CME/SAM

CK5, CK5/6, and Double-Stains CK7/CK5 and p53/CK5 Discriminate In Situ vs Invasive Urothelial Cancer in the Prostate

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Key Words: Urothelial carcinoma; Prostate; Staging; Immunohistochemistry; CK5

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Upon completion of this activity you will be able to:
- list immunohistochemical markers for staining urothelial carcinoma and basal cells within the prostate gland.
- describe the characteristic staining pattern of single and combined immunohistochemical markers for distinguishing in situ from invasive urothelial carcinoma within the prostate.
- diagnose pT4a urothelial carcinoma in the prostate using immunohistochemistry.

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Questions appear on p 305. Exam is located at www.ascp.org/ajcpcme.

Abstract

For primary bladder tumors, distinguishing urothelial carcinoma (UC) invading the fibromuscular stroma of the prostate (pT4a) from in situ UC involving prostatic ducts can be difficult. Immunohistochemical markers (cytokeratin [CK]5/6, CK5, CK7, CK20, p53, p63, high-molecular-weight keratin [HMWK], androgen receptor, prostate-specific antigen [PSA], prostate specific acid phosphatase [PSAP], laminin, CD44s, CD141) were assessed for their usefulness in determining depth of UC invasion in the prostate. In cystoprostatectomy specimens containing in situ UC in prostatic ducts, both CK5/6 and CK5 clearly differentiated prostatic basal cells from in situ UC. The remaining markers were not effective in determining depth of tumor invasion. Double-stain combinations CK7/CK5 and p53/CK5 were performed and robustly color contrasted in situ tumor from surrounding basal cells. The use of CK5/6, CK5, CK7/CK5, or p53/CK5 is recommended to assist in determining the depth of UC invasion in the prostate when histologic findings are equivocal.

Bladder cancer is the fourth most common cancer in men, with 52,760 new cases per year in the United States, and is responsible for an estimated 10,410 male deaths annually.[1] Histologic staging in biopsy and resection specimens is crucial for correct treatment and prognosis. Bladder cancer is staged using the tumor, nodes, metastasis (TNM) classification system.[2] The pT designation is based on depth of tumor invasion, with deeper invasion correlating with worse prognosis.[2] Tumor invading the fibromuscular stroma of the prostate gland is staged as pT4a.[2]

Urothelial carcinoma (UC) involving the prostate is almost always the result of secondary spread from primary bladder UC, because primary prostatic UC is rare.[3] However, tumor extension into the prostate from primary bladder UC is not uncommon, occurring in 12% to 58% of patients.[4-9] UC may manifest in the prostate via the urethra or direct invasion through the bladder muscle wall.[10] In situ UC occurs in the prostatic urethra or prostatic ducts. Invasive tumor extends into the prostatic periurethral subepithelial tissue or fibromuscular stroma. Invasion into the fibromuscular stroma of the prostate forecasts a far more ominous patient prognosis compared with in situ tumor or subepithelial invasion.[8,11,12] Moreover, treatment options may differ, depending on the extent of prostatic involvement seen in biopsy or radical cystoprostatectomy specimens.[13-15] Conservative management consisting of transurethral resection and bacille Calmette-Guérin (BCG) can be considered in patients whose biopsy specimens show UC in situ in the urethra or prostatic ducts, whereas radical cystoprostatectomy is indicated for prostatic fibromuscular stromal invasion.[15] Patients with prostatic fibromuscular stromal invasion in cystoprostatectomy specimens may benefit

from adjuvant chemotherapy.[13,14] Correctly staging the depth of prostatic involvement with UC (in situ in the urethra/ducts vs invasive into the fibromuscular stroma) is beneficial for patient care.

We aimed to identify immunohistochemical (IHC) markers that could be used to differentiate in situ UC in prostatic ducts from fibromuscular stromal invasion, to assist in the staging of bladder cancer.

Materials and Methods

A small number of cases of primary bladder UC involving the prostate were used to preliminarily assess 12 IHC antibody markers (1 to 6 cases tested per antibody). The antibodies were chosen because of their known reactivity for either prostatic tissue or UC. Antibodies used included p63 (clone BC4A4, 1:300 dilution, BioCare Medical, Concord, CA), high-molecular-weight keratin (HMWK; clone 34βE12, 1:250, DAKO, Carpinteria, CA), CD44s (clone DF1485, 1:200, Leica Microsystems, Buffalo Grove, IL), prostate-specific antigen (PSA; polyclonal, 1:10,000, DAKO), prostate specific acid phosphatase (PSAP; clone PASE/4LJ, 1:150, Cell Marque, Rocklin, CA), androgen receptor (AR; clone AR441, 1:200, DAKO), laminin (clone 4C7, 1:50, DAKO), CK7 (clone OV-YL-12/30, 1:600, DAKO), CK20 (clone K20.8, 1:200, DAKO), CD141 (clone 15C8, 1:200, Leica Microsystems), p53 (clone DO-7, 1:1,000, DAKO) and CK5/6 (clone D5/16B4, 1:50, DAKO). A more recently available antibody, CK5 (clone XM26, 1:150 dilution, Leica Microsystems), was also evaluated based on the results from CK5/6. IHC markers unable to differentiate UC from basal cells of the ducts were not further analyzed, whereas markers showing promise were investigated as described herein.

