# Focus on bladder cancer

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## Introduction

The specialized lining of the urinary tract, the urothelium, which extends from the renal pelvis to the urethra, is a source of the 5th most common cancer, responsible for approximately 3% of all cancer-related deaths in the United States (Jemal et al., 2004). In the Western world, cigarette smoking is the most important risk factor, contributing to approximately 50% of all bladder cancers, followed by petrochemical and other industrial exposures. Bladder stones, chronic indwelling Foley catheters (as is common in patients with spinal cord injuries), schistosomiasis (which is especially problematic in Egypt), and chemical irritations are well-documented risk factors. Chronic use of cyclophosphamide, and the use of high-dose alkylator therapy, has led to increased recognition of cancers secondary to the toxic catabolite acrolein. Urothelial cancer is unique among noncutaneous carcinomas in that it is the only common epithelial neoplasm that *usually* presents at a superficial stage which can be readily examined visually and cytologically for diagnostic and followup studies. The dual-track concept postulates that urothelial cancers arise via two distinct but somewhat overlapping pathways: papillary and nonpapillary (Figure 1, Czerniak and Herz, 1995). 80% to 85% of urothelial cancers are exophytic papillary lesions, which arise from hyperplastic epithelium. These tumors tend to recur and only rarely evolve into a higher-grade, invasive cancer. The balance are nonpapillary and invasive at diagnosis, and arise from severe dysplasia or carcinoma

