Current Preclinical Models for the Advancement of Translational Bladder Cancer Research

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Abstract

Bladder cancer is a common disease representing the fifth most diagnosed solid tumor in the United States. Despite this, advances in our understanding of the molecular etiology and treatment of bladder cancer have been relatively lacking. This is especially apparent when recent advances in other cancers, such as breast and prostate, are taken into consideration. The field of bladder cancer research is ready and poised for a series of paradigm-shifting discoveries that will greatly impact the way this disease is clinically managed. Future preclinical discoveries with translational potential will require investigators to take full advantage of recent advances in molecular and animal modeling methodologies. We present an overview of current preclinical models and their potential roles in advancing our understanding of this deadly disease and for advancing care.

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Introduction

Urothelial carcinoma represents the third and eighth most common solid tumor in men and women, respectively. The worldwide incidence of urothelial carcinoma is increasing. As developing countries industrialize and rates of tobacco use increase, this trend is expected to continue (1). However, recent advances in the treatment of bladder cancer are limited. To date, surgery remains the only curative treatment of organ-confined disease. Cisplatin-based combination chemotherapy for more advanced disease is generally not curative and offers a median survival of approximately 15 months with 5-year overall survival a dismal 4% to 20% (2). Bladder cancer is highly chemoresistant upon relapse with no formally approved second-line agents and a median survival of only 6 to 9 months (3–5). Numerous chemotherapeutic and biologics show poor or no activity in the second-line setting. Increased understanding of the molecular biology of this disease and identification of new effective therapeutic agents is essential.

Before the clinical application of an emerging discovery, T1 translational research can be defined as a 2-step process: identification of the most clinically challenging problems and the application of basic science to address them. While there are numerous small-molecule pharmacologic agents with potentially promising anticancer activity, care must be taken about which models are chosen to test these compounds to ensure that they prove relevant to human urothelial carcinoma. With this in mind, this review will discuss the available preclinical models of urothelial carcinoma and their potential applications.

Molecular Biology: Gaps in Knowledge and the Quest to Develop Personalized Therapy

Urothelial carcinoma is a disease of complex etiology and biology (reviewed in refs. 6, 7). The clinical management of urothelial carcinoma presents an array of challenges. Relative to other malignancies, we are still in the early stages of identifying the most important molecular “drivers” of urothelial carcinoma tumorigenesis and progression. Therefore, extensive preclinical studies are necessary to identify novel therapeutic targets and to elucidate and prioritize the development of suitable antitumor agents. A detailed description of identified molecular mechanisms that underlie urothelial carcinoma tumorigenesis and progression is beyond the scope of this review. Interested individuals are referred to more comprehensive reviews on this subject (8). However, a brief discussion of major events in this process is germane. It is currently believed that urothelial carcinoma proceeds along 2 relatively distinct molecular pathways (reviewed in ref. 6). Papillary, low-grade tumors are uniformly superficial and do not progress to invasive lesions.
However, patients with these tumors are at high risk for recurrence as well as progression to high-grade disease in approximately 10% to 15% of cases. Formation of low-grade lesions is associated with molecular aberrations in the oncogene RAS, FGFR3, and deletions of 9q, among others (6). Treatment of low-grade urothelial carcinoma focuses primarily on adequate tumor resection.

High-grade urothelial carcinoma is associated with alterations in p53, retinoblastoma (Rb), and PTEN among others. These tumors also recur with high frequency but unlike low-grade tumors can progress. Muscle invasive urothelial carcinoma is in fact thought to arise from superficial high-grade lesions such as carcinoma in situ (CIS). Clinical management of muscle invasive urothelial carcinoma centers on cystectomy with neoadjuvant or adjuvant chemotherapy for selected individuals. The biology of urothelial carcinoma refractory to surgical and chemotherapeutic intervention is poorly understood, and multiple mechanisms may be operative in this process.

