



Sidi Bou Said, Tunisia, 1999. Courtesy of J. Bryan Murphy, MD, Clearwater, Florida.

A normal transitional cell acquires the malignant phenotype due to the interaction of tumor suppressor genes, oncogenes, growth factors, adhesion molecules, and angiogenic factors.

Molecular Mechanisms and Pathways in Bladder Cancer Development and Progression

Ichabod Jung, MD, and Edward Messing, MD

Background: *The basis for bladder cancer development and progression is complex and involves genetic abnormalities. These abnormalities yield phenotypic changes that allow normal transitional cells to become cancerous and finally acquire the "malignant phenotype."*

Methods: *The authors review the most common genetic alterations in bladder cancer and the molecular mechanisms and pathways involved in the conversion of normal transitional cell into malignant transitional cancer cells.*

Results: *There are several potential genetic changes of the urothelium that eventually cause bladder cancer initiation and tumor progression. Some of these alterations are also found in other malignancies suggesting that key common pathways exist in the development of cancer.*

Conclusions: *As the roles of certain genes or proteins are further elucidated, a better understanding of cancer development can aid in the prevention, diagnosis, and treatment of bladder cancer.*

Introduction

It is estimated that in 2000 in the United States, 53,200 new cases of bladder cancer (BC) will occur, and 12,200 patients will die of this disease.¹ The major-

ity of these new cases, approximately 75%, are limited to the mucosa (stage Ta or Tis) and lamina propria (T1) at presentation, and most of these tumors can be removed by transurethral resection. Recurrence rates are high (30% to 85%), and 10% to 30% of "superficial" tumors (T1 or less) will subsequently progress to muscle invasive disease (stages T2-T4), which has a poorer prognosis.² For the remaining 25%, the initial presentation involves muscle invasive disease that will usually relapse with metastases (as well as localized disease persistence) within a median of 2 years if managed only by transurethral resection and intravesical therapy.³ These data support the heterogeneous nature and malignant potential of transitional cell carcinoma

From the Department of Urology at the University of Rochester Medical Center, Rochester, New York.

Address reprint requests to Edward Messing, MD, University of Rochester Medical Center, 601 Elmwood Avenue, Box 656, MED 1-5336, Rochester, NY 14642. E-mail: edward_messing@urmc.rochester.edu

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(TCC), which represents more than 90% of the BCs that occur in the United States.

How a normal urothelial cell transforms to a malignant cell and then metastasizes is a complex process that involves the interaction of many different genes, proteins, and other molecules. Several areas of molecular research have contributed to our knowledge about the initiation and progression of BC. Loss of tumor suppressor gene function or induction of oncogenes can lead to unregulated cell growth and proliferation. Abnormal expression of growth factors, adhesion molecules, and angiogenic factors are important in the progression of BC. Due to the genomic instability of cancer cells, it has been difficult to identify those genetic, chromosomal, and transcriptional changes found in BC that are fundamental to the malignant process vs those that represent secondary or epigenetic aberrations. In general, identifying the former would be more useful in developing detection and preventive strategies, while the latter may be more valuable for prognostic purposes. Awareness of both is likely needed to develop effective therapeutic approaches.

Cancer Initiation and Promotion

Environmental Factors

Environmental chemicals are thought to play a significant role in BC initiation. Carcinogens derived from occupational exposures, cigarette smoking, and inflam-

matory conditions associated with long-term indwelling foley catheters and schistosomal infections are important factors in initiating BC. Furthermore, prior pelvic irradiation and cyclophosphamide exposure also appear to be important risk factors, possibly from direct mutagenesis. All of these conditions may lead to genetic changes, which irreversibly convert a normal urothelial cell to one with the malignant phenotype.

Cigarette Smoking: Tobacco use is a well-documented risk factor for developing BC, but specific carcinogen(s) and molecular pathway(s) have not been elucidated. Much interest has focused on aromatic amines such as 4-aminobiphenyl (ABP) because they are found not only in cigarette smoke but also in several industrial chemicals. One potential mechanism by which amines cause carcinogenesis is by forming DNA adducts that result in transitional mutations (Fig 1).⁴

