Current Oncology Reports (2019) 21: 23
https://doi.org/10.1007/s11912-019-0772-8

GENITOURINARY CANCERS (DP PETRYLAK AND JW KIM, SECTION EDITORS)

Clinical and Genomic Considerations for Variant Histology in Bladder Cancer

Justin T. Matulay[1] · Vikram M. Narayan[1] · Ashish M. Kamat[1]

Published online: 26 February 2019

Abstract

Purpose of Review Urothelial carcinoma demonstrates remarkable plasticity in its ability to differentiate into divergent histologic subtypes in both a pure and mixed form. This review presents the most current data pertaining to bladder cancer with variant histology.

Recent Findings Recognition of bladder cancer variants has increased profoundly in the past two decades with their inclusion in the pathologic guidelines and increased awareness among pathologists and urologists. Most of the available literature consists of small single-institutional studies, but there is compelling evidence to support deviation from the normal urothelial carcinoma management pathways for certain subtypes. While traditionally diagnosed by microscopic appearance, next-generation sequencing and molecular profiling have enabled identification of genomic markers associated with specific variants that exist in tumors lacking classic histologic hallmarks. This genomic information holds promise for predicting response to specific treatments or even in the development of novel targeted therapies.

Summary Combining increased awareness of variant histology, its impact on clinical outcomes, and genomic data will result in a more nuanced treatment approach to reduce morbidity and optimize oncologic outcomes for our patients.

Keywords Bladder cancer · Variant histology · Molecular profiling

Introduction

Bladder cancer, the world's 10th most common malignancy, is often referred to synonymously with urothelial carcinoma (UC) since this is the histology in roughly 90% of cases [1, 2]. The urothelium of the bladder exhibits a remarkable ability for divergent differentiation resulting in several histologic variants of urothelial cell origin [3]. Classification is made based on microscopic cytoarchitectural appearance, and much of the nomenclature is borrowed from neoplasms of other organ sites with similar morphology [3, 4••]. It is important to define what exactly is meant by the term "variant histology" since it impacts the spectrum of disease and, for our purposes, any non-pure UC bladder cancer is considered a variant (Table 1).

Under the heading of variant histology, there are pure divergent histologies where a single morphologic subtype is present throughout without a distinct urothelial component, e.g., squamous cell carcinoma (SCC) of the bladder [4••]. If the divergent morphology occurs with at least some degree of typical UC, then the tumor is classified as urothelial carcinoma "with divergent differentiation." This distinction is important due to differences in prognosis and management (addressed later). It is worth mentioning that variant histology is not limited to the bladder but has been described throughout the urothelial-lined surfaces of the urinary tract including 9 to 24% of nephroureterectomy specimens; however, the body of literature is very limited for extravesical sites [5–7].

Impact of Variant Histology

The incidence of variant histology appears to be increasing over time; however, this is due in large part to increased recognition of histologic subtypes and improved reporting. On blinded

This article is part of the Topical Collection on *Genitourinary Cancers*

✉ Ashish M. Kamat
akamat@mdanderson.org

Justin T. Matulay
jtmatulay@mdanderson.org

Vikram M. Narayan
vmnarayan@mdanderson.org

[1] Department of Urology, Division of Surgery, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Unit 1373, Houston, TX 77030, USA

Table 1 World Health Organization classification of tumors of the urothelial tract

Infiltrating urothelial tumors	Glandular neoplasms
Typical	Adenocarcinoma, NOS
Micropapillary	Enteric
Plasmacytoid/signet ring cell/diffuse	Mucinous
Sarcomatoid	Mixed
Nested	Villous adenoma
Giant cell	
Microcystic	Urachal carcinoma
Poorly differentiated	
Lipid-rich	Mesenchymal tumors
Clear cell	Rhabdomyosarcoma
	Leiomyosarcoma
Squamous neoplasms	Angiosarcoma
Pure squamous cell carcinoma	Inflammatory myofibroblastic tumor
Verrucous carcinoma	Perivascular epithelioid cell tumor
Squamous cell papilloma	Solitary fibrous tumor
	Leiomyoma
Neuroendocrine tumors	Hemangioma
Small cell	Granular cell tumor
Large cell	Neurofibroma
Well-differentiated neuroendocrine	
Paraganglioma	Urothelial tract hematopoietic and lymphoid tumors
Melanocytic tumors	
Malignant melanoma	
Nevus	
Melanosis	

Reproduced with permission from Moch H, Humphrey PA, Ulbright TM, Reuter VE. World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs. IARC, Lyon, 2016

pathologic re-review of 1211 radical cystectomy (RC) specimens from 1980 to 2005 originally diagnosed with UC, Linder et al. re-classified 33% as variants [8•]. This is consistent with the findings of Moschini et al. who found 31.7% of their RCs contained at least a component of variant histology from 1990 to 2015 [9]. They also noted a rise in the proportion of variant tumors over time, from 21.3% in the 1990s to 35.1% since the year 2000, again lending support to the idea of increased awareness being responsible for the higher rate of diagnosis.

As a group, variant histology carries worse prognosis than pure UC, for both the pure and mixed forms [10, 11]. These tumors present at a higher stage and are more likely to develop metastatic disease. Thus, expedient definitive therapy for a patient diagnosed with variant histology is critical. For example, non-muscle invasive bladder cancer (NMIBC) with variant histology is classified as "very high risk" with a recommendation for immediate RC [12, 13].

Even though it may be accepted that upfront RC should be considered for NMIBC with variant histology, this concept relies on the assumption that the correct diagnosis has been rendered. The vast majority of urologists in practice do not have access to a dedicated genitourinary pathologist who is acutely aware of current trends in the field. Expert genitourinary pathologists at the University of Michigan re-reviewed 589 transurethral resection of bladder tumor (TURBT) specimens from patients who were initially diagnosed in the community and found that the presence of variant histology was missed in 44% of cases [14]. Tumor heterogeneity and undersampling also affect our ability to recognize variant histology. Transurethral resection alone does not appear to provide accurate information with relatively poor concordance between the TURBT specimen and final pathology at RC depending on the histologic subtype. In one multi-institutional Italian study, TURBT only identified variant histology in 6.4% of patients compared to 14.1% at RC [15]. At a single US academic center, both TURBT and RC found variants in 27% of patients; however, the concordance was low ($\kappa = 0.27$).