Cell Lines in Urothelial Carcinoma Research

A large number of cell lines are available representing different grades and stages of urothelial carcinoma, and reflect many of the genetic, morphologic, and gene expression alterations observed in human urothelial carcinoma. The most common applications for cell lines include the study of in vivo tumorigenicity and metastases, and in vitro response to drug treatment. Most studies of urothelial carcinoma cell lines in vitro are conducted with cell monolayers grown on plastic. While simple and cost-effective, this approach has limitations. For example, established cell lines for in vitro use can exhibit metabolic (9) and pharmacokinetic (10) behavior different from in situ normal or tumor tissue. In addition, while 3-dimensional culture systems using extracellular matrix may provide a more physiologically relevant in vitro model for tumor dynamics and tumor "ecology" (11), it must be recognized that urothelial carcinoma tumors are surrounded in situ by supporting mesenchyme, vascular elements, matrix, and other cell types. However, these limitations can be partially overcome by the use of orthotopic xenografting approaches, as well as tissue recombination xenografting (see later). In the current section, we provide a summary of available cell lines, with the data presented being representative rather than exhaustive.

Benign urothelial cell lines

"Normal" urothelial cells represent an important control for urothelial carcinoma research and provide the opportunity to investigate carcinogenesis in vitro (reviewed in ref. 12). Initial work was conducted by Reznikoff and colleagues with documentation of short-term ureteral explant cultures in standard (high calcium) medium supplemented with fetal calf serum (12). One culture of these cells was immortalized with SV40 large T antigen (SV-HUC), but genetic instability on longer passages was observed (14). In addition, when these immortalized cells (which are nontumorigenic at low passage) were treated with carcinogens, sublines with increasing tumorigenicity were generated (15). Later, human papillomavirus (HPV) E6 or E7 were used for immortalization (16), with HPV E7 yielding more genetically stable cells (16). Urothelial cells immortalized with HPV-E6/E7 (17, 18) generate differentiatable, immortalized normal urothelial cell lines. Tamatani and colleagues developed a human urothelial line, 1T1, using this approach (19). Another immortalized benign urothelial cell line in use is UROtsa (20), which used a temperature-sensitive SV40 large T construct. UROtsa cells grow in high calcium-defined medium in the absence of fetal calf serum (21), resulting in stratification and expression of tight and gap junctions. UROtsa has been used to evaluate responses to arsenic, a known bladder carcinogen (22). Others have used human telomerase reverse transcriptase (hTERT) immortalization (23). The advantage of hTERT immortalization is lack of interference with p53 and Rb, as occurs with SV40 large T or HPV E6/E7 (24, 25). However, additional studies show hTERT immortalized cells also have significant genetic changes at higher passage (26).

Recently, normal (nonimmortalized) urothelial cell cultures have been established using defined, low calcium, serum-free medium (27). These cells have characteristics of poorly differentiated urothelial cells but retain a more cobblestone appearance (in contrast to SV-HUC, which appear more fibroblastic) and showed antigenic differences from CIS cells. Urothelial cell cultures established in low calcium, serum-free medium can be induced to differentiate by the addition of calcium alone, serum, or by troglitazone (PPAR-γ) and PD153035 (EGF receptor inhibitor) resulting in stratification, E-cadherin expression, and assembly of functional tight junctions (12, 28, 29). While recognizing the limitations of each type of model, these lines can be useful as "normal" or benign control cells or to study effects of genetic changes.

Malignant (urothelial carcinoma) cells and cell lines

Bladder cancer cells, either isolated or as explants, have been maintained in short-term cultures for study (30). However, most of these cultures will fail in culture over time, reaching the Hayflick limit (31). However, many human urothelial carcinoma immortalized cell lines are available. Two originated from low-grade papillary urothelial carcinoma and have retained well-differentiated characteristics. The first, RT4, is a PTEN, urolplakin, and E-cadherin–positive cell line with wild-type p53 and grows slowly in culture forming small raised "islands" (32). RT4 is a mainstay of urothelial carcinoma culture models as the representative of low-grade disease. A second line, RT112, also retains many differentiated characteristics (33). This cell line is not widely available in the United States but is in use in Europe and Asia. A third low-grade line, UM-UC9, has some differentiated characteristics such as slow growth and expression of AN43/uroplakin but lacks others, including expression and cell contact assembly of E-cadherin (34, 35). These lines are...
ideal candidates to identify and study molecular events that differentiate low from high-grade disease.