Based on the initial findings, CK5/6 and CK5 were tested in 41 cystoprostatectomy specimens containing UC in the prostate. Four-µm sections were dried at 55°C for 3 hours, then subjected to heat-induced epitope retrieval, 30 minutes for CK5/6 and 20 minutes for CK5, at pH 8.0 on a Leica-Bond Autostainer. Bond Polymer Refine Detection kit was used and the sections were counterstained with hematoxylin.

Subsequently, various double-stain combinations using p53, CK5, CK7, and AR were tested in a small number of cases (3 to 4 cases tested per antibody combination) using various combinations of brown and red chromogen. Based on preliminary observations, p53 (brown)/CK5 (red) and CK7 (red)/CK5 (brown) were chosen for further validation on an additional 19 cystoprostatectomy specimens using the following conditions. Four-µm sections were dried at 55°C for 3 hours, then subjected to heat-induced epitope retrieval for 20 minutes at pH 6.0 for p53 (brown)/CK5 (red) and pH 8.0 for CK7 (red)/CK5 (brown), on a Leica-Bond Autostainer. Bond Polymer Refine Detection kits were used sequentially and the sections were counterstained with hematoxylin. Bond Polymer Refine Detection Kit was used as the brown chromogen and Bond Polymer Refine Red Detection Kit was used as the red chromogen.

Immunoreactivity was semiquantitatively evaluated as negative (0, <5% of cells stained), focally positive (1+, 5%-10% of cells stained), positive (2+, 11%-50% of cells stained) or diffusely positive (3+, >50% of cells stained). Staining intensity was graded from 0 to 3 and a mean intensity was calculated. Appropriate positive controls were used based on the antibody tested.

Results

Twelve IHC antibody markers (p63, HMWK, CD44s, PSA, PSAP, AR, laminin, CK7, CK20, CD141, p53, CK5/6) were preliminarily evaluated on a small number of cases for their usefulness in staging primary UC of the bladder with prostatic involvement. As anticipated, prostatic basal cells surrounding in situ UC stained positively with p63, HMWK, and CK5/6. In addition, in situ UC expressed p63, HMWK, and sometimes CK5/6; however, differential staining intensity between in situ tumor and basal cells was seen with CK5/6, but not with p63 or HMWK.

CD44s, PSA, PSAP, AR, and laminin were expected to show positivity in the basal cells or the basement membrane of the prostate gland with no expression in UC within the ducts. CD44s was expressed both in UC in situ and basal cells of involved ducts; in addition, reactivity was seen in the fibromuscular stroma, particularly nerves and nerve twigs, with high nonspecific background staining. PSA and PSAP did not show any positivity in either basal cells of involved glands or in UC in situ within the ducts. AR reacted with the nuclei of basal cells of the involved ducts, and was negative in UC within the ducts, but because of weak to moderate staining intensity as well as high expression in the fibromuscular stroma, AR was somewhat difficult to appreciate. Laminin highlighted the basement membrane underneath the basal cells and was negative in UC within ducts; however, components of the fibromuscular stroma were reactive, with a high background, rendering interpretation difficult.

CK7, CK20, CD141, and p53 were expected to show expression in UC in the ducts but not the basal cells surrounding involved prostatic ducts. CK7 was strongly positive in UC in the ducts, and occasionally had weak expression in basal cells of involved glands. CK20 expression was variable in UC in situ, and was negative in basal cells. CD141 did not show reactivity in either UC in situ or circumferential basal cells. p53 had frequent nuclear positivity in UC within ducts, and did not stain the basal cells of these ducts.