Formation of these DNA adducts is affected by liver enzymes such as cytochrome p450 1A2 (CYP1A2), N-acetyltransferase 2 (NAT2), and glutathione S-transferase M1 (GSTM1). Some of these enzymes are also present in the urothelial cells. CYP1A2 is an inducible enzyme that demethylates aromatic amines and thereby increases DNA adduct formation.⁵ NAT2 is a major acetylating enzyme that detoxifies amines and thus decreases DNA adduct formation.⁶ These enzymes are polymorphic in the general population; that is, there are variants of these enzymes in a population with slightly different molecular structures and biologic activities. Studies have

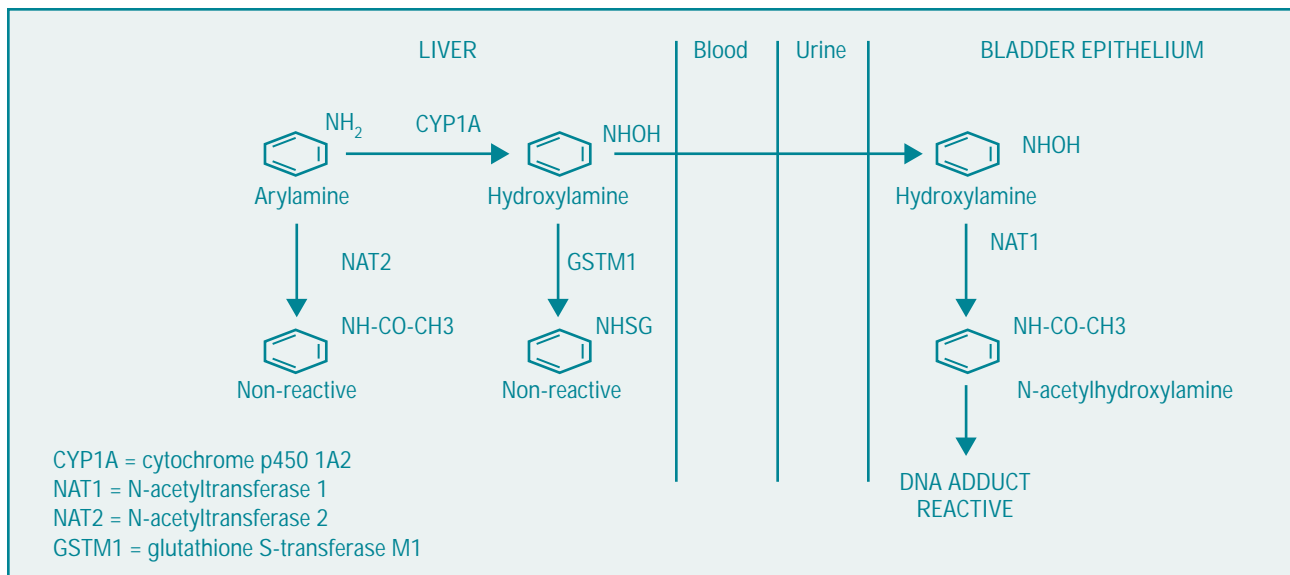


Fig 1. — Arylamine metabolism pathway for bladder carcinogenesis. Arylamines may be N-acetylated by NAT2, which is highly expressed in the liver, rendering them nonreactive. Alternatively, they may be N-hydroxylated in the liver by CYP1A2, transported to the bladder, and taken up by the bladder epithelium. There they may undergo O-acetylation by NAT1, which is highly expressed in the bladder, to form a highly reactive species. Alleles that lead to decreased NAT2 activity and those that lead to increased NAT1 activity would be expected to increase cancer risk from arylamine exposure. Adapted from *Toxicology Letters*, Kadlubar FF, Badawi AF. Genetic susceptibility and carcinogen-DNA adduct formation in human urinary bladder carcinogenesis. 1995;82-83:627-632. With permission from Elsevier Science.

been carried out to determine whether or not individual variation in amine-associated DNA adduct formation is due to a particular phenotype and correlates with risk. These studies indicate that cigarette smokers with slow NAT2/rapid CYP1A2 phenotypes were at higher risk for developing BC than those with rapid NAT2/slow CYP1A2 phenotypes.⁷ Similarly, smokers whose detoxifying enzyme, GSTM1, is homozygously deleted are at an 1.8-fold greater risk for developing BC than smokers with one or two copies.⁸

Occupational Exposure: Exposure to chemicals used in dye,⁹ rubber, and textile¹⁰ manufacturing have been estimated to be responsible for up to 20% of BC cases.¹¹ Most of these chemicals are aromatic amines that take several years to accumulate and thus account for the long latent periods before the development of BC. Aromatic amines from occupational exposures are activated and detoxified through the same reactions that aromatic amines in cigarette smoke are activated and detoxified. Hence, BC susceptibility depends on the cumulative expression profiles of these activating and detoxifying enzymes. It also means that exposures to occupational agents and cigarette smoke may be additive.