Variant Histology of the Urinary Tract

Pure Variants

Squamous Cell Carcinoma

Representing 2–5% of all bladder cancers diagnosed, SCC is the most common non-urothelial subtype in the USA. The histologic appearance is notable for keratinization that resembles epidermis and a strong association with squamous metaplasia. Chronic inflammation of the urothelium from a variety of sources (bladder calculi, indwelling catheters, chronic urinary tract infections, etc.) induces squamous metaplasia that then progresses to invasive carcinoma [16, 17]. This is a distinct etiology from the bilharzial form of SCC caused by the parasitic organism *Schistosoma haematobium*, a significant cause of bladder cancer in endemic regions (Egypt, Middle East, etc.). *S. haematobium* accounted for more than 80% of all bladder cancers diagnosed in Egypt in the latter part of the twentieth century; however, a steady decline the past two decades has been observed as infection rates improve [18, 19].

Given the prevalence of SCC in Egypt, much of the literature on this topic is from institutional cohorts highly enriched for bilharzial disease. Gonheim et al. have reported comparable overall survival (OS) for SCC when compared to UC (HR 0.89, $p = 0.12$) in the largest institutional cohort of more than 2700 muscle invasive bladder cancer patients, > 50% of which are SCC; however, *S. haematobium* eggs were present in 88% of the specimens [20, 21].

In non-endemic regions, such as the USA, SCC comprises a significantly lower proportion of bladder cancers as

compared to UC histology and seems to carry a worse prognosis [22, 23]. Memorial Sloan-Kettering Cancer Center reported on a cohort of 2031 RCs performed at their institution, of which only 78 had pure SCC and found worse overall and disease specific survival outcomes [22]. Two prior studies on bladder SCC have been published using the SEER registry in the past decade. The first from Scoscyrev et al. examined records of non-metastatic bladder cancer patients from 1988 to 2003 and found 1422 SCCs among 106,613 UCs [24]. They noted a very high rate of muscle-invasion at diagnosis (85%), and on stage-for-stage analysis of survival outcomes, SCC was associated with worse survival only among non-organ confined disease (stage III or IV). When expanded beyond localized disease into the metastatic setting, patients with SCC also fared worse than UC with median overall survival of the former about half of the latter (5 months versus 10 months). In an analysis of SEER data from 1988 to 2006, Abdollah et al. focused on 12,311 patients who underwent RC for bladder cancer, subdividing by SCC ($n = 614$) and UC ($n = 11,697$). The rate of pathologic non-organ confined disease ($\geq$ pT3) was 68% in the SCC patients compared to only 47.3% in UC, but there was no statistical difference in survival between groups on a stage-for-stage basis.

Radiotherapy alone is insufficient treatment for SCC of the bladder as demonstrated in older, retrospective trials. The addition of pre-operative radiation has been suggested as a means to control stage T3 or 4 disease prior to RC by reducing positive margin [25, 26].

The utility of neoadjuvant chemotherapy (NAC) for SCC is still an open question based on paucity of data, but what is reported suggests that there is no survival advantage for these patients. At the Cleveland Clinic, a retrospective analysis of 32 pure SCC cases found no pathologic response in the 4 patients who received NAC [27]. Likewise, another study of 21 Japanese patients with SCC of the bladder failed to show an OS benefit from NAC [28]. There are no studies yet to investigate the possible impact that immunotherapy agents might have on bladder variants such as pure SCC.

Adenocarcinoma

Primary adenocarcinoma of the bladder represents fewer than 2% of all bladder cancer cases, with two categories historically described: urachal and non-urachal carcinomas. Bladder adenocarcinomas frequently metastasize and have a tendency to recur locally. Like SCC, bladder adenocarcinoma is more prevalent in developing countries where bilharziasis is common.

Non-urachal adenocarcinoma is generally associated with a poor overall prognosis, with SEER data demonstrating a 5-year overall survival of only 35% [29]. In addition to bilharziasis, bladder exstrophy is a known risk factor, with adenocarcinoma accounting for greater than 90% of carcinomas in exstrophic bladders [30]. Local control is the mainstay of therapy, with radical cystectomy and pelvic lymph node dissection remaining the gold standard.

Adenocarcinoma of the bladder which arises at the dome should be considered to arise from the vestigial urachal remnant and is termed urachal adenocarcinoma. Most cases are mucin producing and the detection of mucin can be a diagnostic clue, along with hematuria and a palpable abdominal mass [31]. Due to the relative infrequency of the condition, prospective randomized controlled trials are lacking. Data from the SEER database have shown that patients with urachal adenocarcinoma have a 5-year cancer-specific survival rate of 57% and an overall survival rate of 51% [32]. With urachal tumors, a partial cystectomy with *en bloc* resection of the bladder dome, urachal ligament, and umbilicus is mandatory and is an effective alternative to radical cystectomy. Radiotherapy may also help with local control. In one retrospective series of 192 patients treated with radical cystectomy, a subset of higher stage (pT3, pT4) and node-positive patients received postoperative radiotherapy and were found to have a higher 5-year disease-free survival than the cystectomy alone group ($61 \pm 6\%$ vs $37 \pm 5\%$, respectively, $p = 0.002$), although the distant metastasis rate was higher in this group [33].

No chemotherapy regimen has been systematically evaluated for efficacy in patients with adenocarcinoma; however, histologic similarities to enteric adenocarcinoma have led to reports of objective responses to agents such as 5-fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) [34], which have been used to treat colorectal cancer. Targeted exome sequencing of a small cohort of urachal adenocarcinoma cases identified TP53 mutations in seven of nine cases, and an EGFR amplification in one patient who subsequently responded to treatment with cetuximab, an anti-EGFR monoclonal antibody [35]. A separate genome-wide analysis of 17 patients confirmed that TP53 was the most commonly altered gene, with an additional 35% of cases demonstrating focal DNA amplifications in FGFR family genes [36].