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Based on the aforementioned workup, CK5/6 was chosen for further testing, along with the more recently available CK5 antibody. CK5/6 and CK5 were further analyzed in prostate specimens from 41 cystoprostatectomy cases (21 cases invasive UC only, 4 cases in situ UC only, and 16 cases both invasive and in situ UC). In these cystoprostatectomy cases, CK5/6 strongly marked the basal cells of involved prostatic ducts (20/20 cases positive: 100% with 3+ immunoreactivity; mean intensity, 3.0) and was weakly reactive within in situ UC cells (12/20 cases positive: 40% with 0, 20% with 1+, 25% with 2+, and 15% with 3+; mean intensity, 0.9) **Image 1**. In addition, UC invasive into the fibromuscular stroma was also highlighted by CK5/6 with a variable pattern (27/37 cases positive: 27% with 0, 8% with 1+, 8% with 2+, and 57% with 3+; mean intensity, 1.8). CK5 showed results similar to those of CK5/6, but overall was a slightly cleaner marker with less in situ UC reactivity. The basal cells of the prostatic ducts were strongly stained with CK5 (20/20 cases positive: 100% with 3+; mean intensity, 3.0), whereas in situ UC in the ducts was much weaker (4/20 cases positive: 80% with 0, 5% with 1+, 5% with 2+, and 10% with 3+; mean intensity, 0.5) **Image 2**. CK5 positivity in UC invasive into the fibromuscular stroma had an all-or-none result (18/37 cases positive: 51% with 0 and 49% with 3+; mean intensity, 1.5). Both CK5/6

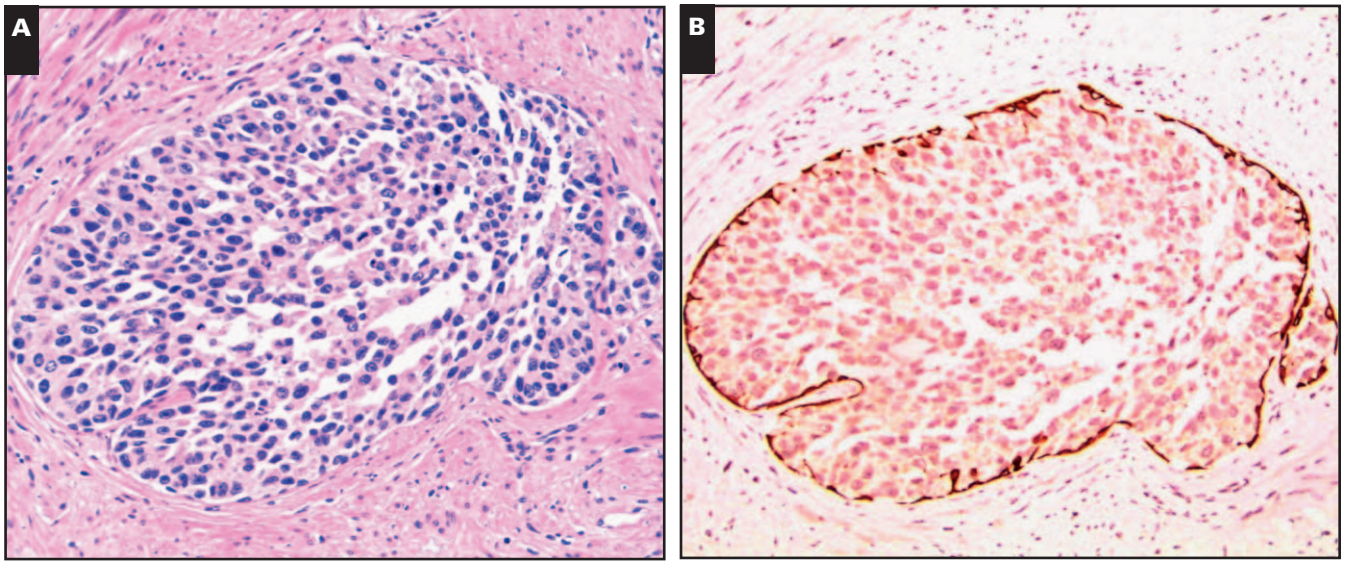

Image 1 H&E (**A**) and cytoplasmic CK5/6 antibody (**B**) highlighting flattened basal cells that circumscribe the weak background reactivity in urothelial cancer (UC) in situ (×20).