Schistosomiasis: Although rare in western countries, schistosomiasis is endemic in other parts of the world where it is a major cause of BC. *Schistosoma haematobium* cystitis appears to be causally related to the development of BC — both squamous cell carcinoma and TCC.¹² While the precise mechanism by which schistosomiasis causes BC is uncertain, recent attention has focused on nitrite and N-nitroso compounds (NNCs).¹³ NNCs can be formed endogenously following the secondary infection by nitrate-reducing bacteria that invariably accompany schistosomal cystitis. NNCs, including nitrosamines and the direct-acting nitrosamides, are carcinogenic, inducing tumorigenic alkylation of specific bases and DNA sequences.

Chronic Cystitis: Long-term indwelling Foley catheters or bladder calculi causing chronic cystitis also are associated with an increased risk for squamous cell carcinoma of the bladder.¹⁴ The exact mechanisms of carcinogenesis are not understood but are likely related to the repeated chronic irritation caused by a foreign object resulting in metaplasia, then dysplasia, and finally carcinoma. One theory relating chronic cystitis to BC, particularly squamous cell carcinoma, implicates nitric oxide and NNCs, whose levels are raised in chronic cystitis associated with chronic indwelling catheterization.¹⁵ Nitric oxide both deaminates 5-methylcytosine and itself is converted to nitrates. These are subsequently reduced by bacteria to nitrites that in turn stimulate endogenous formation of NNCs. NNCs are carcinogenic because they are able to alkylate DNA sequences.

Cyclophosphamide: Patients treated with cyclophosphamide have up to a ninefold increased risk of developing BC.¹⁶ Most of these tumors are TCCs and are high grade, rapidly growing, and muscle invasive at the time of diagnosis.¹⁷ The risk of developing BC appears to be directly related to the total cumulative dose of cyclophosphamide (greater than 50 g).¹⁸ Acrolein, a metabolite of cyclophosphamide, is known to be carcinogenic and is thought to be responsible for the induction of BC.¹⁹ The precise mechanism by which cyclophosphamide or its metabolites (acrolein or phosphoramidate mustard) induce BC remains to be elucidated. Mutations of the p53 gene have recently been found in 9 (43%) of 19 patients with cyclophosphamide-related bladder tumors,²⁰ but it is unknown if these changes have resulted directly from exposure to cyclophosphamide and its metabolites, if they actually cause BC induction or progression, or if they are simply associated with high-grade, high-stage BC.

Radiation Therapy: An increased risk for developing BC has been associated with radiation therapy to the pelvis for ovarian,²¹ cervical,²² and prostatic carcinomas.²³ BC tends to occur 5 to 10 years after radiation and is characteristically high grade and locally advanced at diagnosis.²⁴ The relative risk of secondary bladder malignancy ranges 1.5- to 4-fold and is likely proportional to the dose of radiation given. The precise mechanism by which radiation induces BC is unknown but is likely related to the generation of free radicals that cause direct DNA mutation of important regulatory genes (suppressor and oncogenes).

Hereditary Factors: Familial clustering of BC, especially of relatively young individuals, has provided support to the concept that there may be a genetic component involved in some BCs.²⁵ Furthermore, it has been shown that the risk of upper urinary tract but not bladder TCC is increased more than 10-fold in families with hereditary nonpolyposis colon cancer.²⁶ Kramer and associates,²⁷ in their study of New York State residents, and Goldgar et al,²⁸ in their study of Utah residents, determined relative risks in first-degree relatives of BC patients of 1.9 and 1.5, respectively. In contrast, Kiemeny et al²⁹ in a study of Icelanders found an increased risk in first-degree relatives but found an even greater risk for second- and third-degree relatives, placing into serious question the direct inheritance of "BC predisposition" genes.