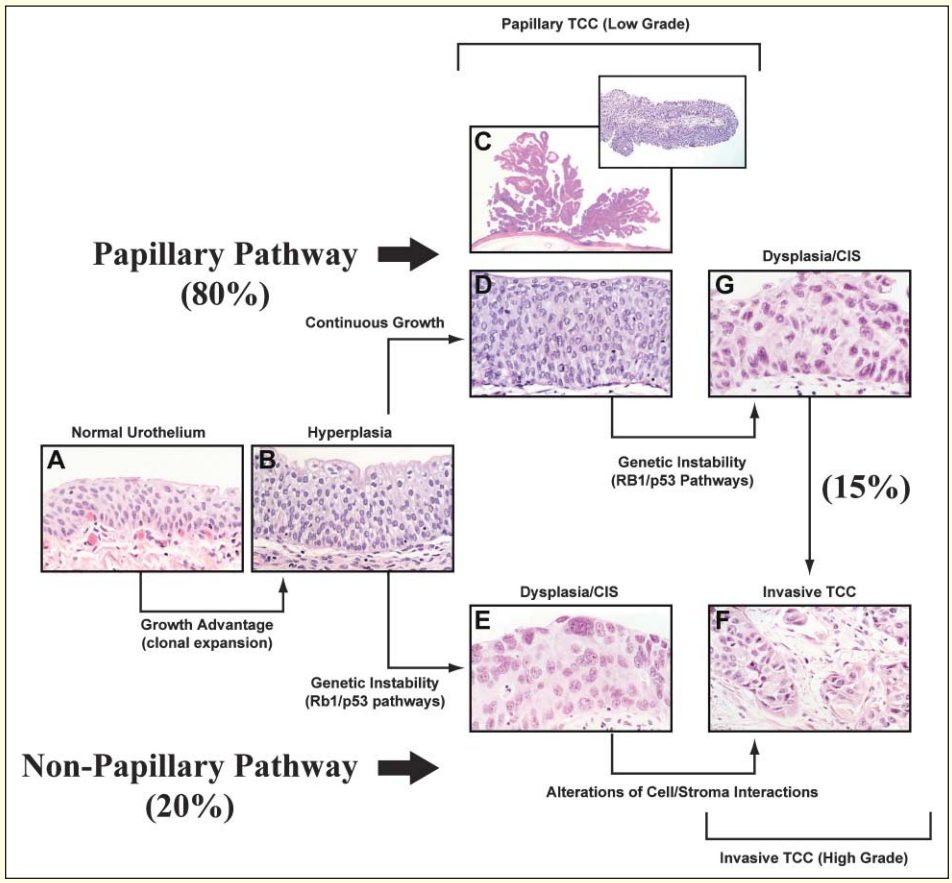


**Figure 1.** Dual tract concept of human bladder carcinogenesis

The expansion of a preneoplastic clone, which shows minimal phenotypic deviation from normal urothelium, is an incipient event of bladder carcinogenesis. The continuous growth of such clone results in the development of low-grade superficial papillary tumors. In a nonpapillary pathway, a successive subclone of the initial hyperplasia develops genetic instability with frequent loss of major tumor suppressors (RB and p53).
**A:** Normal urothelium.
**B:** Urothelial hyperplasia adjacent to a low-grade superficial papillary TCC.
**C and D:** Low-grade superficial tumor with features of hyperplasia.
**E:** Flat severe intraurothelial dysplasia/carcinoma in situ adjacent to a high-grade invasive nonpapillary carcinoma shown in **F**.
**F:** High-grade invasive nonpapillary carcinoma.
**G:** Flat severe intraurothelial dysplasia/carcinoma in situ developing in bladder mucosa adjacent to a low-grade papillary tumor. It is responsible for switching the pathway and progression of some low-grade papillary tumors to high-grade invasive cancers.

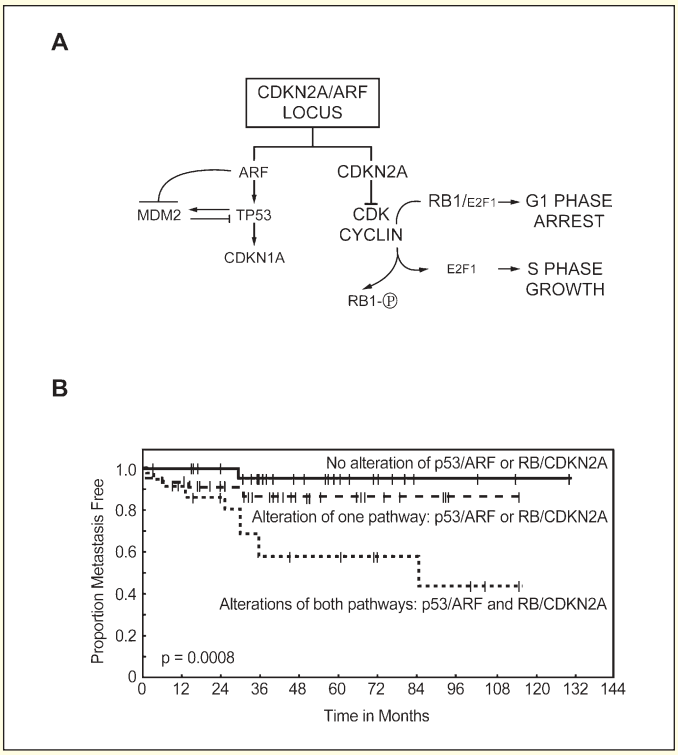


**Figure 2.** Molecular mechanisms of bladder carcinogenesis

**A:** Alterations of p53 and RB pathways that may directly or indirectly involve p53 and RB genes by loss of tandemly linked CDKN2a and ARF genes play a major role in bladder carcinogenesis. They are typically seen in a large proportion of clinically aggressive high-grade nonpapillary cancers. (From Mark and Jones, 1998. Used with permission.)

**B:** Kaplan-Meier analysis of metastasis-free survival of patients with bladder cancer (n = 181) in relation to the inactivation of RB and p53 pathways. The inactivation of RB and p53 pathways has a synergistic effect on metastasis-free survival. (B.C. and W.F.B., unpublished data.)