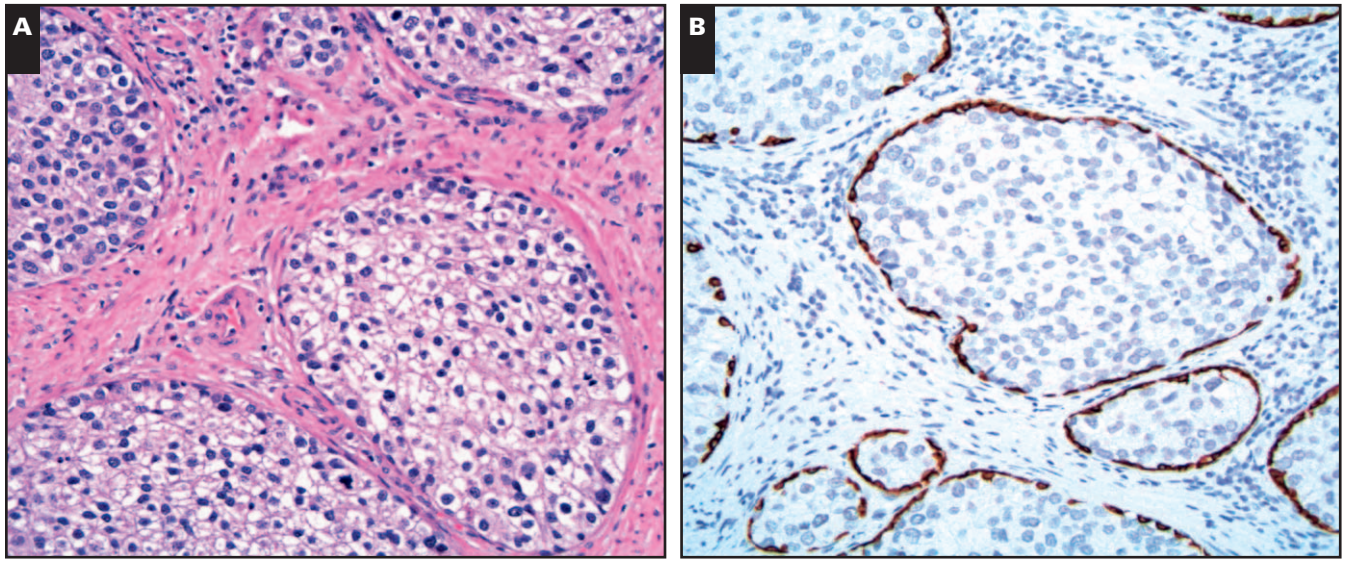

Image 2 H&E (**A**) and cytoplasmic CK5 (**B**) showing similar staining patterns as CK5/6 with no background reactivity (×20).

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and CK5 were negative in the nonneoplastic fibromuscular stroma in all 41 cases. Unremarkable prostatic acinar cells showed minimal reactivity for CK5/6 and CK5.

CK5 (a basal cell marker) and IHC markers known to stain UC, including p53 and CK7, were used in double-stain combinations and evaluated using 19 additional cystoprostatectomy cases of primary bladder UC involving prostate (8 invasive UC only, 7 in situ UC only, and 4 both invasive and in situ UC). The combination of CK7 (red)/CK5 (brown) clearly differentiated in situ from invasive tumor. CK7 strongly stained in situ UC in the ducts (11/11 cases positive: 100% with 3+; mean intensity, 3.0) and was unreactive in basal cells (0/11), while CK5 explicitly demarcated the surrounding basal cells (11/11 cases positive: 100% with 3+; mean intensity, 3.0) and was negative within in situ UC (0/11 cases positive) **Image 3**. CK7 was consistently positive in invasive UC in the fibromuscular stroma (12/12 cases positive: 8.3% with 2+ and 91.7% with 3+; mean intensity, 2.9) and CK5 demonstrated variable positivity (6/12 cases positive: 50.0% with 0, 8.3% with 2+, and 41.7% with 3+; mean intensity, 1.4) **Image 4**. Results using p53 (brown)/CK5 (red) were similar. In situ UC had strong p53 expression (11/11 cases positive: 100% with 3+; mean intensity, 3.0) and basal cells were unreactive (0/11 cases positive), whereas CK5 clearly discriminated the