Numerous cell lines originating from high-grade/advanced-stage urothelial carcinomas are also available. These cells vary in morphology, mutations/deletions, epithelial/mesenchymal characteristics, growth as xenografts in immunodeficient mice, and other characteristics such as metastatic potential (Table 1). When choosing a cell line to model urothelial carcinoma behavior, it is essential that individuals take into account the advantages and disadvantages of cell lines used for subcutaneous and orthotopic studies (10). A resource for information on genetic alterations in these cell lines is the Catalog of Somatic Mutations in Cancer (COSMIC) at the Sanger Institute (Cambridge, United Kingdom; ref. 36).

Urothelial carcinoma cell line misidentification

While the plethora of urothelial carcinoma cell lines allows extensive genetic, morphologic, and functional studies, as well as elegant modeling, there exists an ever-present risk of cell line cross-contamination. Although HeLa cells are notorious contaminants of various cultures, T24 also behaves in a similar manner (37–39). The American Type Culture Collection (ATCC) documents their standard lines using DNA fingerprinting, or short tandem repeat (STR) profiling (38, 39). The Health Protection Agency in the United Kingdom supports a list of misidentified cells (40). While the ATCC currently maintains their database, it only covers the few urothelial carcinoma cell lines they distribute, and therefore, documentation of an STR profile consistent with urothelial origin is essential. The importance of reliable STR is especially clear when it is recognized that uroplakin and keratin expression can be lost in a subset of urothelial carcinoma. Lung-tropic sublines (MGH-U1-m/F1 and the T24T-FL1, Fl2, Fl3 series; refs. 48, 54) of the T24T urothelial carcinoma. Lung-tropic sublines (MGH-U1-m/F1 and the T24T-FL1, Fl2, Fl3 series; refs. 48, 54) of the T24T series of lung metastatic cell lines and the parental T24 cell line led to the identification of a novel metastasis suppressor RhoGDI2 and the association of elevated expression of epiregulin, urokinase-type plasminogen activator (uPA), MMP14 and TIMP-2 with increased risk of lung metastasis (48, 55).

Development of additional metastatic progression sublines from human and mouse bladder carcinomas and integration of more sophisticated genomic, proteomic, and bioinformatic analytic methods may lead to discovery and validation of urothelial carcinoma metastasis genes and signaling pathways. Furthermore, genes identified in functional genomic analysis of animal models need to be tested for their prognostic and therapeutic values using the large collection of microarray profiling data that have been collected from human urothelial carcinoma samples (56, 57).

In Vivo Systems Using Urothelial Carcinoma Cell Lines

Orthotopic murine xenograft models

Orthotopic models for urothelial carcinoma research involve the injection of urothelial carcinoma cells into a host bladder. These models allow for the study of cancer cell behavior within the normal host tissue microenvironment. Technically, single cell suspensions of human urothelial carcinoma cell lines are directly injected into the wall of the bladder of immune-compromised mice via an open abdominal incision or injected into the bladders following catheterization, which requires chemical or mechanical traumatization of the bladder mucosa (41). Mice are monitored with serial examinations for hematuria or a palpable mass or with various imaging techniques to identify growing lesions (42–45). As with all in vivo models, this approach has limitations. For example, experiments designed to identify specific immune responses to therapy cannot be carried out in immune-deficient mice (46), the time frame permitted for the study of metastasis is limited because of death from ureteral obstruction by primary tumors and the sites of metastases do not fully reflect the spectrum of organ tropism typically seen in human urothelial carcinomas such as the high incidence of bone metastasis (47).