Mixed Variants

Micropapillary

Micropapillary bladder cancer was first described from our institution in 1994, with a histologic appearance comprised of narrow, filiform processes or papillary clusters that are similar to papillary serous carcinomas of the ovary [37]. The presence of a micropapillary component predisposes the patient to an advanced clinical presentation, both with advanced local stage and potentially metastatic disease.

The largest consecutive series of micropapillary cases to date was reported by Kamat et al. from the MD Anderson Cancer Center [38•]. In this report, we found that BCG therapy for patients with non-muscle invasive micropapillary disease was ineffective, with 67% of patients developing disease

progression, including 22% who developed metastatic disease [38•]. When comparing patients who underwent immediate cystectomy or NAC, both groups had statistically similar 5-year overall survival: 71% vs 63%, respectively. However, patients treated with NAC had higher rates of non-organ confined disease (68.7% vs 34.8%, $p = 0.016$). Meeks et al. reported on a smaller cohort of patients with micropapillary histology treated with either upfront cystectomy or NAC followed by cystectomy [39]. In 13 out of 29 patients (45%) receiving NAC, downstaging to pT0 was observed, compared to 2 out of 15 patients (13%) of those who underwent cystectomy alone ($p = 0.049$). However, there was no significant difference at 2 years between the groups with respect to rates of recurrence, overall survival, or cancer-specific survival. An updated analysis of the MD Anderson cohort found that risk stratification based on clinical T-stage and the presence of hydronephrosis significantly predicted 5-year disease specific survival of 96%, 51%, and 17% ($p < 0.001$) for low-, intermediate-, and high-risk groups, respectively. Downstaging to pT1 or less, regardless of the administration of NAC, was a strong predictor of survival with 5-year DSS of 96% versus only 45% ($p < 0.001$) in those without downstaging [40].

There are conflicting views on the management of non-muscle invasive micropapillary cases; several small case series have reported 12–60 month disease-free intervals after treatment with intravesical BCG alone [41, 42]. Vourganti et al. performed a SEER study that identified 120 patients over a 7-year period. They found that patients with non-muscle invasive micropapillary bladder cancer had significantly worse survival outcomes than those with similar stage urothelial carcinoma [43]. This difference was not observed in patients with higher stage disease, lending further credence to the idea that early stage micropapillary bladder tumors should be aggressively managed. Additional evidence in support of this was provided by a more recent study by our group, which reviewed a cohort of 72 patients with cT1N0M0 disease with micropapillary bladder cancer. Among those treated with primary intravesical BCG ($n = 40$), we observed 75% to have disease recurrence, 45% to have disease progression, and 35% develop lymph node metastasis [44]. Those treated with initial radical cystectomy had improved 5-year cancer specific survival (100% vs. 60%, $p = 0.006$), and pathologic upstaging was identified in nearly a third of cases who underwent up-front cystectomy.

Currently, for both non-muscle invasive and locally advanced cases of micropapillary bladder cancer, we advocate for upfront radical cystectomy, with NAC for select patients.

The next chapter in micropapillary bladder cancer management, like many other malignancies, lies in targeting unique genetic signatures to deliver highly effective therapy. Overexpression of the drug targetable *ERBB2* oncogene occurs in up to two thirds of micropapillary tumors [45], and phase II data of the small-molecule inhibitor afatinib has shown improvement in progression-free survival [46]. Guo et al. performed whole-genome expression messenger RNA profiling on a cohort of 43 patients with micropapillary bladder cancer, comparing them to a reference set of conventional pure urothelial carcinoma cases [47]. Micropapillary tumors were found to have a predominantly luminal gene expression signature (42 of 43 cases), with a subset of luminal cases also defined as an aggressive, chemoresistant p53-like variant. The downregulation of miR-296 and upregulation of its target genes, including activation of the RUVBL1 pathway, was also described to drive the expression signature of micropapillary cases. These may represent future therapeutic targets that warrant additional investigation [47].

Plasmacytoid

Plasmacytoid tumors exhibit an advanced histologic pattern of invasion into bladder and perivesical tissues. Clinically, patients with this subtype experience poor outcomes. In a series of 31 patients from our center, more than 85% of patients with the plasmcytoid variant presented with ≥ cT2 disease, with almost half presenting with metastatic or locally unresectable disease [48]. Cisplatin-based NAC provided an overall response rate of 53% (8 of 15 patients), although recurrences were common and most frequently manifested as peritoneal carcinomatosis. Overall survival was only 17.7 months. Whole-exome and targeted sequencing performed on plasmacytoid tumors have led to the identification that these cancers often have somatic alterations in the CDH1 gene, which encodes for E-cadherin [49]. This finding was notably specific for the plasmacytoid variant, with no CDH1-truncating mutations identified in the TCGA urothelial carcinoma cohort. The loss of E-cadherin results in enhanced cellular migration and may be an explanation for the tendency of these tumors to recur locally and with peritoneal metastases, resulting in an aggressive clinical course.

Sarcomatoid

The sarcomatoid variant is exceedingly rare, with a prevalence of less than 1% of all bladder cancers [50]. Of note, whereas previously there was a distinction made between carcinosarcoma and sarcomatoid variants, currently these are viewed as a single entity. Sarcomatoid bladder cancers can develop due to exposure to radiotherapy as well as cyclophosphamide. SEER analysis has shown that in non-invasive cases, even when stratified by stage, the presence of sarcomatoid disease was associated with worse survival and twofold higher risk of cancer-specific mortality when compared with pure urothelial carcinoma [51]. Sarcomatoid tumors of the bladder have been shown to have high expression of epithelial-to-mesenchymal transition (EMT)-associated genes, including *FoxC2*, *SNAIL*, and *ZEB1* [52]. Sarcomatoid tumors tend to be chemotherapy

resistant, and immediate radical cystectomy surgery remains the mainstay of treatment.

Squamous and Glandular Differentiation

Urothelial carcinoma with squamous differentiation is the most common histologic entity among the variant subtypes, with an estimated incidence of up to 20–40% of cases. Glandular differentiation is the second most common variant and can be found in up to 18% of invasive tumors [10]. Data regarding optimal management is conflicting, as squamous and glandular differentiation can co-exist with other variant histologic types, including micropapillary, sarcomatoid, and others.