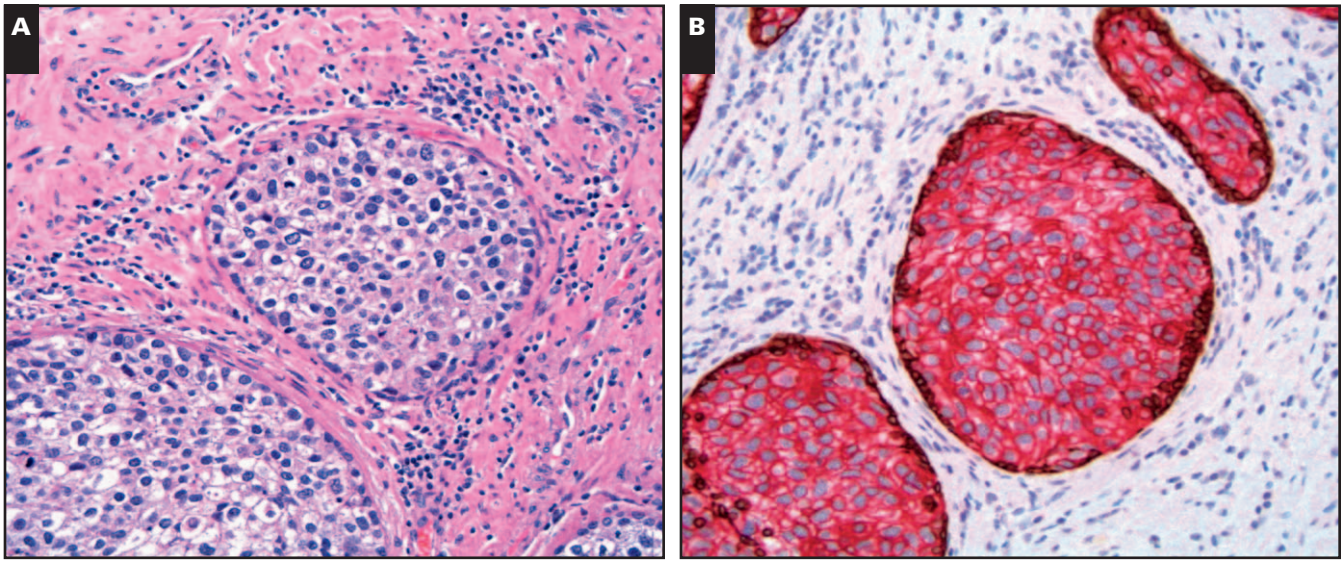

Image3 H&E (**A**) and CK7 (red)/CK5 (brown) double-stain cytoplasmic CK5 (brown) (**B**) highlighting prostatic basal cells, which surround in situ UC highlighted by CK7 (red) (×20).

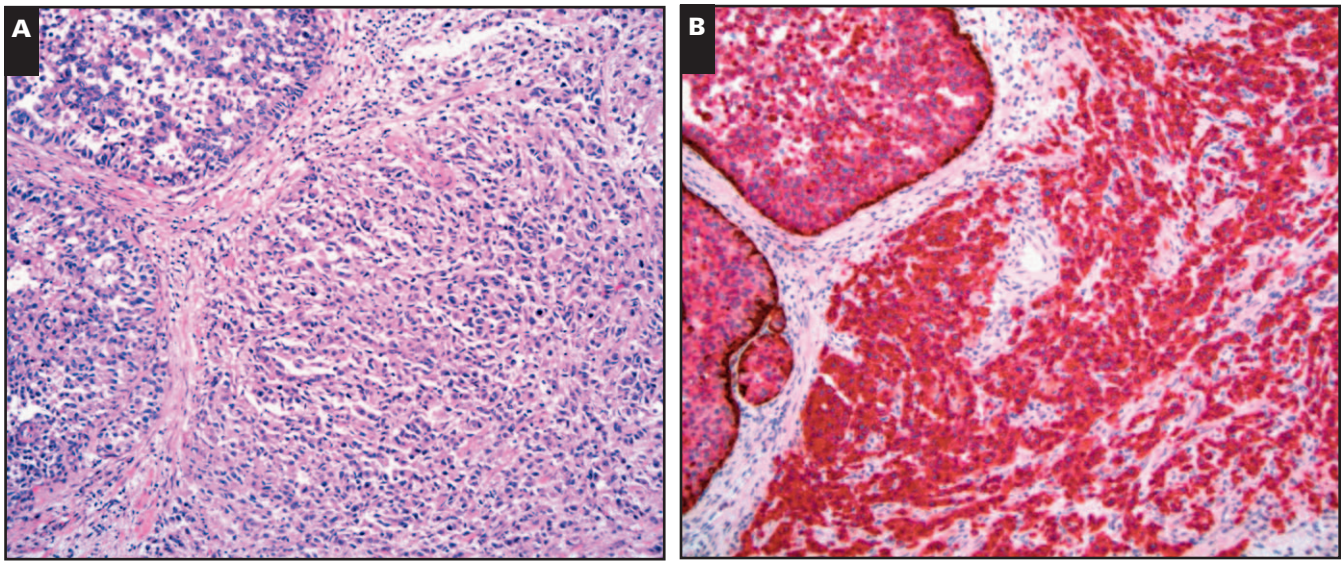

Image 4 H&E (**A**) and double-stain CK7 (red)/CK5 (brown) (**B**) showing in situ UC in red circumscribed by brown basal cells as well as invasive UC with no surrounding brown basal cells (×20).