Metastatic models

Most deaths from urothelial carcinoma are caused by metastatic spread to distant organs, including bone, lung, and liver (7, 47). Highly metastatic variants of urothelial carcinoma cell lines have been isolated through repeated rounds of in vivo selections from metastatic nodules. The route of cell inoculation into host animals (i.e., orthotopic, intracardiac, or tail vein injection) influences the pattern of metastases and the equipment available to monitor metastases dictates experimental design (43, 48–50). The developments of nearly isogenic variants with low and high metastatic potential are valuable resources for the identification and validation of candidate metastasis genes. For example, sublines established from bone metastasis of TSU-Pr1-B1 and -B2 displayed significantly increased metastatic proclivity to bone and expressed elevated level of matrix metalloproteinases (MT1-MMP, MT2-MMP, MMP-9, and fibroblast growth factor receptor 2; refs. 51, 52) and prominent epithelial features, in contrast to the more mesenchymal-like parental cells, suggesting a functional role of mesenchymal-epithelial transition in metastatic colonization (53). Multiple isogenic series of lung-metastatic cell lines have also been established for urothelial carcinoma. Lung-tropic sublines (MGH-U1-m/F1 and the T24T-FL1, Fl2, Fl3 series; refs. 48, 54) of the T24T series of lung-metastatic cell lines and the parental T24 cell line led to the identification of a novel metastasis suppressor RhoGDI2 and the association of elevated expression of epiregulin, urokinase-type plasminogen activator (uPA), MMP14 and TIMP-2 with increased risk of lung metastasis (48, 55).

Murine Models of Bladder Cancer

Genetically engineered mouse models

The development of Genetically engineered mouse models (GEMM) has been made possible by the discovery of uroplakins, a group of integral membrane proteins largely restricted to urothelial cells (58, 59). Oncogenic alterations introduced into mouse urothelium under the control of mouse uroplakin II (UPII) promoter include...
## Table 1. Commonly used bladder cancer cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Morphology</th>
<th>Differentiation</th>
<th>p53</th>
<th>RB</th>
<th>PTEN</th>
<th>CDKN2A</th>
<th>Xenograft (nude mice)</th>
<th>EMT</th>
<th>Other</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>RT4</td>
<td>Epithelial</td>
<td>High</td>
<td>WT</td>
<td>Loss</td>
<td>WT</td>
<td>HD</td>
<td></td>
<td></td>
<td></td>
<td>ATCC:HTB-2</td>
</tr>
<tr>
<td>RT112</td>
<td>Epithelial</td>
<td>High</td>
<td>WT</td>
<td>Normal</td>
<td>WT</td>
<td>Deleted CDKN2A</td>
<td></td>
<td></td>
<td></td>
<td>ATCC:HTB-2</td>
</tr>
<tr>
<td>T24</td>
<td>Mixed epithelial/fibroblastoid</td>
<td>Low</td>
<td>Mutant</td>
<td>Normal</td>
<td>Mutant</td>
<td>Deleted</td>
<td></td>
<td></td>
<td></td>
<td>ATCC: HTB-4</td>
</tr>
<tr>
<td>J82</td>
<td>Mixed epithelial/fibroblastoid</td>
<td>Low</td>
<td>Mutant</td>
<td>Loss</td>
<td>WT</td>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td>ATCC: HTB-1</td>
</tr>
<tr>
<td>5637</td>
<td>Epithelial</td>
<td>Low</td>
<td>Mutant</td>
<td>Loss</td>
<td>WT</td>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td>ATCC: HTB-9</td>
</tr>
<tr>
<td>253J (P)</td>
<td>Epithelial</td>
<td>Low</td>
<td>WT</td>
<td></td>
<td></td>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td>ATCC: HTB-5</td>
</tr>
<tr>
<td>253J (BV)</td>
<td>Epithelial</td>
<td>Low</td>
<td>WT</td>
<td></td>
<td></td>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td>ATCC: HTB-5</td>
</tr>
<tr>
<td>TCCSup</td>
<td>Epithelial</td>
<td>Low</td>
<td>Mutant</td>
<td>Loss</td>
<td>WT</td>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td>ATCC: HTB-5</td>
</tr>
<tr>
<td>SCaBER</td>
<td>Epithelial</td>
<td>Low</td>
<td>Mutant</td>
<td>Normal</td>
<td>HD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ATCC: HTB-5</td>
</tr>
<tr>
<td>UM-UC1</td>
<td>Epithelial</td>
<td>Low</td>
<td>Mutant</td>
<td>Normal</td>
<td>Deleted</td>
<td>HD</td>
<td></td>
<td></td>
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<td>ATCC: CRL-1749</td>
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<tr>
<td>UM-UC3</td>
<td>Spindle</td>
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<td>Normal</td>
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</tr>
<tr>
<td>UM-UC6</td>
<td>Epithelial</td>
<td>Low</td>
<td>WT</td>
<td>Normal</td>
<td>WT</td>
<td>Loss</td>
<td></td>
<td></td>
<td></td>
<td>ATCC: HTB-5</td>
</tr>
<tr>
<td>UM-UC9</td>
<td>Epithelial</td>
<td>Low</td>
<td>WT</td>
<td>Normal</td>
<td>Mutant</td>
<td>Loss</td>
<td></td>
<td></td>
<td></td>
<td>ATCC: HTB-5</td>
</tr>
<tr>
<td>UM-UC10</td>
<td>Epithelial</td>
<td>Low</td>
<td>WT</td>
<td>Normal</td>
<td>Mutant</td>
<td>Loss</td>
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<td></td>
<td></td>
<td>ATCC: HTB-5</td>
</tr>
<tr>
<td>UM-UC11</td>
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<td>Low</td>
<td>WT</td>
<td>Normal</td>
<td>WT</td>
<td>Loss</td>
<td></td>
<td></td>
<td></td>
<td>ATCC: HTB-5</td>
</tr>
</tbody>
</table>