Gender, Race, and Age

Men are approximately three times more likely than women to develop BC. This gender difference in risk exists even after accounting for differences in cigarette smoking and occupational exposure to environ-

mental carcinogens. Men also have traditionally had increased exposures to putative BC carcinogens found in the workplace and in cigarette smoke. However, a variety of social trends over the past quarter century in western countries would predict a relative rise of BC incidence in women, which has not been seen.¹ One hypothesis for the gender difference in BC risk is the difference in CYP1A2 activity.³⁰ Horn et al³⁰ observed elevated CYP1A2 activity as determined by the caffeine breath test in men compared with women, although this increase was not statistically significant. Interestingly, they found a statistically significant difference between parous and nulliparous women ($P=0.03$). Men and parous women had similar caffeine breath test values. Nulliparous women, however, had lower values, suggesting hormones such as progesterone may influence CYP1A2 activity.

There is marked racial-ethnic variation in BC incidence. In the United States, whites have a 37% greater risk than blacks of developing BC. Conversely, localized disease is found in 72% of whites compared to only 50% of blacks. Americans of Asian descent have rates roughly 40% those of whites. These differences cannot be strictly attributed to differences in cigarette smoking or environmental exposure to chemicals. In a study of Los Angeles men,³¹ blacks had a higher smoking prevalence than whites or Asian Americans who had similar smoking rates. Nevertheless, white men

have a BC rate twice that of blacks and nearly 2.5 times that of Asian Americans.³² A possible explanation for this phenomenon is the difference in the acetylator phenotypes (NAT2) in these racial-ethnic groups.³³ The prevalences of the slow acetylator phenotype among these groups were predicted by their incidences of BC (54% of whites were slow acetylators vs 34% of blacks vs 14% of Asians). Slow acetylators exhibited higher mean levels of ABP-hemoglobin adducts than rapid acetylators, regardless of race and cigarette smoking status.

BC is a disease of the elderly. Roughly 80% of newly diagnosed TCCs occur in people 60 years of age and over, with an increasing incidence in both gender and all races with aging.³ The reasons for this remain unclear, but factors that presumably play a role in this phenomenon include the cumulative effects of a lifetime of exposures to carcinogens and procarcinogens, the relative failings of DNA repair mechanisms, yet uncertain immune mechanisms, and perhaps local factors such as urinary retention. Whatever the explanations, BC is the second most prevalent cancer in men 60 years of age and older in the United States. Since the elderly comprise the fastest-growing segment of all western societies, it is likely that without significant efforts at prevention and/or avoidance (from known carcinogenic exposures), the incidence of BC will probably continue to rise.