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circumscribing basal cells (11/11 cases positive; 100% with 3+; mean intensity, 3.0) and was negative in the in situ tumor (0/11 cases positive) **Image 5**. p53 marked invasive UC in the fibromuscular stroma (10/12 cases positive: 16.7% with 0, 16.7% with 1+, 33.3% with 2+, and 33.3% with 3+; mean intensity, 2.0) and CK5 revealed inconsistent staining (6/12 cases positive: 50.0% 0, 50% 3+; mean intensity 1.5) **Image 6**. It also was noted that CK5 had increased reactivity in invasive UC with squamous differentiation in both double-stains **Image 7** and **Image 8**.

Three other double-stain combinations were examined in a limited fashion. First, reversing chromogens to CK7 (brown)/CK5 (red) was difficult to visualize because the brown chromogen of CK7 obscured the red chromogen of CK5 in basal cells. Second, CK7 (red)/AR (brown) was not helpful because AR was a poor marker of basal cells. Finally, p53 (red)/CK5 (brown) was useful, but the unconventional red staining of nuclei was not preferred.

Discussion

Bladder UC is a devastating disease that can involve the prostate gland.[4-9] Primary bladder UC with invasion into the

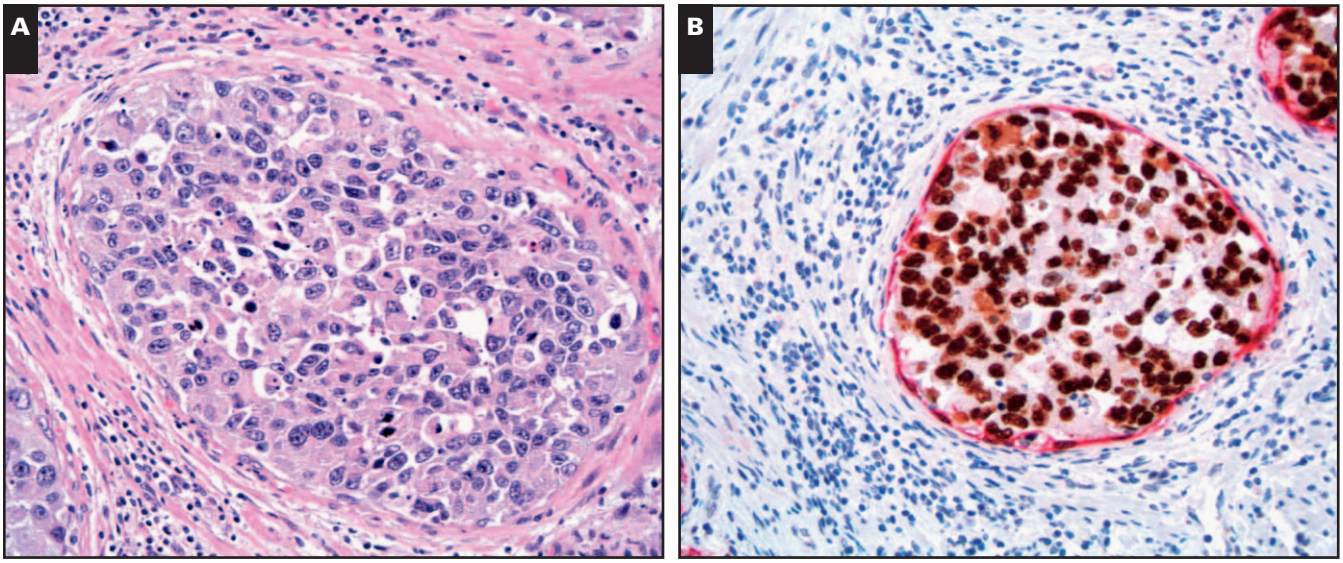

Image 5 H&E (**A**) and p53 (brown)/CK5 (red) double-stain nuclear p53 (brown) (**B**) reacting with in situ UC in an intact prostatic duct with surrounding cytoplasmic CK5 (red) stained basal cells (×20).

