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ELSEVIER

Urologic Oncology: Seminars and Original Investigations ▮ (2016) ▮▮▮–▮▮▮

UROLOGIC ONCOLOGY

Original article

Ulceration in bladder cancer associates with extravesical disease, independent of cell cycle, or hypoxia pathways status Integrating gross morphology and expression profiles in cystectomies

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Received 1 March 2016; received in revised form 16 May 2016; accepted 30 May 2016

Abstract

Objective: Ulceration is common in bladder tumors, but its prognostic role, although intuitive, is not established. We aim to explore the presence of gross ulceration and its relationship with other morphological and biological features classically associated with extravesical disease, in patients submitted to radical cystectomy.

Methods: Tumor size and morphology were noted on 101 cystectomy patients (2000–2010). Papillary, exophytic, and vegetant tumors were grouped as "papillary" and solid/nodular, ulcerated and infiltrative as "nonpapillary." Ulceration was noted grossly in every case as a binary parameter, regardless of morphology. Immunohistochemistry was performed for hypoxia (hypoxia-inducible factor-1α and vascular endothelial growth factor), and cell cycle proteins (pRb, p53, and cyclin D1).

Results: Mean age was 66.7 year, male:female ratio was 2:1, 20 patients received bacillus Calmette-Guerin and 10 neoadjuvant chemotherapy. Upstaging rate was 56.4%. Ulcerated lesions presented mostly as nonpapillary and nonorgan confined (nOC), whereas nonulcerated tumors were often papillary and organ confined (OC). Tumor size was smaller in nonpapillary tumors ($P = 0.002$), but did not associate with altered hypoxia or cell cycle expressions. pRb and cyclin D1 loss and p53 overexpression were more frequent in ulcerated and non-OC tumors as did the phenotype vascular endothelial growth factor-negative/hypoxia-inducible factor-1α-low ($P < 0.001$). On a multivariate model, ulceration was an independent predictor of non-OC and extravesical disease.

Conclusion: Patients with ulcerated tumors were often staged with extravesical disease, independent of other morphologic and biological features known to affect prognosis. Prospective studies are needed to confirm the predictive value of tumor ulceration at cystoscopy, which could improve patient stratification for neoadjuvant chemotherapy.

Graphical abstract: Interactions between clinical and biological parameters of known prognostic relevance in bladder cancer, and its relationship with tumor ulceration.

Keywords: Bladder cancer; Ulceration; Morphology; Hypoxia; Cell cycle; pappilary

1. Introduction

Radical cystectomy is indicated in patients with muscle-invasive bladder cancer (MIBC) and treatment-refractory non–MIBC (NMIBC). Outcome after surgery differs significantly between organ confined (OC) and extravesical

This work was financially supported by FAPESP (grant 2011/18476-6) (São Paulo State Research Support Foundation), Brazil.

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http://dx.doi.org/10.1016/j.urolonc.2016.05.031
1078-1439/

Fig. 1. Anatomical display (coronal cut surface) of a cystoprostatectomy specimen during gross accession. There is a flat ulcerated lesion on the bladder dome, categorized as "Infiltrative with ulceration" which, despite small, was non–organ-confined. (Color version of figure is available online.)

tumors [1], and it seems that patients with extravesical disease would benefit the most from neoadjuvant therapy [2]. Conventional preoperative clinical staging, however, is suboptimal and postsurgical up classification has ranged from 42% to 80% in current series [3,4].

Therefore, there has been an increased interest in developing preoperative models and nomograms to aid this distinction with accuracy approximately 80% [5–8]. Variables include demographics (age and sex), cystoscopy findings (palpable mass under anesthesia, number, and size of lesions), imaging alterations (hydronephrosis, suggested extravesical fat/lymph node involvement), and finally, transurethral resection biopsy (TURB) information (number of biopsies, pathological stage and grade, aggressive histology, lymphovascular invasion, and adjacent carcinoma in situ). Such variety reflects the limited predictive power of individual parameters, and therefore new prognostic tools are welcome. Tumor morphology and ulceration have not been evaluated in this scenario.

College of American Pathologists (CAP) cancer protocols are detailed checklists to capture vital clinical content for all cancer pathology reporting, based on the American Joint Committee on Cancer Union for International Cancer Control/TNM staging. In 2000, the fifth edition on bladder cancer recommended the following 4 morphological gross categories: pure papillary, solid, ulcerated, and indeterminate. The following editions added the flat category and removed the term "pure" from papillary. Furthermore, these descriptions were then considered optional, with a note that they may be clinically important, but not yet validated or regularly used in patient management. In fact, bladder cancer morphology has only recently gained credit by important research that link molecular subtypes with papillary/non-papillary features and squamous differentiation, although accurate definitions have not been provided [9].

The association of ulceration and tumor gross morphology is well established in malignancies of the skin and gastrointestinal tract where they are used for staging and prediction of chemotherapy response, outcome, and survival [10–12]. There have been a few studies on the prognostic significance of bladder cancer gross morphology [13,14] as Kakizoe et al. [15,16] acknowledged the coexistence of papillary and nodular tumors, developing the idea that nodular carcinomas arise de novo or progress from a previous papillary lesion. This concept has been molecularly better defined in the past 30 years, but morphology remains as an intuitive and subjective concept. The protein expression profiles associated with each oncogenic pathway are explored as diagnostic and prognostic tools, especially regarding cell cycle alterations, and also the hypoxia and PTEN/Akt/mTOR pathways [17–20]. Alterations in gene and expression profiles of these pathways have been associated with ulcerative growth in other organs [21,22]. As any tumor growing toward a lumen or surface, bladder cancer is also subject to ulceration.

This is the first study to specifically analyze ulceration in bladder cancer. Here, we investigate its relationship with gross features, and correlate the findings with protein expression profiles of pathways likely related to the process. Finally, we explore the association of ulceration and extravesical disease, along with well-established prognostic parameters.

2. Materials and methods

All patients who underwent open radical cystectomy for bladder cancer, with extended pelvic lymphadenectomy, from January 2000 to October 2010 were initially enrolled. Bladder specimens with no grossly visible tumors were excluded from the cohort, therefore, pathological classifications [23] pT0 and pTis (including patients with complete pathological response to neoadjuvant therapy) were not included. Also, because this represents a historic series, most patients did not undergo re-TURB before surgery. Totally, 27 patients underwent TURB <42 days before cystectomy and 23 had surgery >42 days after TURB (mean $=$ 336.5 d).

2.1. Pathology

Specimens were processed and diagnosed according to the current editions of CAP/American Joint Committee on Cancer full protocols [24] in a checklist system. Accordingly, tumor size was measured as greatest tumor dimension (GTD) and additional 2 dimensions. The ellipsoid equation ($W^2 \times L \times 0.52$) was applied to calculate tumor volume.