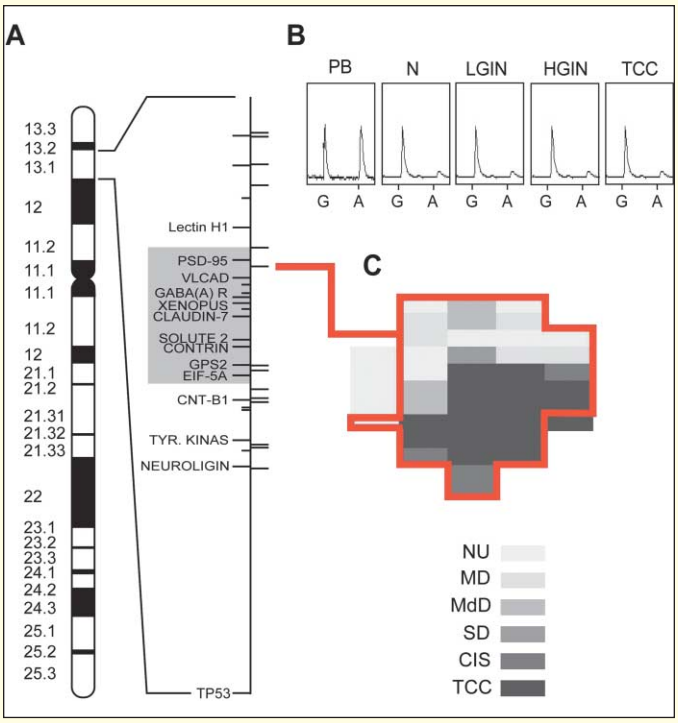


**Figure 3.** An example of chromosomal region associated with clonal expansion of intraurothelial preneoplasia identified by whole organ mapping with SNPs

**A:** Genome sequence map of 2.5 Mb segment telomeric to p53 is shown. The bars on the left side of the map depict known (long bars) and predicted (short bars) genes. The bars on the right side of the map depict all informative SNPs identified in the peripheral blood of the same patient. The chromosomal region subjected to fine mapping is depicted in the chromosomal diagram on the left side of the gene sequence map. An area of allelic loss associated with clonal expansion of preneoplasia is depicted as a shadowed bar defined by testing of SNPs with loss of polymorphism in the entire bladder mucosa.

**B:** An example of informative SNP showing loss of G/A polymorphism in mucosal samples corresponding to normal urothelium (NU), low-grade dysplasia (LGIN), high-grade dysplasia (HGIN), and invasive bladder cancer (TCC).

**C:** Histologic map of cystectomy showing the distribution of preneoplastic in situ lesions and invasive cancer. The distribution of allelic loss in the bladder mucosa is outlined by the continuous red line and depicts a plaque of clonally expanding preneoplastic urothelial cells in association with a loss of approximately 0.1 Mb segment outlined by a shadowed area in **A**. This segment contains nine known and three predicted candidate genes that may provide growth advantage and drive clonal expansion of a preneoplastic clone. (B.C. and T.T., unpublished data.)

in situ (CIS). The vast majority of invasive bladder cancers occur in patients without a prior history of papillary tumors.

## Molecular genetics

Virtually every newly discovered transforming and tumor suppressor gene has been studied in urothelial cancer (Cordon-Cardo et al., 1995; Czerniak and Herz, 1995). The human *ras* genes represent an important prototypical family of cellular transforming genes originally identified in the T24 human urothelial cancer cell line (Reddy et al., 1982). Two mechanisms for *ras* gene transformation have been discovered. The most frequent is mutation of codons 12, 13, 59, or 61, which affect the enzymatic activity of the protein. In the second mechanism, internal splicing within the last intron mediates *ras* gene expression. Mutations of the coding sequence of the H-*ras* gene, especially at codon 12, are relatively frequent, appearing in approximately 30%–40% of urothelial malignancies (Czerniak et al., 1992). Though concurrent mutations within the splicing mechanism and the coding sequence resulting in the overexpression of the transforming gene product were found exclusively in high-grade and high-stage tumors, they could be documented in less than 10% of urothelial tumors. The exact role of the *ras* genes and their significance as diagnostic markers of urinary bladder carcinoma are still unclear.

