available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Platinum Priority – Brief Correspondence

Editorial by Jon L. Griffin on pp. 23–24 of this issue

Intratumoral Heterogeneity of Bladder Cancer by Molecular Subtypes and Histologic Variants

Joshua I. Warrick[a,b,*], *Gottfrid Sjödahl*[c], *Matthew Kaag*[b], *Jay D. Raman*[b], *Suzanne Merrill*[b], *Lauren Shuman*[a], *Guoli Chen*[a], *Vonn Walter*[d], *David J. DeGraff*[a,b]

[a] *Department of Pathology—Penn State College of Medicine, Hershey, PA, USA;* [b] *Department of Surgery, Division of Urology—Penn State College of Medicine, Hershey, PA, USA;* [c] *Division of Urological Research, Department of Translational Medicine, Lund University, Skåne University Hospital, Malmö, Sweden;* [d] *Department of Public Health Sciences, Penn State College of Medicine, Hershey, PA, USA*

Article info

Article history:
Accepted September 1, 2018

Associate Editor:
James Catto

Statistical Editor:
Andrew Vickers

Keywords:
Molecular subtype
Histologic variant
Intratumoral heterogeneity

EU ✱ ACME
www.eu-acme.org/europeanurology

Please visit www.eu-acme.org/europeanurology to answer questions on-line. The EU-ACME credits will then be attributed automatically.

Abstract

Molecular subtyping may inform on prognosis and treatment response in bladder cancer. However, intratumoral molecular heterogeneity is not well studied in this disease and could complicate efforts to use molecular subtyping to guide patient management. To investigate intratumoral heterogeneity in bladder cancer, we examined molecular subtypes in a consecutive, retrospective cystectomy series of histologic variant bladder cancers and conventional urothelial carcinomas co-occurring with them. Molecular subtypes were assigned as per the approach reported by Lund University, an approach that incorporates cell cycle alterations and markers of differentiation, to give the urothelial-like, genomically unstable, basal-squamous, mesenchymal-like, and neuroendocrine-like subtypes. The majority (93%) of tumors were classified as urothelial like, genomically unstable, or basal squamous. Among patients with more than one tumor histology, 39% demonstrated molecular heterogeneity among the different tumor histologies. This was greatest for the basal-squamous subtype, 78% of which co-occurred with either urothelial-like or genomically unstable carcinoma (among cases with multiple histologies). In contrast, there was no co-occurrence of urothelial-like and genomically unstable carcinoma in the same patient. The findings indicate that bladder cancer is often molecularly heterogeneous, particularly in the basal-squamous subtype. This raises the concern for sampling error in laboratory tests that guide therapy based on molecular subtyping.

Patient summary: In this report, we investigated molecular diversity among different areas from the same tumor in patients with bladder cancer. We found that different areas from the same tumor are often molecularly different. We conclude that this biological diversity must be taken into account when interpreting clinical molecular tests performed on bladder cancer samples.

© 2018 European Association of Urology. Published by Elsevier B.V. All rights reserved.

* Corresponding author. Department of Pathology, Milton S. Hershey Medical Center, Penn State College of Medicine, 500 University Drive, Hershey, PA 17033, USA. Tel. +1-717-531-8246; Fax: +1-717-531-5311.
E-mail address: jwarrick@pennstatehealth.psu.edu (J.I. Warrick).

Muscle-invasive bladder cancer can be grouped into discreet molecular subtypes based on gene expression signatures [1–5]. Molecular subtyping is potentially clinically important, because it may predict patient survival [3], and response to conventional neoadjuvant chemotherapy [6] and system checkpoint inhibitors [7]. The majority of subtyping schema broadly classify tumors as either luminal, which express markers of urothelial differentiation, or basal squamous, which express markers of basal cell or squamous differentiation. Additional subtypes are neuroendocrine,

https://doi.org/10.1016/j.eururo.2018.09.003
0302-2838/© 2018 European Association of Urology. Published by Elsevier B.V. All rights reserved.

mesenchymal, and inflammatory. Uniquely, a subtyping schema developed at Lund University includes markers of cell cycle regulation, including RB1 and p16 (*CDKN2A*), in addition to markers of urothelial and basal/squamous differentiation [4,5]. This schema classifies tumors as urothelial like, genomically unstable, basal squamous, mesenchymal like, or small cell/neuroendocrine like (detailed in Supplementary Table 2).