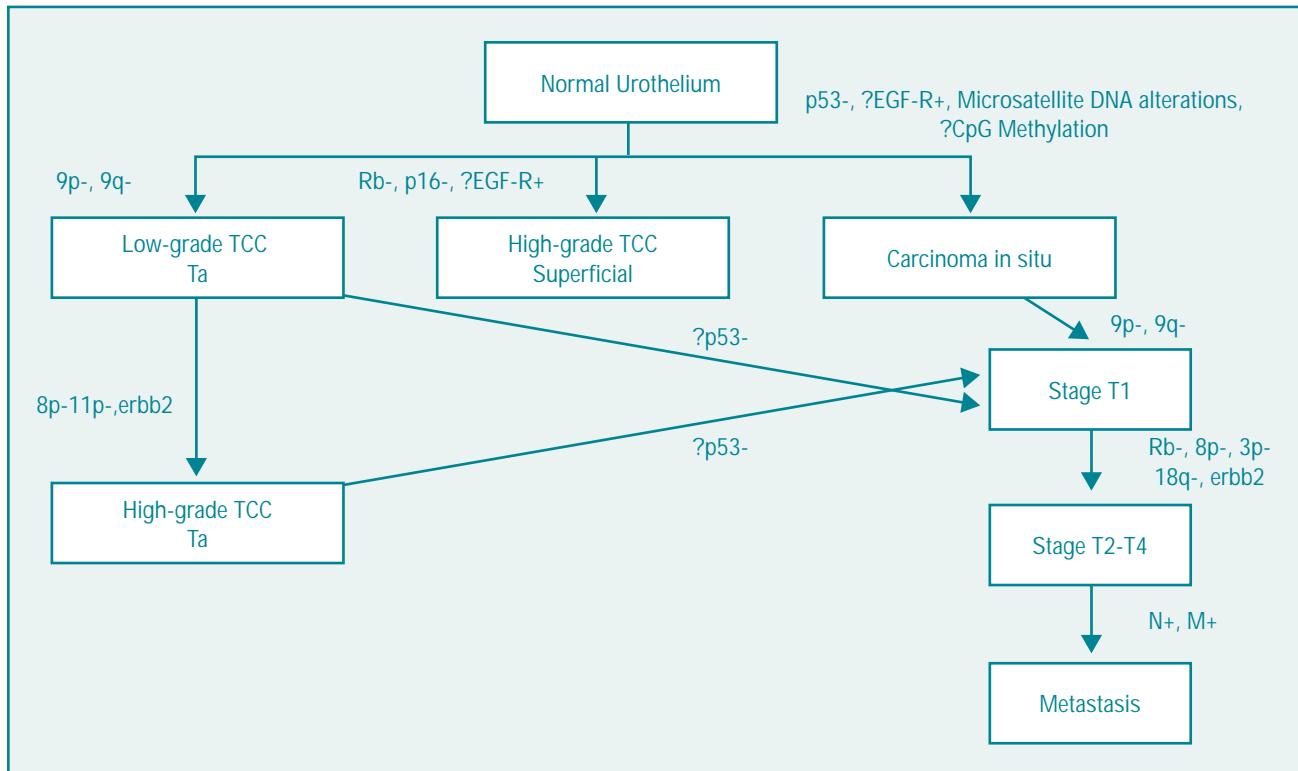


Fig 2. — Model for TCC development and progression. Common genetic alterations clinically correlate with specific stage and grade. From Knowles MA. Molecular genetics of bladder cancer. *Br J Urol.* 1995;75:57-66. Adapted with permission.

Cancer Development

Tumor Suppressor Genes

Studies on loss of heterozygosity using polymorphic markers (ie, for a given gene, an individual inherits a maternal allele and a paternal allele that are not identical) have identified specific allelic deletions in many BCs that are not present in the DNA from normal tissues.³⁴ Deletions or inactivation of specific genes in these genetic regions may lead to cancer development (Fig 2). Many of these deleted regions are common to a variety of neoplasms, implicating a fundamental role in malignant transformation and progression. Usually, such genes encode proteins that regulate cell division, direct cells to programmed death (apoptosis), and/or correct or guard against the propagation of genetic mutations. The retinoblastoma (Rb) gene on chromosome 13 (13q) and the p53 gene on chromosome 17 (17p) are the best studied tumor suppressor genes. They play an important role in the progression of BC³⁵ and possibly its development. Additionally, since deletions on chromosome 9 are found in more than 60% of BCs,³⁶ there is strong evidence that at least two BC suppressor genes are present on that chromosome. While other putative suppressor genes have been identified, they have not been as well studied, and their role in clinical prognostication has yet to be determined.

Retinoblastoma Tumor Suppressor Gene: The Rb gene encodes a nuclear phosphoprotein (pRb) that functions as a cell cycle regulator. Unphosphorylated pRb negatively regulates E2F, a protein transcription factor, by binding with it. When pRb is phosphorylated by the cyclin/CDK complex, the transcription factor E2F-1 is released and switches on genes (eg, thymidine synthetase) whose products drive cells into the DNA synthesis (S) phase of the cell cycle. Normal cells express the Rb protein, while mutations or gene deletions, which often result in lack of protein expression, may be identified by the lack of Rb expression. Loss of heterozygosity at the Rb locus associates strongly with the absence of Rb protein expression. Rb gene mutations are seen in approximately 30% of BCs.³⁷ Inability to detect pRb immunohistochemically is associated with increased tumor grade and stage, especially muscle invasion.³⁸ Additionally, since a phosphorylated pRb cannot bind E2F-1, hyperphosphorylated pRb, which can be identified immunohistochemically as intense nuclear staining, carries with it a similarly ominous prognosis as absence of Rb staining.^{39,40}

The p53 Tumor Suppressor Gene: The p53 gene encodes a 53kDa transcription factor with a critical role in DNA repair and apoptosis. Mutated p53 protein has a much longer half-life than wild-type p53, thus

allowing its detection by immunohistochemistry. Approximately 50% of muscle-invasive TCCs show nuclear overexpression of p53 indicating presence of a mutated protein. This is associated with increased stage and grade.⁴¹ It appears that in superficially invasive disease (T1), although p53 mutant expression occurs less frequently, it is associated with poorer outcome and a higher rate of disease progression. Altered p53 status is also a predictor of decreased survival. In muscle-invasive TCC, it has been associated with a doubling in the risk of death from BC.⁴² Furthermore, altered p53 is frequently associated with carcinoma in situ and its poorer outlook, thus supporting the argument that p53 has a role in a path to BC development and progression in high-grade TCCs.