Antunes et al. reported that when compared with pure urothelial carcinoma, the presence of squamous differentiation ($n = 25$) was associated with higher rates of disease recurrence (34% vs 64%) and mortality (16% vs 40%) [53]. In contrast, in a larger cohort of variant histology patients (including 132 patients with squamous differentiation and 41 patients with glandular differentiation), Kim et al. reported that the presence of squamous/glandular differentiation portends no significant 10-year cancer specific survival difference when compared with pure urothelial carcinoma cases (52% vs 51%, $p = 0.71$), despite worse pathology at the time of radical cystectomy [54]. Thus, in general, when patients present with either squamous or glandular differentiation, we recommend that they be managed as appropriate for regular urothelial carcinoma at that stage, with a recognition that the biologic behavior might be more aggressive than not.

Nested

The nested variant is histologically characterized by small nests of cells infiltrating the lamina propria or muscularis propria of the bladder. They can sometimes be confused for von Brunn's nests or nephrogenic adenoma [51]. Like other histologic cases, the nested variant is associated with advanced pathological features on presentation, although these features may not necessarily translate into worse long-term outcomes when compared to stage matched pure urothelial cases. Linder et al. reported on a cohort of 52 patients with the nested variant who were treated with radical cystectomy; when matched with cases of pure urothelial carcinoma and after a median follow-up of almost 11 years, patients with the nested variant did not have statistically significant differences in local recurrence-free survival or cancer-specific survival. However, patients with the nested variant did have a high rate of adverse pathologic features, with 69% having pT3-T4 disease and 19% having lymph node involvement at the time of cystectomy [55]. Mally et al. reported on a non-muscle invasive cohort of 30 cT1 nested variant cases and compared them to matched cases of pure urothelial carcinoma; no statistically significant differences were observed in cancer specific survival ($p = 0.2$). However, the rate of upstaging in those who underwent cystectomy in this study (within 3 months of a restaging TUR) to either the bladder and/or lymph nodes was as high as 54% [56].

Lymphoepithelioma-Like

Lymphoepithelioma-like carcinoma (LELC) of the bladder has a < 1% incidence and is histologically similar to nasopharyngeal LELC, with small aggregates of high-grade tumor cells featuring irregular nuclear borders and prominent nucleoli. Inflammatory cells are also frequently seen [57]. Although the Epstein–Barr virus (EBV) has been associated with LELC in other organs, to date, it has not been shown to be associated with LELC of the bladder. A systematic review by Yang et al. of LELC of the bladder identified 140 reported cases in the literature, with radical cystectomy associated with the highest disease-free survival rate (67.8%), when compared with TURBT alone or partial cystectomy [58]. LELC frequently co-presents with other histologic variants. These tumors tend to respond favorably to platinum-based chemotherapy, particularly in pure and predominantly LELC cases.

Molecular Markers and Variant Histology

In the era of readily accessible next-generation sequencing technology, an enormous amount of genetic data is now available and increasing daily. Several large-scale sequencing studies have been conducted over the past 6 years on both muscle invasive and non-muscle invasive tumors, leading to recognition of patterns in molecular profiles for both prognostic and predictive purposes [59–65, 66•, 67].

The current muscle-invasive bladder cancer (MIBC) subtyping nomenclature is broken down into five major groups (Fig. 1)—luminal, luminal-papillary, luminal-infiltrated, basal-squamous, and neuronal—which derive their names from the histologic appearance most often associated with a particular expressional profile [68, 69]. For instance, the neuroendocrine subtype is so-named because this particular profile is enriched with tumors exhibiting a small cell carcinoma appearance (small, round blue cells). Even for those tumors not histologically identified as neuroendocrine, the characteristic neuronal molecular profile includes loss of *TP53* and *RB1* as well as *E2F3* amplification, with the worst overall prognosis of all [65]. Using knowledge gained from treatment regimens that have shown good activity for neuroendocrine cancers in the past, one might hypothesize that the addition of etoposide or ifosfamide to cisplatinum chemotherapy would be the best way to approach these tumors [70, 71].

The genetic signature of the basal-squamous subtype is highly enriched for both basal markers (*CD44*, *KRT6A*, etc.) and squamous markers (*DSC3*, *GSDMC*, etc.) while