Bladder cancer is often histologically heterogeneous within a given patient. This is well demonstrated by the frequent co-occurrence of conventional urothelial carcinoma and named histologic variants of bladder cancer, such as squamous and micropapillary. Recent data indicate that molecular subtype may also be heterogeneous [8], an observation that may complicate translating molecular subtyping into patient care. To investigate this, we studied molecular subtypes in a series of histologic variant bladder cancers—specifically squamous, glandular, micropapillary, nested, plasmacytoid, and sarcomatoid—and conventional urothelial carcinomas co-occurring with these variants. In brief, we assembled a consecutive retrospective series of 309 cystectomy cases performed for bladder cancer. Histology slides were re-reviewed, and cases with variant histology were selected ($n = 83$). Molecular subtyping was performed on the histologic variants and any co-occurring conventional urothelial carcinoma, using the immunohistochemistry-based method developed at Lund University [4].

The vast majority (93%) of tumors in our study were classified as basal squamous, urothelial like, or genomically unstable. Tumors with the same subtype clustered together using agglomerative methods, as shown in Figure 1A, corroborating the utility of the Lund approach. Clustering was driven largely by differences in expression of markers of the basal-squamous subtype, and differences in expression of p16 and RB1, which are inversely expressed in urothelial-like (p16–/RB1 +) and genomically unstable tumors (p16+/RB1–). Expression of p16 and RB1 was mutually exclusive ($p < 0.0001$, Fisher's exact test); 93% of tumors were negative for at least one of these markers. Specific histologic variants tended toward specific subtypes, as shown in Figure 1B, keeping with prior studies [3,9].

As shown in Figure 2A, 39% of cases with multiple tumor histologies demonstrated molecular heterogeneity among the different histologies ($n = 41$ cases). This followed discernable patterns. Specifically, squamous-basal tumors had the greatest heterogeneity, with 78% co-occurring with either genomically unstable or urothelial-like carcinoma (among cases with squamous and another histology). In contrast, there was no co-occurrence of urothelial-like and genomically unstable carcinoma in the same patient. Stated differently, while co-occurring tumors often differed in markers of basal-squamous differentiation, tumors rarely differed in markers of cell cycle regulation, including loss of p16 or RB1expression (Supplementary Table 5 and Supplementary Fig. 1). This finding suggests that loss of either p16 or RB1 occurs early in bladder cancer evolution, while basal-squamous differentiation occurs later. To further test if loss of p16 and RB1 occurs early, we subtyped 60 noninvasive carcinomas, including noninvasive papillary urothelial carcinoma and flat urothelial carcinoma in situ. We found that all typable cases were either urothelial like or genomically unstable, and RB1 and p16 losses were mutually exclusive ($p = 0.002$, Fisher's exact test), although only 73% of cases lost one of these markers, a smaller fraction than in the invasive tumors (Supplementary Fig. 2). These findings corroborate that RB1 and p16 losses are early events in bladder cancer evolution.

There was a significantly lower basal-squamous cancer burden in bladders treated with neoadjuvant chemotherapy compared with those receiving upfront cystectomy ($p = 0.02$, Wilcoxon; see the Supplementary material). Burden of neither urothelial-like nor genomically unstable cancer differed by neoadjuvant chemotherapy status.

The findings of this study have important clinical implications. Prior studies have shown that response to platinum-based neoadjuvant chemotherapy is most effective in muscle-invasive bladder cancer with a basal-squamous molecular subtype [6]. Indeed, we found burden of basal-squamous disease was lower in patients receiving neoadjuvant chemotherapy, keeping with this finding. Laboratory tests for a basal-squamous signature have thus been developed to predict response to neoadjuvant chemotherapy. However, as we found that basal-squamous carcinomas often co-occur with carcinoma of another molecular subtype, our findings raise concern for a sampling error in molecularly heterogeneous tumors. In such cases, molecular subtyping from limited samples could misinform on chemotherapy response and treatment recommendations. Separately, if all invasive carcinoma within a patient's bladder is driven by a common genetic event—namely, loss of p16 or RB1 – treatment strategies that exploit this homogeneity may be effective. For example, tumors with p16 loss may respond uniformly to the CDK4/6 inhibitor abemaciclib.

