walsh PC, Retik AB, Vaughan ED, Wein AJ, editor. Campbell's urology 8th edn. Pennsylvania: Saunders. pp 2750–2751.

Morrison AS, Buring JE, Verhoek WG, Aoki K, Leck I, Ohno Y, Obata K. 1984. An international study of smoking and bladder cancer. J Urol 131:650–654.

Nebert DW. 1994. Drug-metabolizing enzymes in ligand-modulated transcription. Biochem Pharmacol 47:25–37.

Nocito A, Bubendorf L, Maria Tinner E, Suess K, Wagner U, Forster T, Kononen J, Fijan A, Bruderer J, Schmid U, Ackermann D, Maurer R, Alund G, Knonagel H, Rist M, Anabitarte M, Hering F, Hardmeier T, Schoenenberger AJ, Flury R, Jager P, Luc Fehr J, Schraml P, Moch H, Mihatsch MJ, Gasser T, Sauter G. 2001. Microarrays of bladder cancer tissue are highly representative of proliferation index and histological grade. J Pathol 194:349–357.

Nuovo GJ, Plaia TW, Belinsky SA, Baylin SB, Herman JG. 1999. In situ detection of the hypermethylation-induced inactivation of the p16 gene as an early event in oncogenesis. Proc Natl Acad Sci USA 96:12754–12759.

Olumi AF, Tsai YC, Nichols PW, Skinner DG, Cain DR, Bender LI, Jones PA. 1990. Allelic loss of chromosome 17p distinguishes high grade from low grade transitional cell carcinomas of the bladder. Cancer Res 50:7081–7083.

Orlow I, LaRue H, Osman I, Lacombe L, Moore L, Rabbani F, Meyer F, Fradet Y, Cordon-Cardo C. 1999. Deletions of the INK4A gene in superficial bladder tumors. Association with recurrence. Am J Pathol 155:105–113.

Peltomaki P, de la Chapelle A. 1997. Mutations predisposing to hereditary nonpolyposis colorectal cancer. Adv Cancer Res 71:93–119.

Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB. 1994. Human glutathione *S*-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 300:271–276.

Ponder BA. 2001. Cancer genetics. Nature 411:336–341.

Presti JC, Jr., Reuter VE, Galan T, Fair WR, Cordon-Cardo C. 1991. Molecular genetic alterations in superficial and locally advanced human bladder cancer. Cancer Res 51: 5405–5409.

Rao J, Seligson D, Visapaa H, Horvath S, Eeva M, Michel K, Pantuck A, Belldegrun A, Palotie A. 2002. Tissue microarray analysis of cytoskeletal actin-associated biomarkers gelsolin and E-cadherin in urothelial carcinoma. Cancer 95:1247–1257.

Razin A, Riggs AD. 1980. DNA methylation and gene function. Science 210:604–610.

Ribeiro-Filho LA, Franks J, Sasaki M, Shiina H, Li LC, Nojima D, Arap S, Carroll P, Enokida H, Nakagawa M, Yonezawa S, Dahiya R. 2002. CpG hypermethylation of promoter region and inactivation of E-cadherin gene in human bladder cancer. Mol Carcinog 34:187–198.

Richter J, Wagner U, Kononen J, Fijan A, Bruderer J, Schmid U, Ackermann D, Maurer R, Alund G, Knonagel H, Rist M, Wilber K, Anabitarte M, Hering F, Hardmeier T, Schonenberger A, Flury R, Jager P, Fehr JL, Schraml P, Moch H, Mihatsch MJ, Gasser T, Kallioniemi OP, Sauter G. 2000. High-throughput tissue microarray analysis of cyclin E gene amplification and overexpression in urinary bladder cancer. Am J Pathol 157:787–794.

Robertson KD. 2001. DNA methylation, methyltransferases, and cancer. Oncogene 20:3139–3155.

Salem C, Liang G, Tsai YC, Coulter J, Knowles MA, Feng AC, Groshen S, Nichols PW, Jones PA. 2000. Progressive increases in de novo methylation of CpG islands in bladder cancer. Cancer Res 60:2473–2476.

Sanchez-Carbayo M, Socci ND, Lozano JJ, Li W, Charytonowicz E, Belbin TJ, Prystowsky MB, Ortiz AR, Childs G, Cordon-Cardo C. 2003. Gene discovery in bladder cancer progression using cDNA microarrays. Am J Pathol 163:505–516.

Sandberg AA, Berger CS. 1994. Review of chromosome studies in urological tumors. II. Cytogenetics and molecular genetics of bladder cancer. J Urol 151:545–560.

Sandberg AA, Berger CS, Haddad FS, Kerr D, Hecht F. 1986. Chromosome change in transitional cell carcinoma of ureter. Cancer Genet Cytogenet 19:335–340.

Sardi I, Bartoletti R, Occhini I, Piazzini M, Travaglini F, Guazzelli R, Montali E. 1999. Microsatellite alterations in superficial and locally advanced transitional cell carcinoma of the bladder. Oncol Rep 6:901–905.

Sasaki M, Nakajima K, Perinchery G, Fujino T, Oh BR, Fujimoto S, Dahiya R. 2000. Frequent genotype changes at −308 of the human tumor necrosis factor-alpha promoter region in human uterine endometrial cancer. Oncol Rep 7:369–373.

Sathyanarayana UG, Maruyama R, Padar A, Suzuki M, Bondaruk J, Sagalowsky A, Minna JD, Frenkel EP, Grossman HB, Czerniak B, Gazdar AF. 2004. Molecular detection of noninvasive and invasive bladder tumor tissues and exfoliated cells by aberrant promoter methylation of laminin-5 encoding genes. Cancer Res 64:1425–1430.