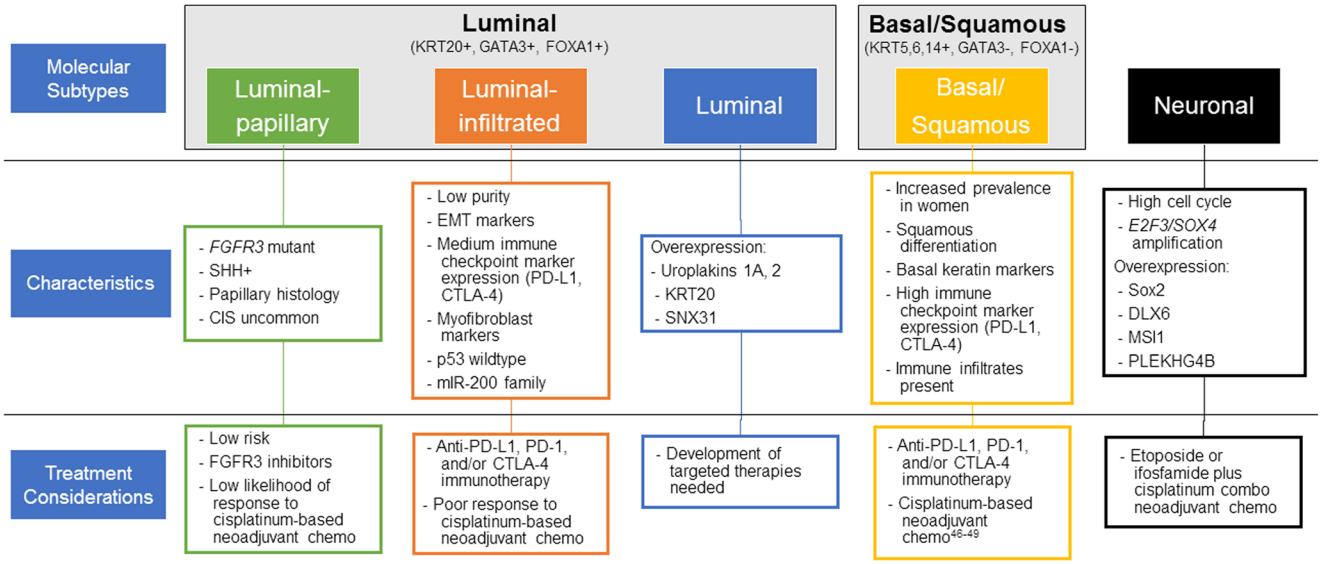

Fig. 1 Molecular subtypes of muscle invasive bladder cancer. Characteristics and specific treatment considerations are listed for each category. Reprinted from *Cell*, Volume 171, Issue 3, Robertson AG, Kim J, Al-Ahmadie H, et al., *Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer*, pages 540–556 e525, ©2017, with permission from Elsevier

containing a large proportion of histologically identified SCCs [65]. This subgroup also carries a poor prognosis but has been associated with the best response to neoadjuvant cisplatinum-based chemotherapy [66•]. An intriguing development from the most recent Cancer Genome Atlas publication is the finding that immune markers (*CXCL11*, *L1CAM*, *IDO1*, *CD274*) are also highly expressed in this group and could be an indicator of response to the immune checkpoint inhibitors [65].

By incorporating mutational profiling into our diagnostic paradigm, rather than relying on microscopic analysis, we will have moved into a new era of defining urothelial variants by more than their histologic appearance and perhaps improve clinical outcomes with a more targeted approach to therapy.

Conclusions

Recognition of histologic variants in bladder cancer has increased over the past several decades. While challenges still remain, proper identification of a urothelial variant is an important part of modern risk stratification in the management of bladder cancer. Certain subtypes may benefit from a very different treatment approach than the standard bladder cancer pathways, such as moving directly to cystectomy for SCC rather than using NAC. As a group, variant histology is indicative of more aggressive disease; however, there are some notable exceptions to keep in mind (i.e., lymphoepithelioma-like). Investigation into the molecular underpinnings of bladder cancer has led to the discovery that histology alone cannot always properly classify tumors that may look dissimilar but share specific mutations that relate to prognosis and response to treatment.

Compliance with Ethical Standards

Conflict of Interest Justin T. Matulay and Vikram M. Narayan declare that they have no conflict of interest. Ashish M. Kamat has received research funding from FKD, Merck, BMS, Photocure, Heat Biologics & Telesta; serves/has served on the advisory board for Merck, BMS, Photocure, Theralase, Medac, Astra Zeneca, Ferring, Taris, Combat Medical, Synergo; is President of International Bladder Cancer Network (IBCN) and International Bladder Cancer Group (IBCG); and holder of patent, CyPRIT (Cytokine Predictors of Response to Intravesical Therapy) jointly with UT MD Anderson Cancer Center.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424. https://doi.org/10.3322/caac.21492.
2. Hansel DE, Amin MB, Comperat E, Cote RJ, Knuchel R, Montironi R, et al. A contemporary update on pathology standards for bladder cancer: transurethral resection and radical cystectomy specimens. Eur Urol. 2013;63(2):321–32. https://doi.org/10.1016/j.eururo.2012.10.008.