Experimental data and numerous clinical studies indicate that alterations of both the p53 and RB gene pathways (Shariat et al., 2004) play a major role in human bladder carcinogenesis (Figure 2). The most frequent alteration involves exons 5–11 of p53 and the loss of RB gene function (Mark and Jones, 1998). This mechanism was identified in approximately 60% of the human urinary bladder carcinoma cell lines tested. The second, less frequent mechanism, observed in approximately 20% of the cell lines, represents an independent synchronous loss of the tandemly linked CDKN2a (p16ink4a) and ARF (p19ink4b) in a single step that inactivates the 9p21 locus and results in transformation and immortalization of urothelial cells, presumably by indirectly impairing the p53 and RB pathways. A third mechanism, which was also observed in approximately 20% of cell lines, involved alterations of

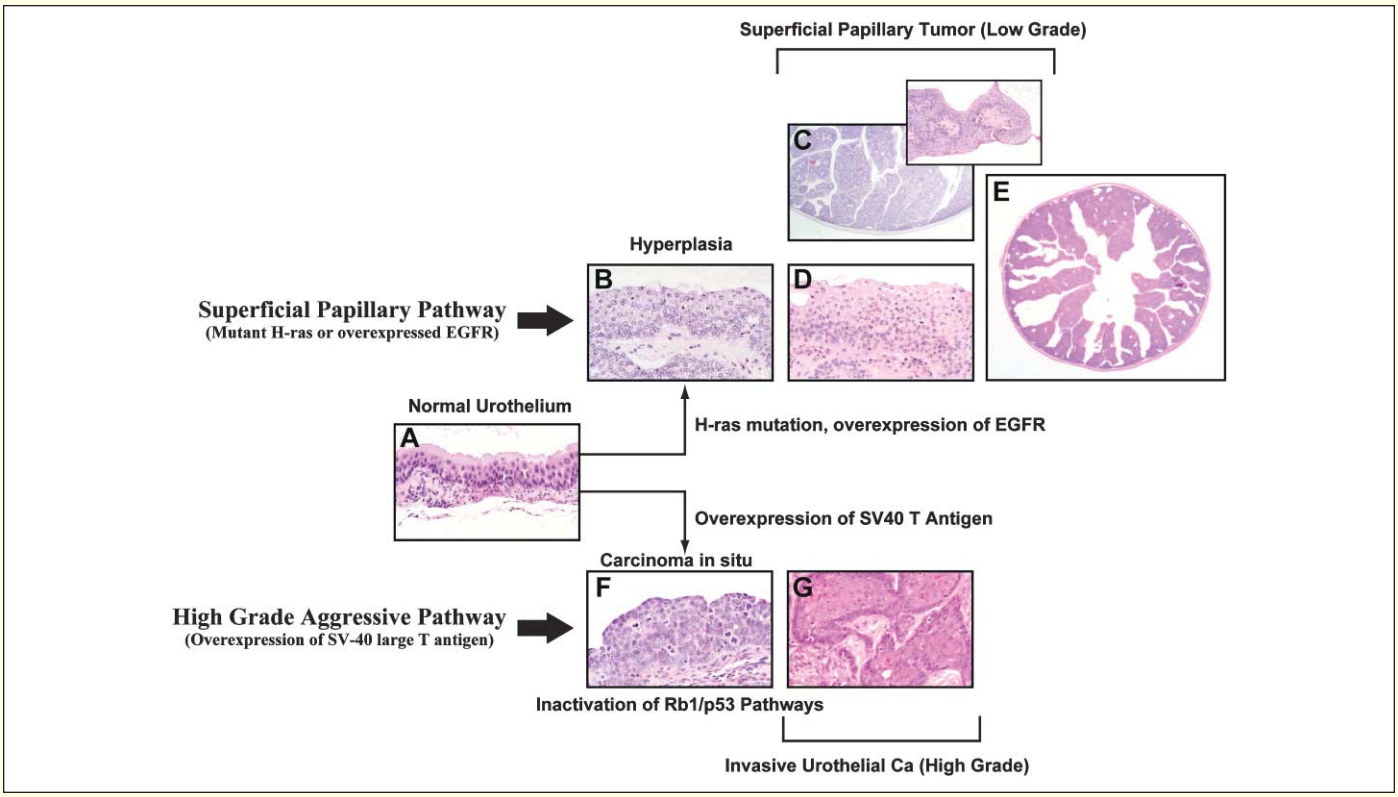


**Figure 4.** A mouse model of urothelial carcinogenesis recapitulating dual tract pathogenesis of human bladder cancer

**A:** Normal mouse urothelium.
**B:** The uroplakin promotor was used to drive expression of mutant ras or EGFR, which resulted in urothelial hyperplasia and development of superficial papillary tumor of low histologic grade.
**C and D:** Low-grade superficial papillary tumor with features of urothelial hyperplasia developed after overexpression of mutant H-ras.
**E:** Whole-mount histologic preparation of mouse bladder showing diffuse involvement of the mucosa by a superficial low-grade papillary tumor.
**F and G:** In contrast, the uroplakin driven expression of SV 40 large T antigen simultaneously inactivating p53 and RB pathways resulted in the development of high-grade in situ neoplasia, which progressed to a high-grade invasive cancer. (Knockout animals were generously provided by Drs. X.R. Wu and T.T. Sun, Department of Urology, Kaplan Comprehensive Cancer Center, New York University School of Medicine, New York.)

p53 in exons 1–4 followed by loss of CDKN2a and ARF function. Thus, patterns of p53 mutations may be a prerequisite for subsequent, distinctive molecular events in other genes such as RB and CDKN2a and ARF locus (Mark and Jones, 1998).

