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Original contribution

Clinicopathologic features and utility of immunohistochemical markers in signet-ring cell adenocarcinoma of the bladder☆

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Summary Signet-ring adenocarcinoma is an aggressive form of primary bladder adenocarcinoma that has been associated with poor outcomes. The utility of immunohistochemical markers in tumors with signet-ring morphology may vary from more typical adenocarcinomas arising at the same location, although this has not been examined in bladder adenocarcinoma. We examined a series of bladder adenocarcinomas to determine the impact of signet-ring cell features on clinical outcomes and immunohistochemical findings. We identified 25 patients with bladder adenocarcinoma, ranging in age from 28 to 78 years (mean, 57 years) and with a male-female ratio of 18:7. Six cases were urachal in origin. Signet-ring cells occurred in 19 of 25 bladder adenocarcinomas (76%) and ranged from 5% to 100% of tumor volume, with most tumors demonstrating more than 60% signet-ring cell differentiation (15/19), when present. Regional lymph node metastases were present in 8 of 19 patients (42%) who underwent cystectomy. The percentage of tumor containing signet-ring cells was significantly associated with the presence of *adverse pathologic features* (defined as unresectable primary tumor or regional lymph node metastasis, $P = .013$) and decreased overall survival ($P = .034$), and the latter remained significant in multivariable analysis after adjusting for positive soft tissue margins ($P = .026$). A comparison between immunohistochemical markers frequently used to analyze bladder adenocarcinoma demonstrated decreased expression of several markers in signet-ring (n = 9) versus colonic-type (n = 8) morphology, including CDX-2, β-catenin, and E-cadherin, although these results did not reach statistical significance. In summary, the extent of signet-ring differentiation in bladder adenocarcinoma is associated with worsened survival and higher stage disease; the utility of immunohistochemical analysis in foci consisting of predominant signet-ring cells may be limited, although further studies that address this finding are needed.

1. Introduction

Primary adenocarcinoma of the bladder accounts for less than 2% of all bladder carcinomas and may arise from

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urachal remnants present in the dome of the bladder or, more commonly, from the bladder urothelium at other sites [1-3]. Outcomes for patients with bladder adenocarcinoma are generally poor [4], with increased pathologic stage significantly correlating with worsened overall survival in several studies [5,6]. Although some series have reported that adenocarcinomas of urachal origin may demonstrate more favorable long-term outcomes [3,5], this finding has been controversial and may be associated with the younger age of presentation of urachal adenocarcinomas. Extensive intestinal metaplasia, either alone or in the setting of bladder extrophy, has been proposed to represent a risk factor in the development of bladder adenocarcinoma [7,8], although these findings have been challenged in recent years [9]. Additional risk factors include villous adenoma and the presence of a cystocele [2,10].

Multiple histologic subtypes of bladder adenocarcinoma exist and include colonic-type adenocarcinoma, mucinous adenocarcinoma, signet-ring cell carcinoma, clear cell carcinoma, hepatoid carcinoma, and adenocarcinoma not otherwise specified, as well as mixed forms [5,11-15]. The morphology of these lesions demonstrates significant overlap between urachal and nonurachal bladder adenocarcinomas, although urachal-derived adenocarcinomas may more frequently demonstrate a mucinous appearance [5]. In general, the diagnosis of a urachal primary can be difficult and is generally based on the presence of a lesion at the dome of the bladder, association with urachal remnants or involvement of the urachal tract, lack of a separate lesion within the bladder, and sharp demarcation from the surrounding urothelium [16], rather than the presence of specific morphologic findings.

Finally, the distinction between primary adenocarcinomas of the bladder and those secondarily involving the bladder either by direct extension or metastatic spread has been the focus of many immunohistochemical studies [17]. Specifically, most markers examined have concentrated on the differentiation between colon carcinoma and colonic-type bladder adenocarcinoma because of the morphologic similarity between these 2 entities. Of numerous markers examined, including cytokeratin (CK) 7, CK20, villin-1, CDX-2, β-catenin, and others, absence of CDX-2 and villin-1 expression appears to be the most robust markers in the determination of primary bladder origin [18-20]. However, the utility of these markers in other variants of bladder adenocarcinoma is unclear, with studies from other anatomical locations suggesting that the immunohistochemical profiles of signet-ring cell carcinoma, for example, may vary from more typical adenocarcinomas arising at the same site [21].

We examined all cases of primary bladder adenocarcinoma identified at our institution to evaluate the extent of signet-ring differentiation within these lesions and to evaluate the utility of currently used immunohistochemical stains in this morphologic subtype.

2. Materials and methods

2.1. Patient specimens

This study was performed with approval from the Institutional Review Board. Archives from 1980 to 2007 were searched for all cases of primary bladder adenocarcinoma, including urachal and nonurachal bladder origin. A retrospective review of patient records was performed to exclude patients with a prior or subsequent history of adenocarcinoma at any other anatomical site, including the gastrointestinal tract, breast, and prostate. Specifically, all patients diagnosed with adenocarcinoma of the bladder were subsequently evaluated by chest radiograph, mammogram, colonoscopy, and upper endoscopy to exclude primaries at other locations. Any case classified as urothelial carcinoma with glandular differentiation was excluded from further study. In total, bladder adenocarcinoma specimens accounted for approximately 1% of all bladder neoplasms identified during this period and included 6 partial cystectomies with pelvic lymph node dissection, 13 radical cystectomies with pelvic lymph node dissection, and 6 biopsies/transurethral resection (TUR) specimens from patients with unresectable disease. All hematoxylin and eosin material was entirely reviewed to determine pathologic stage, morphology of the invasive adenocarcinoma component, extent of signet-ring cell differentiation, and associated superficial component. Prior biopsy or TUR material was available for review on 12 of 19 patients who underwent subsequent partial or radical cystectomy and was reviewed both to exclude patients with invasive urothelial carcinoma with glandular differentiation, as well as to correlate the morphology between the biopsy/TUR and resection specimen.

2.2. Immunohistochemical analysis

Tissue microarrays were constructed using formalin-fixed, paraffin-embedded tissue from all specimens represented in this study, with utilizable material ultimately available from 17 cases because of loss of tissue or sampling error. The 17 invasive bladder adenocarcinoma cases included 5 urachal and 12 nonurachal adenocarcinomas. Each specimen was represented by three to four 1.5-mm cores to obtain adequate representation of different regions of neoplastic cells to assess for heterogeneity of protein expression, and emphasis was placed on the comparison of signet-ring cell to colonic-type morphology present within these specimens. Additional nonneoplastic tissue from other histologic sites was included on the tissue microarrays to serve as immunohistochemical controls. Immunohistochemical analysis was performed using the avidin-biotin complex immunoperoxidase technique. Antibodies used for study included CK20 (1:20; DAKO, Carpinteria, CA), CK7 (1:40, DAKO), CDX-2 (1:10; Biogenex, San Ramon, CA), β-catenin (1:250; BD Biosciences, San Jose, CA), E-cadherin (1:500; Zymed, San Francisco, CA), α-methylacyl