3. Amin MB. Histological variants of urothelial carcinoma: diagnostic, therapeutic and prognostic implications. Mod Pathol. 2009;22(Suppl 2):S96–S118. https://doi.org/10.1038/modpathol.2009.26.
4.•• Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO classification of tumours of the urinary system and male genital organs—part B: prostate and bladder tumours. Eur Urol. 2016;70(1):106–19. https://doi.org/10.1016/j.eururo.2016.02.028. **A review of the most recent update to the WHO classification for urothelial malignancies. This version contains information regarding reporting standards for pathologists, such as inclusion of the percentage of divergent histology present in a specimen. Changes to the nomenclature of specific variant histologic subtypes can be found in this document.**
5. Kim JK, Moon KC, Jeong CW, Kwak C, Kim HH, Ku JH. Variant histology as a significant predictor of survival after radical nephroureterectomy in patients with upper urinary tract urothelial carcinoma. Urol Oncol. 2017;35(7):458 e9–e15. https://doi.org/10.1016/j.urolonc.2017.02.010.
6. Perez-Montiel D, Wakely PE, Hes O, Michal M, Suster S. High-grade urothelial carcinoma of the renal pelvis: clinicopathologic study of 108 cases with emphasis on unusual morphologic variants. Mod Pathol. 2006;19(4):494–503. https://doi.org/10.1038/modpathol.3800559.
7. Rink M, Robinson BD, Green DA, Cha EK, Hansen J, Comploj E, et al. Impact of histological variants on clinical outcomes of patients with upper urinary tract urothelial carcinoma. J Urol. 2012;188(2):398–404. https://doi.org/10.1016/j.juro.2012.04.009.
8.• Linder BJ, Boorjian SA, Cheville JC, Sukov WR, Thapa P, Tarrell RF, et al. The impact of histological reclassification during pathology re-review—evidence of a Will Rogers effect in bladder cancer? J Urol. 2013;190(5):1692–6. https://doi.org/10.1016/j.juro.2013.05.040. **A review of the most recent update to the WHO classification for urothelial malignancies. This version contains information regarding reporting standards for pathologists, such as inclusion of the percentage of divergent histology present in a specimen. Changes to the nomenclature of specific variant histologic subtypes can be found in this document.**
9. Moschini M, Dell'Oglio P, Luciano R, Gandaglia G, Soria F, Mattei A, et al. Incidence and effect of variant histology on oncological outcomes in patients with bladder cancer treated with radical cystectomy. Urol Oncol. 2017;35(6):335–41. https://doi.org/10.1016/j.urolonc.2016.12.006.
10. Black PC, Brown GA, Dinney CP. The impact of variant histology on the outcome of bladder cancer treated with curative intent. Urol Oncol. 2009;27(1):3–7. https://doi.org/10.1016/j.urolonc.2007.07.010.
11. Wasco MJ, Daignault S, Zhang Y, Kunju LP, Kinnaman M, Braun T, et al. Urothelial carcinoma with divergent histologic differentiation (mixed histologic features) predicts the presence of locally advanced bladder cancer when detected at transurethral resection. Urology. 2007;70(1):69–74. https://doi.org/10.1016/j.urology.2007.03.033.
12. Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. Eur Urol. 2017;71(3):447–61. https://doi.org/10.1016/j.eururo.2016.05.041.
13. Chang SS, Boorjian SA, Chou R, Clark PE, Daneshmand S, Konety BR, et al. Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline. J Urol. 2016;196(4):1021–9. https://doi.org/10.1016/j.juro.2016.06.049.
14. Shah RB, Montgomery JS, Montie JE, Kunju LP. Variant (divergent) histologic differentiation in urothelial carcinoma is under-recognized in community practice: impact of mandatory central pathology review at a large referral hospital. Urol Oncol. 2013;31(8):1650–5. https://doi.org/10.1016/j.urolonc.2012.04.009.
15. Moschini M, Shariat SF, Freschi M, Soria F, D'Andrea D, Abufaraj M, et al. Is transurethral resection alone enough for the diagnosis of histological variants? A single-center study. Urol Oncol. 2017;35(8):528.e1–528.e5. https://doi.org/10.1016/j.urolonc.2017.03.024.
16. Clouston D, Lawrentschuk N. Metaplastic conditions of the bladder. BJU Int. 2013;112(Suppl 2):27–31. https://doi.org/10.1111/bju.12378.
17. Fernandez MI, Brausi M, Clark PE, Cookson MS, Grossman HB, Khochikar M, et al. Epidemiology, prevention, screening, diagnosis, and evaluation: update of the ICUD-SIU joint consultation on bladder cancer. World J Urol. 2018. https://doi.org/10.1007/s00345-018-2436-y.
18. Gouda I, Mokhtar N, Bilal D, El-Bolkainy T, El-Bolkainy NM. Bilharziasis and bladder cancer: a time trend analysis of 9843 patients. J Egypt Natl Canc Inst. 2007;19(2):158–62.
19. Salem HK, Mahfouz S. Changing patterns (age, incidence, and pathologic types) of Schistosoma-associated bladder cancer in Egypt in the past decade. Urology. 2012;79(2):379–83. https://doi.org/10.1016/j.urology.2011.08.072.
20. Ghoneim MA, Abdel-Latif M, el-Mekresh M, Abol-Enein H, Mosbah A, Ashamallah A, et al. Radical cystectomy for carcinoma of the bladder: 2,720 consecutive cases 5 years later. J Urol. 2008;180(1):121–7. https://doi.org/10.1016/j.juro.2008.03.024.
21. Ghoneim MA, el-Mekresh MM, el-Baz MA, el-Attar IA, Ashamallah A. Radical cystectomy for carcinoma of the bladder: critical evaluation of the results in 1,026 cases. J Urol. 1997;158(2):393–9.
22. Ehdaie B, Maschino A, Shariat SF, Rioja J, Hamilton RJ, Lowrance WT, et al. Comparative outcomes of pure squamous cell carcinoma and urothelial carcinoma with squamous differentiation in patients treated with radical cystectomy. J Urol. 2012;187(1):74–9. https://doi.org/10.1016/j.juro.2011.09.056.
23. Kassouf W, Spiess PE, Siefker-Radtke A, Swanson D, Grossman HB, Kamat AM, et al. Outcome and patterns of recurrence of nonbilharzial pure squamous cell carcinoma of the bladder: a contemporary review of the University of Texas M D Anderson Cancer Center experience. Cancer. 2007;110(4):764–9. https://doi.org/10.1002/cncr.22853.
24. Scosyrev E, Yao J, Messing E. Urothelial carcinoma versus squamous cell carcinoma of bladder: is survival different with stage adjustment? Urology. 2009;73(4):822–7. https://doi.org/10.1016/j.urology.2008.11.042.
25. Ghoneim MA, Ashamallah AK, Awaad HK, Whitmore WF Jr. Randomized trial of cystectomy with or without preoperative radiotherapy for carcinoma of the bilharzial bladder. J Urol. 1985;134(2):266–8.
26. Swanson DA, Liles A, Zagars GK. Preoperative irradiation and radical cystectomy for stages T2 and T3 squamous cell carcinoma of the bladder. J Urol. 1990;143(1):37–40.
27. Zahoor H, Elson P, Stephenson A, Haber G-P, Kaouk J, Fergany A, et al. Patient characteristics, treatment patterns and prognostic factors in squamous cell bladder cancer. Clin Genitourin Cancer. 2018;16(2):e437–e42. https://doi.org/10.1016/j.clgc.2017.10.005.
28. Makino T, Izumi K, Natsagdorj A, Iwamoto H, Kadomoto S, Naito R, et al. Significance of perioperative chemotherapy in squamous cell carcinoma of the upper and lower urinary tract. Anticancer Res. 2018;38(4):2241–5. https://doi.org/10.21873/anticanres.12467.
29. Wright JL, Porter MP, Li CI, Lange PH, Lin DW. Differences in survival among patients with urachal and nonurachal adenocarcinomas of the bladder. Cancer. 2006;107(4):721–8. https://doi.org/10.1002/cncr.22059.