**Abbreviations:** E, epithelial; EMT, epithelial-mesenchymal transition; GM-CSF, granulocyte macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; HD, homozygous deletion; M, mesenchymal; WT, wild-type.
SV40 large T antigen (60), activated Ha-ras (61), dominant-negative p53 (62), and EGF receptor (63). In addition, the development of a UPII-driven Cre strain (64), has allowed the removal of loxP-flanked tumor suppressors from mouse urothelium, such as p53 and/or pRb (65). A wealth of information, some leading to paradigm-changing concepts, has been obtained from these transgenic and knockout studies. These include the molecular definition of the divergent phenotypic pathways of urothelial carcinoma (6), the biologic potential of genetic alterations in initiating bladder tumors (65–67), the unique context of urothelium in tumorigenesis (68, 69), the critical roles of oncogene dosage in dictating whether and when urothelial tumors arise (60, 61) and the identification of molecular targets that are strongly associated with urothelial carcinoma formation and progression for therapeutic intervention (61).

Despite the caveat that tumors arise from murine and not human cells, the fact that GEMMs harbor well-defined, initial genetic alterations is a strength making GEMMs an invaluable tool for therapeutic testing. Given that bladder tumors develop and evolve in the GEMMs in their natural microenvironment endowed with a tumor vasculature, epithelial–stromal signaling and tumor–immune cell interactions, therapeutic responses in GEMMs may have higher predictive value than other model systems.

In addition, tumor formation kinetics and progression in GEMMs are in general highly predictable, making it easy to pinpoint the specific effects of tumor inhibition. GEMMs, in particular transgenic models, can be made in in-bred strains, thus minimizing the effects of divergent genetic backgrounds on drug metabolism, tumor response, and drug resistance. Existing GEMMs exhibit the entire spectrum of tumor evolution from precursor lesions, to benign lesions, to full-fledged tumors and metastases. Therapeutic as well as preventive strategies can therefore be tailored to target different stages of tumor development (68).

Although GEMMs have been underused for evaluating drug targets and efficacy, the situation is expected to rapidly improve with the increasing awareness of their availability, the understanding of their pivotal role in novel therapeutic testing and the continued refinement of these models to the extent that they faithfully represent the human counterpart. The recent development of tetracycline-inducible and urothelium-specific gene and knockout systems should offer considerable flexibility for the next-generation of urothelial carcinoma models (70).

Chemically induced carcinogen models

De novo urothelial carcinoma can be induced predominantly in rodents with the use of several chemical carcinogens. The majority of these agents have aromatic amine components. The most widely used carcinogen belongs to the nitrosamine family being N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN). BBN is the carcinogen of choice, given its lack of systemic toxicity and exclusive development of urothelial carcinoma (71). BBN, a viscous yellow emulsion, is administered orally, by gavage or as an emulsion in drinking water, and degraded to N-butyl-N-(3-carboxypropyl)-nitrosamine, which has proven carcinogenic effects on the bladder when cleared in the urine. In mice pathologic findings, in sequence are intense submucosal inflammation followed by dysplasia, with or without varying degrees of urothelial metaplasia, then CIS and invasive tumors (72). Metastasis is not typically seen as animals die from obstructive uropathy before spread. When given to rats, the findings are similar, however, the resultant pathology is almost exclusively, low-grade, noninvasive papillary disease. The duration of treatment ranges from 4 to 25 weeks, with some strains showing shorter (A/Jax; ref. 73) and others longer (CD-1; authors experience) periods for tumor development. Given the genetic and pathologic similarity to human disease, this model represents an adequate system to study correlates of human urothelial carcinoma. As such it is well suited to study the impact of specific genes on the development of tumors with the use of transgenic knockout mice (74) and to evaluate the antitumorigenic activity of various agents (75–77).