Although it appears that altered p53 status is associated with a poorer prognosis,⁴² the practical clinical implications need to be further elucidated. For example, it is uncertain whether p53 status is helpful in predicting responses to chemotherapy or surgery. Sarkis et al⁴³ showed that altered p53 was an independent prognostic marker for survival and an indicator of treatment failure in patients with invasive BC treated with neoadjuvant M-VAC (methotrexate, vinblastine, doxorubicin, and cisplatin) therapy. Conversely, Cote and coworkers⁴⁴ found that the only group of patients with local and regionally extensive TCC who benefited from cisplatin-based adjuvant chemotherapy had abnormal p53 expression. Moreover, Glick et al⁴⁵ could not predict by p53 status which patients would eventually suffer recurrence or die after cystectomy and thus could not stratify patients to those who would benefit from a cystectomy. Further confounding the widespread adoption of p53 status as a determinant of treatment is disagreement about how many tumor cells need to display nuclear overexpression of p53 to have prognostic utility. Similarly, technical factors including antibodies used to detect p53, means of tissue preservation (eg, formalin fixed or frozen), and methods of enhancing antigen detection and quantification have differed from study to study.

Chromosome 9: Deletions on chromosome 9 not only appear to occur in greater than 60% of BC across all grades and stages, but also are likely an initiating event.³⁶ Cytogenetic and molecular evidence has shown that it is often the only chromosomal aberration in early disease. Deletions of chromosome 9 alone, detected as monosomy on image analysis, are seldom associated with progression but are often associated with recurrence. Indeed, at least one of the regions on the long arm of chromosome 9 (9q) is deleted primarily in low-grade superficial TCCs, which suggests a different molecular pathway in urothelial tumorigenesis than that which occurs with p53 or pRb inactivation.

The p21 region of chromosome 9 (9p21) has been found to be mutated in a variety of malignancies suggesting the presence of a common tumor suppressor gene.⁴⁶ Evidence points to the CDKN2 or p16 locus as the gene since it encodes a cyclin-dependent kinase inhibitor⁴⁷ that prevents the phosphorylation of Rb, thereby maintaining an active Rb and blocking the exit from the G₁ phase of the cell cycle. Loss of function of p16, by permitting Rb phosphorylation, results in unregulated cell growth as the cell is able to escape into S phase. It has been shown that transfecting wild-type p16 into BC cell lines that are deficient in p16 produces growth arrest, thus supporting its role as a tumor suppressor gene.⁴⁸

One potential mechanism by which p16 or CDKN2 function is lost is by gene silencing via methylation of the promoter region of p16.⁴⁹ Interestingly, another cycle regulatory gene, p14, is also located on chromosome 9p21 and shares part of the same coding region as p16, in the INK4a locus. Because of an alternative reading frame, p14 encodes for a different protein, which when overexpressed leads to cell cycle arrest in both G₁ and G₂ phases.⁵⁰ Gonzalgo et al⁴⁹ have shown that the formation of the p16 transcript, but not p14, can be stopped in BC cell lines by methylation. This methylation appears reversible as the p16 gene can be transcribed after adding a demethylating agent, 5-aza-2-deoxycytidine. Methylation of the p16 promoter region may play an important role in the loss of CDKN2 function and subsequently may allow cell growth and transformation by inactivating (phosphorylating) pRb. Interestingly, p14 appears to exert its effect primarily on p53 by blocking Mdm2-mediated degradation of the p53 protein⁵¹ (thus allowing intact p53 to slow uninhibited cell growth and send cells with altered DNA towards apoptosis).

There are two regions on 9q (9q11-13 and 9q33-34) that also are selectively deleted in a significant proportion of BCs. The functional proteins from these two regions remain to be identified, as do the specific locations of these two putative tumor suppressor genes. However, their deletion is more commonly associated with low-grade cancers.

Microsatellite Instability: Within the human genome are repetitive sequences of DNA — usually 1 to 4 bases long — that in many types of cancers, including BC, are lost. This phenomena is known as microsatellite instability. Microsatellite DNA sequences vary from individual to individual but, being inherited, are identical in all of an individual's cells. However, within cancer cells, there are often variations in many of the sequences caused by errors in DNA replication. Since microsatellite DNA repeats are almost exclusively

found within introns (noncoding segments of DNA), it is uncertain how these DNA replicative errors generate mutations that provide a survival advantage resulting in clonal expansion. What may be more important is that microsatellite instability represents DNA replication errors that also occur in exons, the coding segments of DNA (which go unrecognized in standard microsatellite stability analyses because of the paucity of microsatellite repeats in exons) are expressed as mutated tumor suppressor genes or oncogenes leading to tumor growth and progression.