The findings also offer insight into the biology of bladder cancer evolution, particularly the order of occurrence of major genomic events as bladder cancer evolves through stage and histology. Prior studies have shown that specific histologic variants arise from conventional urothelial carcinoma, consequent to characteristic molecular alterations. For example, plasmacytoid urothelial carcinoma arises from conventional urothelial carcinoma through loss of e-cadherin function, often via mutation [10]. A proposed framework for molecular and histomorphologic evolution is presented in Figure 2B. In short, we propose that bladder cancer typically begins as a precursor with intact cell cycle regulation, such as urothelial dysplasia or early noninvasive, low-grade papillary neoplasia. These precursors then lose either p16 or RB1 expression, invade as conventional urothelial carcinoma with a urothelial-like or genomically unstable molecular subtype, and then progress to basal-squamous carcinoma and histologic variants.

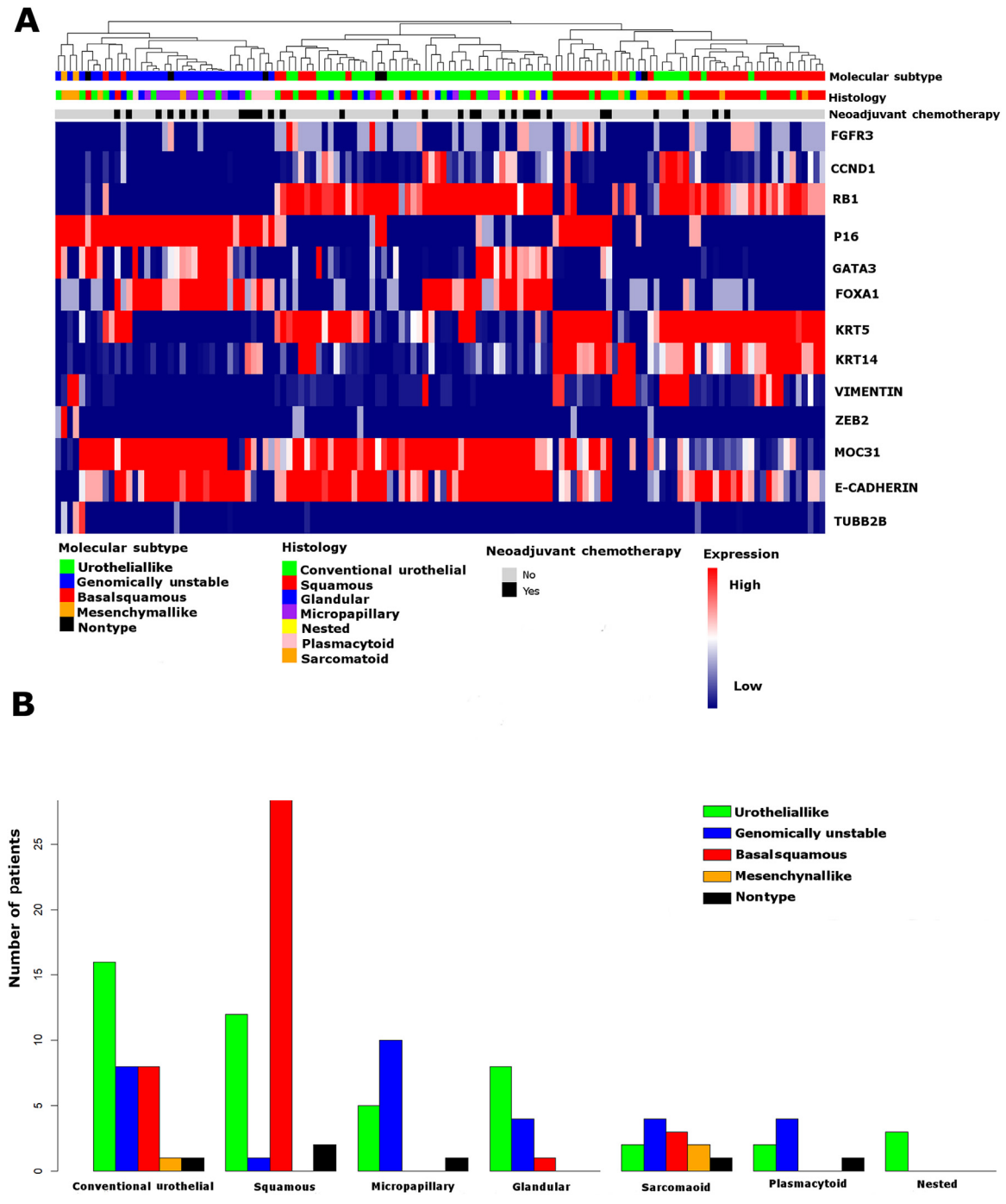

Fig. 1 – Molecular subtypes in histologic variants and conventional urothelial carcinomas. (A) A heatmap of marker expression shows molecular subtypes clustered robustly by agglomerative methods. Expression of p16 and RB1 was mutually exclusive, including in basal-squamous carcinomas. Rows are genes; columns are patients. (B) Specific histologic variants tended to have specific molecular subtypes. The barplot shows histologic variants and the number of cases with each molecular subtype.