30. Kathopoulis N, Thomakos N, Mole I, Papaspirou I, Ntai S, Rodolakis A. Anterior pelvic exenteration for exstrophic bladder adenocarcinoma: case report and review. Int J Surg Case Rep. 2016;25:13–5. https://doi.org/10.1016/j.ijscr.2016.05.022.
31. Sheldon CA, Clayman RV, Gonzalez R, Williams RD, Fraley EE. Malignant urachal lesions. J Urol. 1984;131(1):1–8.
32. Mylonas KS, Malley PO, Ziogas IA, El-Kabab L, Nasioudis D. Malignant urachal neoplasms: a population-based study and systematic review of literature. Urol Oncol. 2017;35(1):33 e11–9. https://doi.org/10.1016/j.urolonc.2016.07.021.
33. Zaghloul MS, Nouh A, Nazmy M, Ramzy S, Zaghloul AS, Sedira MA, et al. Long-term results of primary adenocarcinoma of the urinary bladder: a report on 192 patients. Urol Oncol. 2006;24(1):13–20. https://doi.org/10.1016/j.urolonc.2005.05.027.
34. Yanagihara Y, Tanji N, Miura N, Shirato A, Nishimura K, Fukumoto T, et al. Modified FOLFOX6 chemotherapy in patients with metastatic urachal cancer. Chemotherapy. 2013;59(6):402–6. https://doi.org/10.1159/000362400.
35. Collazo-Lorduy A, Castillo-Martin M, Wang L, Patel V, Iyer G, Jordan E, et al. Urachal carcinoma shares genomic alterations with colorectal carcinoma and may respond to epidermal growth factor inhibition. Eur Urol. 2016;70(5):771–5. https://doi.org/10.1016/j.eururo.2016.04.037.
36. Lee S, Lee J, Sim SH, Lee Y, Moon KC, Lee C, et al. Comprehensive somatic genome alterations of urachal carcinoma. J Med Genet. 2017;54(8):572–8. https://doi.org/10.1136/jmedgenet-2016-104390.
37. Amin MB, Ro JY, el-Sharkawy T, Lee KM, Troncoso P, Silva EG, et al. Micropapillary variant of transitional cell carcinoma of the urinary bladder. Histologic pattern resembling ovarian papillary serous carcinoma. Am J Surg Pathol. 1994;18(12):1224–32.
38.• Kamat AM, Dinney CP, Gee JR, Grossman HB, Siefker-Radtke AO, Tamboli P, et al. Micropapillary bladder cancer: a review of the University of Texas M. D. Anderson Cancer Center experience with 100 consecutive patients. Cancer. 2007;110(1):62–7. https://doi.org/10.1002/cncr.22756. **Among the earliest papers to describe the clinical course of micropapillary bladder cancer. This retrospective, single-institution review found that attempts to treat with intravesical and/or systemic therapies resulted in worse survival when compared to immediate radical cystectomy.**
39. Meeks JJ, Taylor JM, Matsushita K, Herr HW, Donat SM, Bochner BH, et al. Pathological response to neoadjuvant chemotherapy for muscle-invasive micropapillary bladder cancer. BJU Int. 2013;111(8):E325–30. https://doi.org/10.1111/j.1464-410X.2012.11751.x.
40. Fernandez MI, Williams SB, Willis DL, Slack RS, Dickstein RJ, Parikh S, et al. Clinical risk stratification in patients with surgically resectable micropapillary bladder cancer. BJU Int. 2017;119(5):684–91. https://doi.org/10.1111/bju.13689.
41. Amin A, Epstein JI. Noninvasive micropapillary urothelial carcinoma: a clinicopathologic study of 18 cases. Hum Pathol. 2012;43(12):2124–8. https://doi.org/10.1016/j.humpath.2012.04.013.
42. Gaya JM, Palou J, Algaba F, Arce J, Rodriguez-Faba O, Villavicencio H. The case for conservative management in the treatment of patients with non-muscle-invasive micropapillary bladder carcinoma without carcinoma in situ. Can J Urol. 2010;17(5):5370–6.
43. Vourganti S, Harbin A, Singer EA, Shuch B, Metwalli AR, Agarwal PK. Low grade micropapillary urothelial carcinoma, does it exist?—analysis of management and outcomes from the surveillance, Epidemiology and End Results (SEER) database. J Cancer. 2013;4(4):336–42.
44. Willis DL, Fernandez MI, Dickstein RJ, Parikh S, Shah JB, Pisters LL, et al. Clinical outcomes of cT1 micropapillary bladder cancer. J Urol. 2015;193(4):1129–34. https://doi.org/10.1016/j.juro.2014.09.092.
45. Isharwal S, Huang H, Nanjangud G, Audenet F, Chen YB, Gopalan A, et al. Intratumoral heterogeneity of ERBB2 amplification and HER2 expression in micropapillary urothelial carcinoma. Hum Pathol. 2018;77:63–9. https://doi.org/10.1016/j.humpath.2018.03.015.
46. Choudhury NJ, Campanile A, Antic T, Yap KL, Fitzpatrick CA, Wade JL 3rd, et al. Afatinib activity in platinum-refractory metastatic urothelial carcinoma in patients with ERBB alterations. J Clin Oncol. 2016;34(18):2165–71. https://doi.org/10.1200/JCO.2015.66.3047.
47. Guo CC, Dadhania V, Zhang L, Majewski T, Bondaruk J, Sykulski M, et al. Gene expression profile of the clinically aggressive micropapillary variant of bladder cancer. Eur Urol. 2016;70(4):611–20. https://doi.org/10.1016/j.eururo.2016.02.056.
48. Dayyani F, Czerniak BA, Sircar K, Munsell MF, Millikan RE, Dinney CP, et al. Plasmacytoid urothelial carcinoma, a chemosensitive cancer with poor prognosis, and peritoneal carcinomatosis. J Urol. 2013;189(5):1656–61. https://doi.org/10.1016/j.juro.2012.11.084.
49. Al-Ahmadie HA, Iyer G, Lee BH, Scott SN, Mehra R, Bagrodia A, et al. Frequent somatic CDH1 loss-of-function mutations in plasmacytoid variant bladder cancer. Nat Genet. 2016;48(4):356–8. https://doi.org/10.1038/ng.3503.
50. Chalasani V, Chin JL, Izawa JI. Histologic variants of urothelial bladder cancer and nonurothelial histology in bladder cancer. Can Urol Assoc J. 2009;3(6 Suppl 4):S193–8.
51. Beltran AL, Cheng L, Montironi R, Blanca A, Leva M, Rouprêt M, et al. Clinicopathological characteristics and outcome of nested carcinoma of the urinary bladder. Virchows Arch. 2014;465(2):199–205. https://doi.org/10.1007/s00428-014-1601-y.
52. Sanfrancesco J, McKenney JK, Leivo MZ, Gupta S, Elson P, Hansel DE. Sarcomatoid urothelial carcinoma of the bladder: analysis of 28 cases with emphasis on clinicopathologic features and markers of epithelial-to-mesenchymal transition. Arch Pathol Lab Med. 2016;140(6):543–51. https://doi.org/10.5858/arpa.2015-0085-OA.
53. Antunes AA, Nesrallah LJ, Dall'Oglio MF, Maluf CE, Camara C, Leite KR, et al. The role of squamous differentiation in patients with transitional cell carcinoma of the bladder treated with radical cystectomy. Int Braz J Urol. 2007;33(3):339–45 discussion 46.
54. Kim SP, Frank I, Cheville JC, Thompson RH, Weight CJ, Thapa P, et al. The impact of squamous and glandular differentiation on survival after radical cystectomy for urothelial carcinoma. J Urol. 2012;188(2):405–9. https://doi.org/10.1016/j.juro.2012.04.020.
55. Linder BJ, Frank I, Cheville JC, Thompson RH, Thapa P, Tarrell RF, et al. Outcomes following radical cystectomy for nested variant of urothelial carcinoma: a matched cohort analysis. J Urol. 2013;189(5):1670–5. https://doi.org/10.1016/j.juro.2012.11.006.
56. Mally AD, Tin AL, Lee JK, Satasivam P, Cha EK, Donat SM, et al. Clinical outcomes of patients with T1 nested variant of urothelial carcinoma compared to pure urothelial carcinoma of the bladder. Clin Genitourin Cancer. 2017;16:e23–7. https://doi.org/10.1016/j.clgc.2017.07.002.
57. Williamson SR, Zhang S, Lopez-Beltran A, Shah RB, Montironi R, Tan PH, et al. Lymphoepithelioma-like carcinoma of the urinary bladder: clinicopathologic, immunohistochemical, and molecular features. Am J Surg Pathol. 2011;35(4):474–83. https://doi.org/10.1097/PAS.0b013e31820f709e.
58. Yang AW, Pooli A, Lele SM, Kim IW, Davies JD, LaGrange CA. Lymphoepithelioma-like, a variant of urothelial carcinoma of the urinary bladder: a case report and systematic review for optimal treatment modality for disease-free survival. BMC Urol. 2017;17(1):34. https://doi.org/10.1186/s12894-017-0224-4.