Microsatellite instability has been advocated as a means to detect BC. Mao et al⁵² identified microsatellite instability in urine sediments from 19 of 20 patients who were diagnosed with BC. In all of the 15 patients who underwent tumor biopsy, the microsatellite alterations detected in the urine corresponded to those in the primary tumor. Steiner et al⁵³ correctly diagnosed 20 of 21 patients being followed for BC recurrence (n = 11 recurrence; n = 10 no recurrence) using microsatellite analysis with 20 markers in a blinded fashion. Mourah and colleagues⁵⁴ reported similar results by detecting microsatellite instability in 10 of 12 patients with BC. While these results are promising, assessment of microsatellite analyses in a larger study, particularly including many subjects without BC, are needed before this technique can be used for clinical diagnosis and early detection efforts. Of additional concern is the unknown impact of environmental agents on microsatellite repeats in individuals who do not develop cancer.

Oncogenes

Oncogenes may contribute to transformation and progression by being either overexpressed (eg, *c-erb-B2*) or mutated to produce an oncoprotein (eg, *c-H-ras*). One of the more important mechanisms by which oncogenes are overexpressed in BC is through gene amplification (multiple copies of the gene).

c-erb-B2 (HER-2/neu)

The proto-oncogene *c-erb-B2* encodes a transmembrane receptor-like protein similar to the epidermal growth factor receptor (EGFR). In a subset of TCCs, *c-erb-B2* is overexpressed; this appears to be due to gene amplification. The overexpressed *c-erb-B2* has tyrosine kinase activity similar to that of the activated EGFR and the ability to stimulate cellular growth. Although some investigators have shown a relationship with high grade and/or stage in tumors that overexpressed *c-erb-B2*,⁵⁵ others found no such correlation.⁵⁶ In view of these conflicting results, fur-

ther evaluation is required to determine the prognostic value of detecting *c-erb-B2* in BC. Nevertheless, *c-erb-B2* illustrates a potential mechanism by which oncogenes contribute to malignant transformation. Additionally, BCs that overexpress this membrane-bound oncoprotein may be the target for immunological therapeutic strategies that recognize it.

H-ras

H-ras, which codes for a protein anchored to the cytoplasmic side of the cell membrane that is involved in signal transduction, may play a role in BC genesis. *H-ras* mutations, as detected by polymerase chain reaction (PCR) and oligonucleotide-specific hybridization, have been found in up to 36% of bladder tumors.⁵⁷ Most mutations are single-point mutations involving codon 12 (guanine to adenine) but other mutations have been described involving codon 13 (guanine to thymine) and codon 61 (adenine to thymine). Fontana et al⁵⁸ have described a relationship between *H-ras* overexpression and early recurrence in superficial BC. Currently, detection of *H-ras* mutations by PCR is limited to the research setting but may prove fruitful as a prognostic marker.

Cancer Progression

BC cells require the acquisition of certain properties prior to being able to grow rapidly, invade, and metastasize. These properties include uncontrolled growth and cellular mobility, mediated at least in part via EGF and EGFRs, expression or loss of expression of specific cell adhesion molecules, and overproduction of angiogenic factors.

EGF and EGFRs

Abnormal expression or function of receptors for growth factors can enhance the proliferative capacity of malignant cells. EGF is a potent mitogen excreted in high concentrations in human urine in a biologically active form⁵⁹ but is found in lower concentrations in urine of BC patients.⁶⁰ This may reflect increased ligand binding as EGFRs, which are normally found only in the basal layer of the urothelium, become abnormally expressed throughout the entire urothelium in TCC, including those superficial cells directly exposed to urine. EGFR expression as detected by immunohistochemistry correlates with increased grade and stage and with poor prognosis.⁶¹ Abnormal EGFR expression is also found in cystoscopically normal-appearing urothelium in patients with TCCs elsewhere in the bladder,⁶² thus supporting the notions of the existence of an urothelial field defect⁶³ and that abnormal EGFR

expression may be an early event in BC tumorigenesis. Additionally, because ligands that work through EGFRs not only induce mitogenesis but also cellular motility, stimulation of EGFRs in malignant urothelium may encourage transepithelial motility and tumor invasion as well as proliferation.⁶⁴