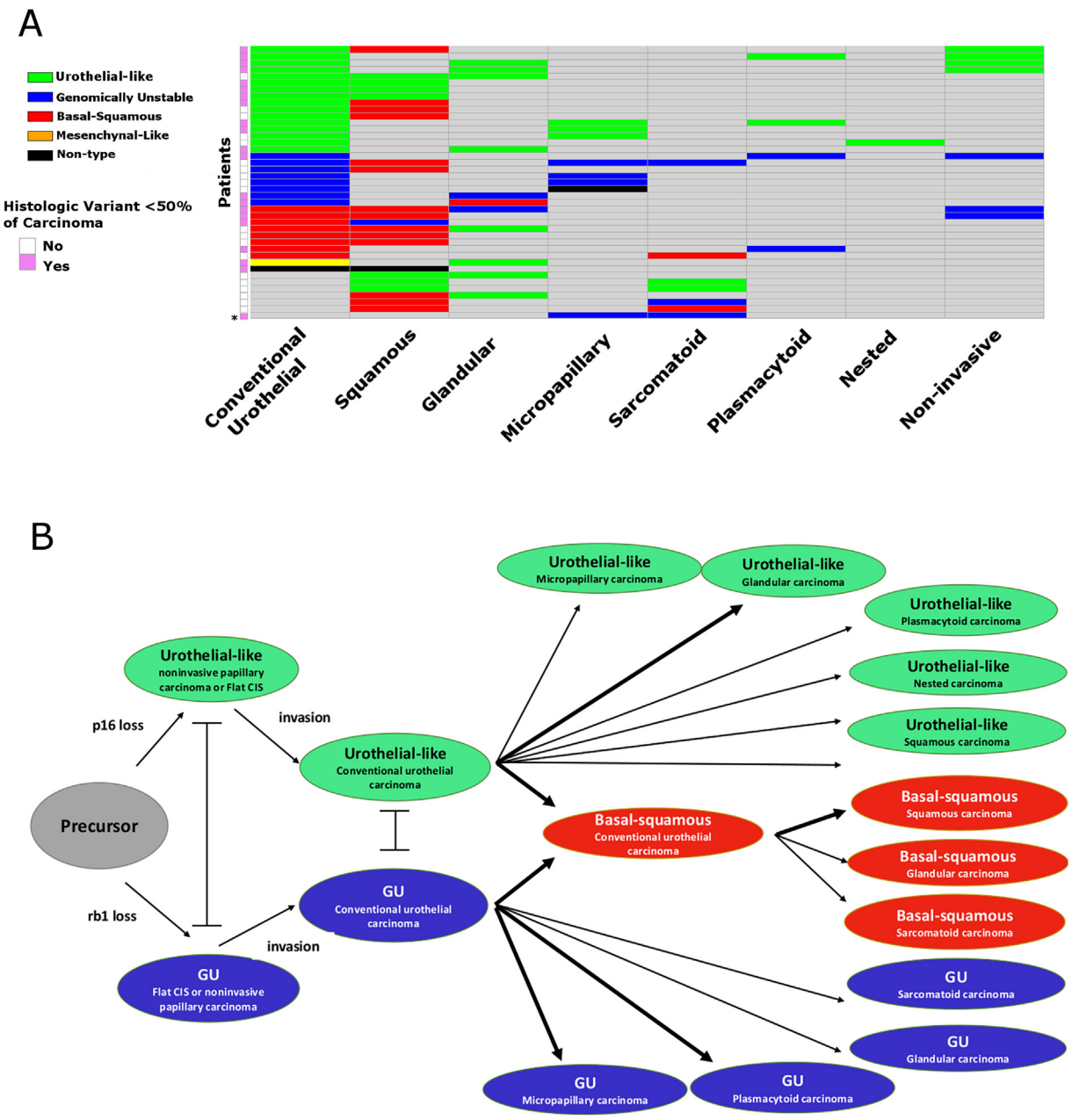

Fig. 2 – Molecular and histomorphologic heterogeneity in bladder cancer. (A) Histologic and molecular heterogeneity presented for each patient with multiple histologies; each row is an individual patient, and each column represents a different histology. Also indicated is whether histologic variant accounted for <50% of tumor burden, an observation relevant to inclusion in clinical trials. Noninvasive = either flat urothelial carcinoma in situ or noninvasive papillary urothelial carcinoma. *This cancer contained >50% conventional urothelial carcinoma, but immunohistochemistry failed for this histology and it was thus not subtyped. (B) Theoretical framework for evolution of bladder cancer. In this model, bladder cancer begins as early, noninvasive neoplasia. This precursor then loses expression of a major cell cycle regulator, either p16 or RB1. The tumor then invades as conventional urothelial carcinoma, either urothelial like or genomically unstable, either of which may evolve to basal-squamous carcinoma. Histologic variants evolve from these, through additional genomic alterations. Greater arrow thickness indicates increased probability that a case will follow the given evolutionary step. CIS = carcinoma in situ; GU = genomically unstable.

In conclusion, through evaluating histologic variants and co-occurring conventional urothelial carcinomas, we have shown that molecular heterogeneity is common in muscle-invasive bladder cancer and occurs in a somewhat predictable manner. This knowledge may guide future utilization of molecular subtyping in the care of patients with bladder cancer and particularly suggests caution in using genomic classifiers based on limited tissue sampling.

Author contributions: Joshua I. Warrick had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Warrick, DeGraff, Sjödahl.
Acquisition of data: Warrick, Chen, Kaag, Shuman, DeGraff.
Analysis and interpretation of data: Warrick, Sjödahl, DeGraff, Walter.