Schraml P, Kononen J, Bubendorf L, Moch H, Bissig H, Nocito A, Mihatsch MJ, Kallioniemi OP, Sauter G. 1999. Tissue microarrays for gene amplification surveys in many different tumor types. Clin Cancer Res 5:1966–1975.

Seidegard J, Pero RW, Miller DG, Beattie EJ. 1986. A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer. Carcinogenesis 7: 751–753.

Seripa D, Parrella P, Gallucci M, Gravina C, Papa S, Fortunato P, Alcini A, Flammia G, Lazzari M, Fazio VM. 2001. Sensitive detection of transitional cell carcinoma of the bladder by microsatellite analysis of cells exfoliated in urine. Int J Cancer 95:364–369.

Shen M, Hung RJ, Brennan P, Malaveille C, Donato F, Placidi D, Carta A, Hautefeuille A, Boffetta P, Porru S. 2003. Polymorphisms of the DNA repair genes XRCC1, XRCC3, XPD, interaction with environmental exposures, and bladder cancer risk in a case-control study in northern Italy. Cancer Epidemiol Biomarkers Prev 12: 1234–1240.

Sinues B, Perez J, Bernal ML, Saenz MA, Lanuza J, Bartolome M. 1992. Urinary mutagenicity and *N*-acetylation phenotype in textile industry workers exposed to arylamines. Cancer Res 52 :4885–4889.

Soulitzis N, Sourvinos G, Dokianakis DN, Spandidos DA. 2002. p53 codon 72 polymorphism and its association with bladder cancer. Cancer Lett 179:175–183.

Stern MC, Johnson LR, Bell DA, Taylor JA. 2002a. XPD codon 751 polymorphism, metabolism genes, smoking, and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 11:1004–1011.

Stern MC, Umbach DM, Lunn RM, Taylor JA. 2002b. DNA repair gene XRCC3 codon 241 polymorphism, its interaction with smoking and XRCC1 polymorphisms, and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 11:939–943.

Tada Y, Wada M, Taguchi K, Mochida Y, Kinugawa N, Tsuneyoshi M, Naito S, Kuwano M. 2002. The association of death-associated protein kinase hypermethylation with early recurrence in superficial bladder cancers. Cancer Res 62:4048–4053.

Tchou J, Kasai H, Shibutani S, Chung MH, Laval J, Grollman AP, Nishimura S. 1991. 8-oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. Proc Natl Acad Sci USA 88:4690–4694.

Thibodeau SN, Bren G, Schaid D. 1993. Microsatellite instability in cancer of the proximal colon. Science 260:816–819.

Thykjaer T, Christensen M, Clark AB, Hansen LR, Kunkel TA, Orntoft TF. 2001. Functional analysis of the mismatch repair system in bladder cancer. Br J Cancer 85: 568–575.

Toruner GA, Akyerli C, Ucar A, Aki T, Atsu N, Ozen H, Tez M, Cetinkaya M, Ozcelik T. 2001. Polymorphisms of glutathione *S*-transferase genes (GSTM1, GSTP1 and GSTT1) and bladder cancer susceptibility in the Turkish population. Arch Toxicol 75:459–464.

Tsai YC, Nichols PW, Hiti AL, Williams Z, Skinner DG, Jones PA. 1990. Allelic losses of chromosomes 9, 11, and 17 in human bladder cancer. Cancer Res 50:44–47.

Uchida T, Wang C, Wada C, Iwamura M, Egawa S, Koshiba K. 1996. Microsatellite instability in transitional cell carcinoma of the urinary tract and its relationship to clinicopathological variables and smoking. Int J Cancer 69:142–145.

Valenzuela MT, Galisteo R, Zuluaga A, Villalobos M, Nunez MI, Oliver FJ, Ruiz de Almodovar JM. 2002. Assessing the use of p16 (INK4a) promoter gene methylation in serum for detection of bladder cancer. Eur Urol 42:622–630.

Wang L, Habuchi T, Takahashi T, Mitsumori K, Kamoto T, Kakehi Y, Kakinuma H, Sato K, Nakamura A, Ogawa O, Kato T. 2002. Cyclin D1 gene polymorphism is associated with an increased risk of urinary bladder cancer. Carcinogenesis 23:257–264.

Warzocha K, Ribeiro P, Bienvenu J, Roy P, Charlot C, Rigal D, Coiffier B, Salles G. 1998. Genetic polymorphisms in the tumor necrosis factor locus influence non-Hodgkin's lymphoma outcome. Blood 91:3574–3581.

Wong IH, Lo YM, Zhang J, Liew CT, Ng MH, Wong N, Lai PB, Lau WY, Hjelm NM, Johnson PJ. 1999. Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients. Cancer Res 59:71–73.

Yu MC, Skipper PL, Taghizadeh K, Tannenbaum SR, Chan KK, Henderson BE, Ross RK. 1994. Acetylator phenotype, aminobiphenyl-hemoglobin adduct levels, and bladder cancer risk in white, black, and Asian men in Los Angeles, California. J Natl Cancer Inst 86:712–716.

Zhang X, Ma X, Zhu QG, Li LC, Chen Z, Ye ZQ. 2003. Association between a C/A single nucleotide polymorphism of the E-cadherin gene promoter and transitional cell carcinoma of the bladder. J Urol 170:1379–1382.