Recent studies confirm that some molecular events are common to both pathways of bladder cancer development. These events precede the development of microscopically recognizable disease and are antecedent to the involvement of major tumor suppressors such as *RB1* and *p53*. Mapping studies have shown that bladder cancer develops in association to losses of chromosomal regions critical for expansion of in situ preneoplasia (Figure 3). In general, these regions have mapped contiguously to the known major tumor suppressor genes, such as RB1 and p53 (Czerniak et al., 2000).

## Preclinical models

### *Cell lines*

Bladder cancer researchers benefit from the availability of a large number of human cell lines. Cordon-Cardo's group compared the expression profiles of 9 of them using cDNA microarrays. Importantly, novel targets identified in the screen were later validated by a tissue microarray of primary TCC's (Sanchez-Carbayo et al., 2002).

### *Transgenic mice*

The uroplakin promoter has been used successfully to create transgenic mice that recapitulate the dual-track pathways of human bladder cancer development (Figure 4). In the first, the uroplakin promoter was used to drive expression of the SV-40 large T antigen in the mouse bladder. This simultaneously inactivates the p53 and Rb pathways, resulting in the emergence of CIS and highly invasive tumors that share the molecular abnormalities and histopathology of the aggressive lesions observed in humans (Zhang et al., 1999). Alternatively, the uroplakin promoter has also been used to drive expression of mutant Ras or EGFR in the mouse bladder urothelium. The Ras transgenic mice developed superficial papillary lesions that only became invasive when animals were crossed with p53 null mice. Overexpression of the EGFR similarly resulted in simple urothelial hyperplasia, and animals expressing both mutant Ras and EGFR were indistinguishable in phenotype from those bearing mutant Ras alone. However, overexpression of the EGFR converted tumors in the SV-40 low copy number animals into high grade TCCs.