Extracellular Matrix and Cell Adhesion Molecules

Extracellular matrix and cell adhesion molecules have been extensively studied because they form natural barriers to tumor metastases. Since cell adhesion molecules and other components of the extracellular matrix bind urothelial cells both to each other and to the underlying basal lamina, such connections have to be altered to permit some semblance of cellular disaggregation and local motility. Furthermore, many of these molecules are involved in cell-cell communication, often regulate the expression and function of membrane bound growth factor receptors, and can influence expressions and activities of molecules that regulate the cell cycle and other processes pertinent to urothelial carcinogenesis (eg, angiogenesis).

E-Cadherin

The cadherins comprise a family of transmembrane glycoproteins involved in calcium-dependent cell-cell adhesion. Reduced expression of an epithelial subclass (E-cadherin) has been associated with increased tumor recurrence and invasiveness⁶⁵ and decreased overall survival of BC patients.⁶⁶ Loss of cadherin-associated molecules (alpha, beta, and gamma catenins) has also been shown to be associated with advanced tumor grade and stage. In addition, the presence of multiple abnormalities in the E-cadherin-catenin complex has been correlated with advanced tumor grade and stage and with poor survival of patients with BC.⁶⁷

Possible mechanisms accounting for the decreased function of E-cadherin in BC cells have been investigated. These include suppression or mutation of the E-cadherin gene, allelic loss of 16q, decreased E-cadherin protein expression, and increased protease-mediated degradation. However, the mechanisms by which expression of E-cadherin is decreased still are unknown.

Integrins

The integrins are a family of transmembrane heterodimeric proteins that function as receptors for each other and for components of the extracellular matrix, such as laminin, fibronectin, and collagen. The alpha-6 beta-4 integrin normally associates with collagen VII on

the urothelium's basement membrane forming a hemidesmosomal anchoring complex, an effective barrier to cell migration. The association of alpha-6 beta-4 integrin with collagen VII is lost in BC cells, perhaps explaining defects in the urothelial barrier function that occur with malignancy.⁶⁸ These events may be particularly important if disruption of the structure or function of integrins also enhances the ability of intraluminal urinary constituents with angiogenic, locomotive, mitogenic, or mutagenic properties to gain unusual access to the interstices of the bladder wall.

Angiogenesis

Tumor growth and metastasis require neovascularization or angiogenesis.⁶⁹ This may be achieved either by secreting angiogenesis inducers such as vascular endothelial growth factor (VEGF) or by secreting substances that activate inducers such as basic fibroblast growth factor (b-FGF). These factors may be produced by the tumor cells or released by the surrounding extracellular matrix or tumor associated stromal cells, or they may be products of inflammatory cells that infiltrate tumors.⁷⁰ Using immunohistochemistry, increased microvascular density as a surrogate for angiogenesis has been found to be associated with tumor progression and decreased overall survival in BC patients.⁷¹ Furthermore, increased urinary excretion of b-FGF,⁷⁰ angiogenic factors such as autocrine motility factor (AMF),⁷² AMF receptor,⁷³ and hyaluronic acid and its degradation products⁷⁴ have been found in BC patients and have been proposed as means of detection and tumor monitoring.

There also appears to be potent inhibitors of angiogenesis that are expressed in abnormally low levels in BC compared with normal urothelium. Thrombospondin-1 is an extracellular matrix glycoprotein whose decreased expression in the BC patients' bladders has been correlated with higher recurrence rates and decreased survival compared with patients whose bladders have moderate or high expression.⁷⁵

Conclusions

The molecular mechanisms of BC development and progression are complicated but likely involve the interaction of tumor suppressor genes, oncogenes, growth factors, adhesion molecules, and angiogenic factors that lead a normal transitional cell to acquire the malignant phenotype. BC initiation and development involve an initial insult causing genetic derangements that often leads to either a negation of tumor suppressor genes or an induction of oncogenes. BC progression relies on the transformed cell acquiring the prop-

erties needed to induce further growth (growth and angiogenic factors), invade through the lamina propria (cell adhesion molecules, motility factors), and establish metastatic deposits (all of these). However, further research is needed to better understand these mechanisms and pathways and thereby prevent and clinically alter the diagnosis and treatment of patients with bladder cancer.

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