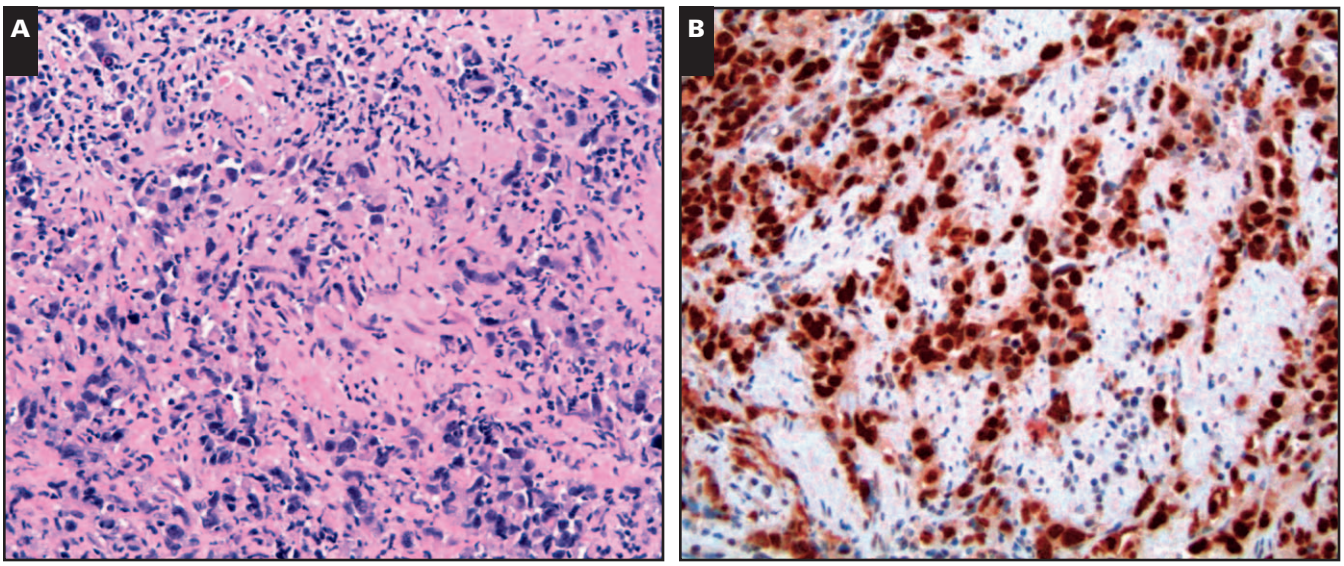

Image 6 H&E (**A**) and p53 (brown)/CK5 (red) double-stain nuclear p53 (brown) and cytoplasmic CK5 (red) (**B**) both reacting with invasive UC in the fibromuscular stroma (×20).

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fibromuscular stroma of the prostate gland is staged as pT4a and has a poor prognosis.[2,8,11,12] Difficulty in histologically determining depth of tumor invasion in the prostate (ie, in situ vs invasive UC) is a current problem affecting the accuracy of bladder cancer staging.[12] Correctly staging bladder UC as pT4a in cystoprostatectomy specimens is important in determining treatment (adjuvant chemotherapy) and prognosis.[13-15] To our knowledge, no previous reports have used IHC to differentiate in situ from invasive UC in the prostate.

Our investigation evaluated CK5/6 and CK5 in cystoprostatectomy specimens from patients with primary bladder UC involving the prostate. Prostatic basal cells surrounding in situ UC were diffusely and strongly positive for CK5/6 and CK5, consistent with the literature reporting CK5/6 as a useful marker to evaluate for the presence of basal cells in foci suspicious for prostatic adenocarcinoma.[16] While we are aware of no prior studies using CK5 for prostate specimens, CK5 has been shown to be superior to CK5/6 for identifying myoepithelial cells in the breast.[17] Both CK5/6 and CK5 were able to reliably and consistently differentiate in situ from invasive UC by highlighting the presence or absence of prostatic basal cells.[16] In situ UC had CK5/6 expression in approximately half of all cases, but the intensity of staining was weak and did not interfere with the detection of basal cells. Using