Adenovirus delivery of transgene or Cre recombinase to rodent urothelium

Another method to study the contribution of altered urothelial gene expression during the development and progression of urinary bladder urothelial carcinoma is through the use of adenovirus-mediated gene delivery. This involves catheterization of female rodents (prostate anatomy with difficult catheterization precludes the use of male mice) to deliver adenovirus encoding a gene of interest into the bladder. Following viral delivery and subsequent viral transduction of urothelial cells, tissue can be harvested at appropriate time points and analyzed (78). Advantages of this approach include the ability to deliver a transgene (79, 80) or a virus encoding Cre recombinase for deletion of rodent genes flanked by flox/flox sites (78), and the relative low cost of this in vivo approach. Disadvantages of adenovirus-mediated gene delivery into the urinary bladder include incomplete transduction of the urothelium and, depending on transduction efficiency, long latency time required for phenotype development. However, pretreatment detergents such as dodecyl-β-D-maltoside (DDM) and SDS have been identified (80) that greatly enhance viral transduction efficiency perhaps by disrupting/disabling these structures.

Mixed Models

Tissue recombination

Cancer is a disease of pathologic alterations in tissue architecture whose precise nature has significance in terms of disease progression (8). Physiologically relevant tissue recombination models offer an advantage over in vitro cell culture systems because they enable the determination of the influence of the microenvironment...
and the discovery and impact of molecular alterations on tumor growth, and the activity of novel therapeutic interventions.

For bladder studies, tissue recombination involves the isolation of embryonic bladder mesenchyme (EBLM) from animals and subsequent recombination with human or murine urothelial carcinoma cells (81), transgenic urothelium, or benign but genetically manipulated urothelial cells. Following recombination, tissue grafts are inserted under the kidney capsule of either immune-compromised or syngeneic hosts and harvested at specific times for analysis (81). One of the major strengths of tissue recombination is the recapitulation of normal multilayered bladder transitional epithelium indicating relatively restricted lineage commitment and subsequent differentiation. However, its use in the study of urothelial carcinoma has been surprisingly limited, probably because of a limited number of benign urothelial or urothelial carcinoma cell lines potentially suitable for use (see cell line section). Thus, most human urothelial carcinoma cell lines (with exceptions, such as RT4, SV-HUC, RT112, and UM-UC9) would be difficult to use for the identification of pathways that induce tumor progression as high-grade, late-stage tumors have usually progressed beyond the point of response to inductive mesenchyme, resulting in the inability to permit the formation of recombinants with any tissue architecture. Therefore, while tissue recombination with benign urothelial components enables us to identify perturbations that promote urothelial carcinoma progression, it is unlikely that highly malignant urothelial carcinoma cell lines would be capable of responding in such a manner.

One cell that is suitable for use in tissue recombination experiments is the RT4 line. In a recent study, RT4 cells were used in tissue recombination experiments to explore the implications of the discovery that p53 alterations and PTEN loss occur in urothelial carcinoma and are significantly associated with poor clinical outcome (78). We recently reported our use of the tissue recombination system to determine the influence of decreased FOXA1 expression for urothelial tumorigenesis (82). FOXA1 expression is detected in normal urothelium, and the presence of FOXA1 expression is correlated with urothelial differentiation, suggesting a potential role for FOXA1 loss in bladder tumor initiation and/or tumor progression (reviewed in ref. 8). Interestingly, FOXA1 loss was detected in 40% of urothelial carcinoma and 80% of squamous cell carcinoma and in keratinizing squamous metaplasia, a precursor to squamous cell carcinoma. We showed FOXA1 expression was significantly diminished with increasing tumor stage. To determine the influence of decreased FOXA1 expression on bladder cancer cell proliferation, we conducted tissue recombination experiments with RT4 cells engineered to exhibit decreased FOXA1 expression. Resulting recombinants exhibited significantly increased RT4 proliferation and tumor volume. Therefore, the tissue recombination technique can allow researchers to perturb a system, verify the influence of this perturbation on the tissue microenvironment, and to pursue potential mechanistic studies important for tumor progression.