59. Choi W, Porten S, Kim S, Willis D, Plimack ER, Hoffman-Censits J, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell. 2014;25(2):152–65. https://doi.org/10.1016/j.ccr.2014.01.009.
60. Damrauer JS, Hoadley KA, Chism DD, Fan C, Tiganelli CJ, Wobker SE, et al. Intrinsic subtypes of high-grade bladder cancer reflect the hallmarks of breast cancer biology. Proc Natl Acad Sci U S A. 2014;111(8):3110–5. https://doi.org/10.1073/pnas.1318376111.
61. Hedegaard J, Lamy P, Nordentoft I, Algaba F, Hoyer S, Ulhoi BP, et al. Comprehensive transcriptional analysis of early-stage urothelial carcinoma. Cancer Cell. 2016;30(1):27–42. https://doi.org/10.1016/j.ccell.2016.05.004.
62. Hurst CD, Alder O, Platt FM, Droop A, Stead LF, Burns JE, et al. Genomic subtypes of non-invasive bladder cancer with distinct metabolic profile and female gender bias in KDM6A mutation frequency. Cancer Cell. 2017;32(5):701–15 e7. https://doi.org/10.1016/j.ccell.2017.08.005.
63. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, et al. Mutational landscape and significance across 12 major cancer types. Nature. 2013;502(7471):333–9. https://doi.org/10.1038/nature12634.
64. Pietzak EJ, Bagrodia A, Cha EK, Drill EN, Iyer G, Isharwal S, et al. Next-generation sequencing of nonmuscle invasive bladder cancer reveals potential biomarkers and rational therapeutic targets. Eur Urol. 2017;72(6):952–9. https://doi.org/10.1016/j.eururo.2017.05.032.
65. Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell. 2017;171(3):540–56 e25. https://doi.org/10.1016/j.cell.2017.09.007.
66.• Seiler R, Ashab HAD, Erho N, van Rhijn BWG, Winters B, Douglas J, et al. Impact of molecular subtypes in muscle-invasive bladder cancer on predicting response and survival after neoadjuvant chemotherapy. Eur Urol. 2017;72(4):544–54. https://doi.org/10.1016/j.eururo.2017.03.030. **Seminal article on responsiveness of the various molecular subtypes of muscle-invasive bladder to neoadjuvant chemotherapy. This work demonstrates the future of personalized medicine where genomic data will be used for designing an optimal treatment plan.**
67. Yap KL, Kiyotani K, Tamura K, Antic T, Jang M, Montoya M, et al. Whole-exome sequencing of muscle-invasive bladder cancer identifies recurrent mutations of UNC5C and prognostic importance of DNA repair gene mutations on survival. Clin Cancer Res. 2014;20(24):6605–17. https://doi.org/10.1158/1078-0432.CCR-14-0257.
68. McConkey DJ, Choi W. Molecular subtypes of bladder cancer. Curr Oncol Rep. 2018;20(10):77. https://doi.org/10.1007/s11912-018-0727-5.
69. Lerner SP, McConkey DJ, Hoadley KA, Chan KS, Kim WY, Radvanyi F, et al. Bladder cancer molecular taxonomy: summary from a consensus meeting. Bladder Cancer. 2016;2(1):37–47. https://doi.org/10.3233/BLC-150037.
70. Siefker-Radtke AO, Dinney CP, Shen Y, Williams DL, Kamat AM, Grossman HB, et al. A phase 2 clinical trial of sequential neoadjuvant chemotherapy with ifosfamide, doxorubicin, and gemcitabine followed by cisplatin, gemcitabine, and ifosfamide in locally advanced urothelial cancer: final results. Cancer. 2013;119(3):540–7. https://doi.org/10.1002/cncr.27751.
71. Matulay JT, Kamat AM. Advances in risk stratification of bladder cancer to guide personalized medicine. F1000Res. 2018;7. https://doi.org/10.12688/f1000research.14903.1.