Drafting of the manuscript: Warrick.
Critical revision of the manuscript for important intellectual content:

Warrick, Sjödahl, Kaag, Merrill, Raman, Chen, Walter, Shuman, DeGraff.
Statistical analysis: Warrick.
Obtaining funding: Warrick.
Administrative, technical, or material support: Shuman.
Supervision: Warrick, DeGraff, Sjödahl.
Other: None.

Financial disclosures: Joshua I. Warrick certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2018.09.003.

References

[1] Choi W, Porten S, Kim S, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell 2014;25:152–65.
[2] Damrauer JS, Hoadley KA, Chism DD, et al. Intrinsic subtypes of high-grade bladder cancer reflect the hallmarks of breast cancer biology. Proc Natl Acad Sci U S A 2014;111:3110–5.
[3] Robertson AG, Kim J, Al-Ahmadie H, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell 2017;171, 540–56 e25.
[4] Sjodahl G, Eriksson P, Liedberg F, Hoglund M. Molecular classification of urothelial carcinoma: global mRNA classification versus tumour-cell phenotype classification. J Pathol 2017;242:113–25.
[5] Sjodahl G, Lauss M, Lovgren K, et al. A molecular taxonomy for urothelial carcinoma. Clin Cancer Res 2012;18:3377–86.
[6] Seiler R, Ashab HAD, Erho N, et al. Impact of molecular subtypes in muscle-invasive bladder cancer on predicting response and survival after neoadjuvant chemotherapy. Eur Urol 2017;72:544–54.
[7] Mariathasan S, Turley SJ, Nickles D, et al. TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature 2018;554:544–8.
[8] Thomsen MBH, Nordentoft I, Lamy P, et al. Comprehensive multiregional analysis of molecular heterogeneity in bladder cancer. Sci Rep 2017;7:11702.
[9] Guo CC, Dadhania V, Zhang L, et al. Gene expression profile of the clinically aggressive micropapillary variant of bladder cancer. Eur Urol 2016;70:611–20.
[10] Al-Ahmadie HA, Iyer G, Lee BH, et al. Frequent somatic CDH1 loss-of-function mutations in plasmacytoid variant bladder cancer. Nat Genet 2016;48:356–8.