### *Xenograft models*

Orthotopic human tumor xenografts have also been developed that recapitulate the properties of the two major subtypes of

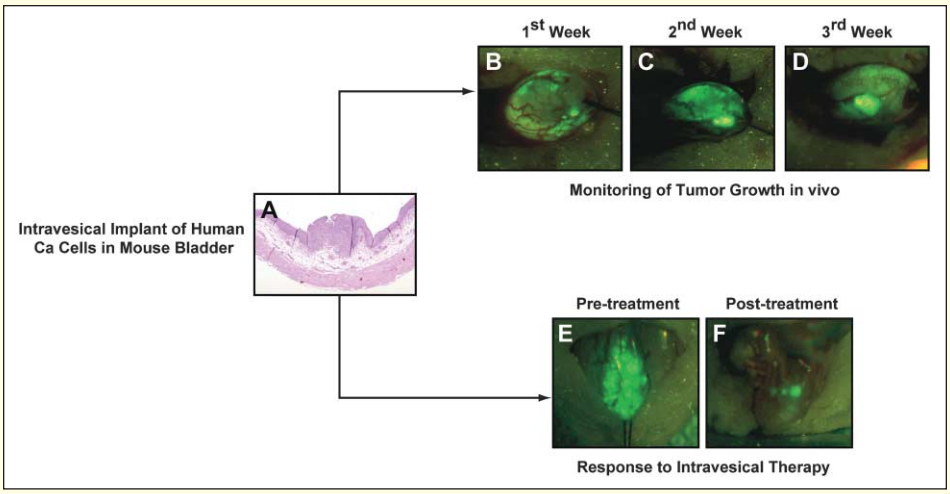


**Figure 5.** Implantation of human bladder cancer cells into mouse bladder

**A:** Superficial KU7 human bladder tumor growing in mouse bladder two weeks after intravesical installation of cancer cells.
**B–D:** A KU7 bladder tumor containing the green fluorescent protein grows in the bladder 1–3 weeks after intravesical installation of tumor cells.
**E:** Pretreatment KU7 bladder tumor growing two weeks after cell injection.
**F:** Marked tumor regression seen three weeks after a one-hour treatment with the intravesical installation of an adenoviral construct containing a human interferon α insert. (Modified from Watanabe et al. [2000]. Used with permission. http://www.nature.com/cgt.)

urothelial cancer. In the first, superficial lesions are created by direct instillation of human KU-7 cells into the nude mouse bladder following gentle trypsinization (Figure 5). Tumors rapidly form in nearly 100% of the mice (Watanabe et al., 2000). In the second, human urothelial cancer cells were "recycled" by selection for orthotopic growth within the bladder wall, resulting in the isolation of variants that display aggressive local growth and metastasis.

**Molecular epidemiology**

Numerous studies have assessed associations between polymorphisms of various genes involved in xenobiotic (i.e., drug) metabolism and bladder cancer risk. However, interpreting such studies requires a detailed knowledge of the metabolism of particular carcinogens. For example, in the metabolism of aromatic amines, a large class of carcinogens implicated in bladder cancer, N-acetyltransferase 2 (NAT-2)-mediated N-acetylation is a detoxification step for some, but an activation step for others (Rothman et al., 1996).

DNA repair capacity varies substantially within the human population, and is plausibly related to cancer susceptibility. In the context of bladder cancer, two variant alleles of the X-ray repair, cross-complementing group 1 (*XRCC1*) gene and a variant allele of *XRCC3*, are associated with a decreased risk of bladder cancer. Using a mutagen challenge comet assay to measure latent genetic instability, Schabath et al. reported a 2-fold increased risk of bladder cancer in individuals susceptible to benzo[*a*]pyrene diol epoxide- (BPDE, a tobacco mutagen) and γ radiation-induced DNA damage in peripheral blood lymphocytes (Schabath et al., 2003), strongly suggesting that individuals better able to resist or repair DNA damage are less susceptible to malignant transformation.

Telomeres also contribute to maintaining genetic stability, and telomere shortening is an early event in carcinogenesis. Using a novel laser scanning cytometry-based quantitative fluorescence in situ hybridization method to determine telomere length distribution in individual cells, it was discovered that individuals with shorter telomeres have a 6-fold elevated risk for bladder cancer and exhibit a supra-additive interaction between smoking status and telomere length (Wu et al., 2003).