Another major defining strength of the tissue recombination model is the ability to use genetically manipulated EBLM derived from transgenic mice for tissue recombination, which can influence urothelial carcinoma growth (83, 84). Therefore, as studies of the tumor microenvironment become increasingly important, EBLM isolated from transgenic mice, and applying approaches used in the study of prostate differentiation and tumor progression through the isolation of transgenic urogenital sinus mesenchyme (85, 86), is certain to aid in the future identification of important stromal targets for cancer therapy.

**Primary human tissue xenografts**

One model that does not suffer from the drawbacks of using cell lines passaged in vitro for decades, coupled with the issue of cell line cross-contamination, involves the subcutaneous xenografting of primary human tumor tissues from patients. This involves placing tumor tissue in an immune-compromised mouse, which uses stromal and angiogenic contributions from the mouse to foster tumor growth. This process can also be applied in a tissue recombination setting using rodent or human smooth muscle components.

Drawbacks of the use of primary human tissue xenografts include (i) the subcutaneous take rate for a given urothelial carcinoma, whereas much better than that seen in many other tumor types, is only approximately 35% (87) and is dependent on multiple poorly characterized intrinsic and environmental factors; (ii) drawbacks about use of a immunocompromised host and finite ability to serially transplant tumors; (iii) heterogeneous nature of human genetics, requiring modest increase in the number of replicates for statistical comparison; (iv) the time to tumor establishment may be long; (v) issues inherent to subcutaneous xenografting experiments, such as host infiltrate and poor pharmacokinetics for therapeutic experiments. Therefore, primary human xenografts may be less ideal for preliminary studies aimed at determining the underlying molecular mechanisms behind a particular process, but better suited for determining the response of human tumors to novel treatments and/or the role of an identified protein/molecule in clinical applications. However, He and colleagues (88) showed that a primary human tissue xenograft showed a similar differentiation pattern, as assessed by conventional histopathologic analysis and immunohistochemical staining, to an established cell line (SW780), suggesting that there may not always be an advantage to using primary tissue.

**Conclusions and Future Directions**

When appropriately used, preclinical models increase our understanding of human disease and enhance all aspects of translational research. Over the past decades, multiple models have been developed to study malignant disease, which now affords us ways to address the most clinically relevant problems in urothelial carcinoma. In
addition, preclinical models of urothelial carcinoma also provide the tools needed to further understand cellular biochemistry that will reveal new clinical targets. The caveat is that preclinical systems, while they provide guidance, cannot completely capture the clinical path of cancer in human subjects, and ultimately will need clinical validation.

Although urothelial carcinoma is a leading cause of cancer-related deaths, and remains an important public health concern worldwide, research funding directed toward increased understanding of the molecular factors that influence the biology of this malignancy is relatively low. Accordingly, research progress in this area has lagged far behind that being achieved in other cancers. Recent data including unexpected results from GEMM models (65), indicates a need for renewed efforts to identify alternative/additional genetic defects driving bladder tumor formation and progression. New imaging technologies for animal models, therapeutic compounds, and existing models covering the spectrum of human urothelial carcinoma characteristics should foster significant progress in the coming years. While a number of oncogenic molecules are being targeted, a single critically important target has not emerged. Further preclinical research into the fundamental biology of urothelial carcinoma will yield better targets and facilitate rational and personalized therapy even in early clinical trials.

Disclosure of Potential Conflicts of Interest

G. Sonpavde has commercial research grant from Celgene, Teva, Novartis, Incyte, and Bellicum; has honoraria from Speakers Bureau of Sanofi-Aventis, Novartis, GSK, Amgen, and Janssen; and is a consultant/ advisory board member of Novartis, Pfizer, and Astellas. No potential conflicts of interest were disclosed by the other authors.

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