**Diagnosis**

The diagnosis of urothelial cancer follows from endoscopic examination coupled with histologic evaluation of material obtained by transurethral biopsy. Unfortunately, cystoscopy can fail to detect the most important indicator of dangerously unstable urothelium: CIS. Areas of CIS can be visually indistinguishable from the surrounding normal bladder. Furthermore, fluorescence cystoscopy (still an investigational technique) demonstrates that conventional cystoscopy can miss not only CIS but small papillary lesions as well.

A variety of biomarkers have been used to enhance the detection of bladder cancer. The "gold standard" and oldest such biomarker is urine cytology. Urine cytology can accurately detect occult CIS, and a positive cytology in absence of a positive cystoscopy is used as an indication for random bladder biopsies. A variety of newer markers have been developed that generally have higher sensitivity but lower specificity than urine cytology. Tests are being developed to evaluate a variety of proteins including cytokeratins, nuclear matrix proteins (BLCA4), extracellular matrix proteins (hyaluronic acid and hyaluronidase), and antiapoptotic proteins (e.g., survivin). Advances in molecular biology have provided the opportunity to take advantage of genomic and proteomic changes in cancer cells to enhance their detection. Microsatellite analysis is promising and is currently undergoing validation through the National Cancer Institute Early Detection Research Network (http://www3.cancer.gov/prevention/cbrg/edrn/).

**Therapy**

***Superficial bladder cancer***

Transurethral resection (TUR) is sufficient therapy for most low-grade noninvasive tumors. The majority of these will recur within a 5-year period, but will rarely invade or result in death from bladder cancer. The recurrence rate of low-grade TCC is decreased by a single post-TUR intravesical instillation of chemotherapy (Sylvester et al., 2004). Although a variety of intravesical therapies (chemotherapy and immunotherapy) are used, bacillus Calmette-Guerin (BCG) remains the most effective intravesical treatment (Bohle et al., 2003). Unfortunately, although BCG is quite an effective therapy in terms of response and short-term control, long-term followup studies reveal that the majority of high-grade invasive cancers eventually recur (Cookson et al., 1997). Efforts to improve the efficacy of BCG by adding interferon-α are ongoing (O'Donnell et al., 2001). At this time, there are no validated markers to predict response to intravesical therapy; however, proinflammatory cytokines

released during T1 helper lymphocyte response modulate the therapeutic effects of BCG (Saint et al., 2002).

***Muscle-invasive disease***

Patients with muscle invasion (clinical stage T2) have a potentially life-threatening disease. However, the notion that such patients have a 50% survival is simply not true. In modern surgical series (Cheng et al., 2000), only about 20% of patients with clinical T2 disease are found to have more advanced stage at cystectomy, and these patients with higher pathologic stage do have about a 50% cause-specific survival. Importantly, however, patients that have pathologically confirmed organ-confined bladder cancer have 80% to 85% long-term disease-free survival with radical cystectomy and pelvic lymphadenectomy. A subset of patients with muscle invasion can be treated with less radical therapy. This subset is currently characterized by solitary lesions with only focal muscle invasion, tumors that are relatively small (<5 cm), are completely resectable by TUR, are located away from the uretero-vesical junctions and the bladder outlet, and are not associated with diffuse CIS or hydronephrosis. For these patients, a therapeutic TUR, a partial cystectomy, or chemoradiation may be adequate (Michaelson et al., 2004). Even with these caveats, however, patients managed with bladder preservation require long-term surveillance, and many will eventually require cystectomy.

***Locally advanced disease***

Patients presenting with cancers unlikely to be cured by surgery alone have improved outcome by the addition of multiagent chemotherapy (Grossman et al., 2003). Perioperative chemotherapy can be given prior to (neoadjuvant) or after (adjuvant) cystectomy, and limited available data do not suggest a significant difference in outcome based on sequence (Millikan et al., 2001). Combination chemotherapy is quite toxic, so that the improved outcome with multimodal therapy is accompanied by a risk for increased toxicity, and patient selection is critical.

***Unresectable and metastatic disease***

For patients with grossly metastatic disease, contemporary chemotherapy regimens produce reliable symptom palliation and median survival in the range of 13 to 18 months. Although few responses are durable, large trials from major centers consistently show about 10% to 15% long-term disease-free survival after combination chemotherapy. The combination of methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) was introduced 20 years ago (Sternberg et al., 1985), and there are at least 10 randomized comparisons of MVAC with other combinations. No incremental improvement from the results obtained with MVAC has been reported, and currently reported phase II and phase III results with various combinations incorporating gemcitabine and taxanes (van der Maase et al., 2000) do not foreshadow improved survival. It is intriguing that several lines of evidence suggest that bladder cancers with altered TP53 are more susceptible to chemotherapy. A national trial to formally test this hypothesis is underway. There is a pressing need to expand the cytotoxic paradigm. Unfortunately, despite promising preclinical data, there are no human data suggesting improved outcome by the integration of newer, targeted therapies.

## Therapeutic prospects

Deregulation of cell cycle and apoptotic pathways via mutation or altered expression of p53, p21/WAF-1, pRB, p27, and INK4A (p16) are necessary for uroepithelial transformation. In addition, members of the erbB family, vascular epidermal growth factor (VEGF), NF$\kappa$B, Akt, PTEN, and cyclooxygenase/2 (COX-2), are also implicated in the progression of the disease (Cote and Datar, 2003). All of these molecules are potential targets for novel therapies.

Both EGFR and Her2/neu are highly expressed by urothelial cancer, and Phase II trials evaluating the efficacy of these growth factor antagonists are ongoing. Recently it was discovered that a small subset of patients with non-small cell lung carcinoma who responded favorably to gefitinib harbored an activating somatic mutation within the tyrosine kinase domain of the EGFR (Lynch et al., 2004). Screening for this mutation in bladder cancer may identify appropriate patients for gefitinib therapy. Recent clinical studies suggest that VEGF overexpression in prechemotherapy samples from patients with locally advanced urothelial cancer undergoing cystectomy and chemotherapy is a strong predictor for recurrence and death from bladder cancer (Slaton et al., 2004). This observation puts forward the important hypotheses that VEGF expression is mechanistically relevant to clinically aggressive bladder cancer and provides rationale for clinical investigation of interventions capable of blocking VEGF signaling. Increased prostaglandin production correlates positively with cancer risk, and COX-2, the inducible rate-limited enzyme for prostaglandin synthesis, is upregulated in bladder cancers. Therapeutic inhibitors of COX-2 (NSAIDs) are under active clinical investigation as a chemopreventive agent.

The development of intravesical gene therapy for superficial bladder cancer has been hindered by limitations in gene delivery to the urothelium. Various approaches to enhance gene transfer are under study, including the administration of the polyamide Syn3, which enhanced adenoviral-mediated gene transfer of interferon-$\alpha$ gene, resulting in the regression of established human xenografts growing in athymic nude mice (Benedict et al., 2004; Figure 5).

## Summary

Urothelial cancer is common and has ample lethal potential. However, most patients present before the disease is clinically beyond the bladder, and tools to interrogate the biologic potential of the urothelium would be expected to produce significant advances in the management of this disease. Likewise, the combination of available chemotherapy with surgery clearly results in an improved cure fraction for patients with locally advanced disease. Thus, significant improvements in overall mortality could be expected from better patient selection for systemic therapy and incremental advances in the activity of systemic therapy.

To this end, the multichannel characterization of genomic and proteomic features of various clinical phenotypes, including benefit from existing therapies, dominates current research directions. In addition, the ready availability of the urothelium should provide an extremely valuable clinical research tool to investigate minimal residual disease, gene therapy, and other novel clinical directions that will be of substantial interest from the perspective of epithelial cancers generally.

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