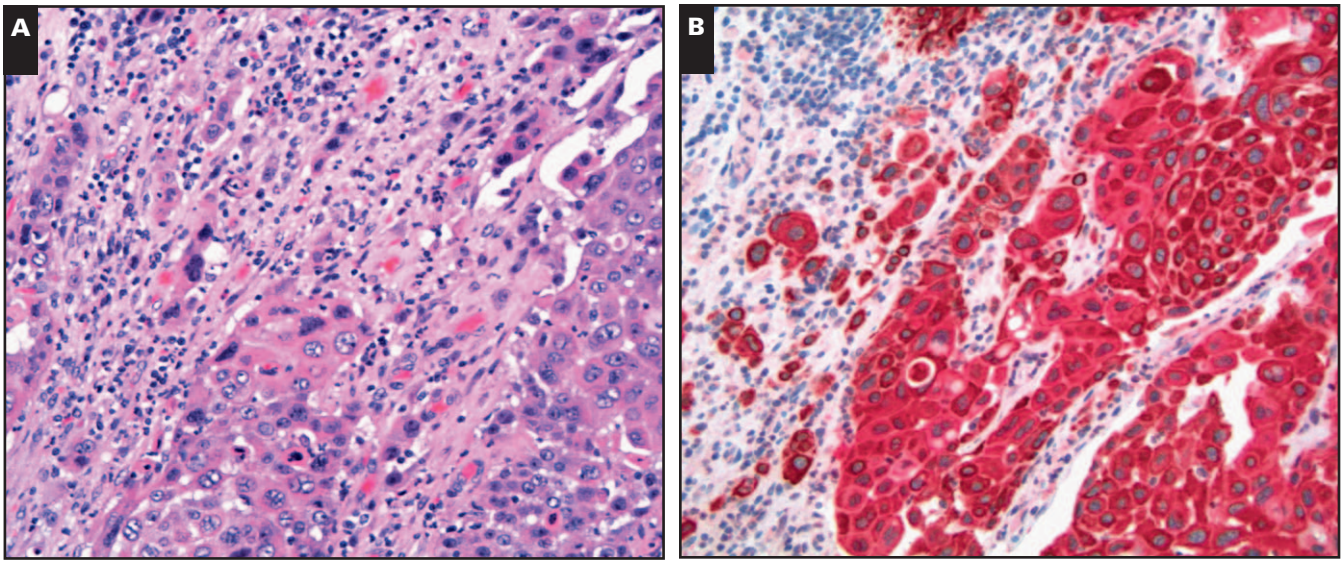

Image 7 H&E (**A**) and double-stain CK7 (red)/CK5 (brown) (**B**) reacting with squamous differentiated invasive UC in the fibromuscular stroma (×20).

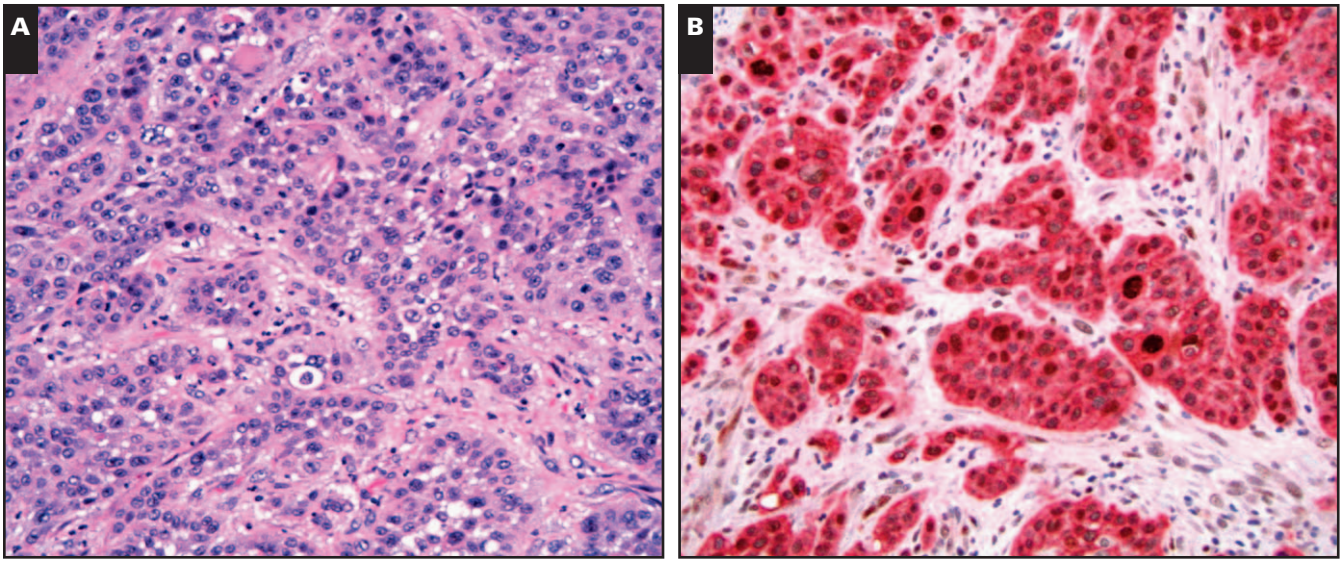

Image 8 H&E (**A**) and double-stain p53 (brown)/CK5 (red) (**B**) staining squamous differentiated invasive UC in the fibromuscular stroma (×20).

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CK5, in situ UC was predominantly negative, thus offering slightly greater distinction between in situ UC and prostatic basal cells compared with CK5/6. Both CK5/6 and CK5 had higher expression in invasive than in situ UC with increased reactivity noted in cases containing squamous differentiation. Although we did not identify past reports of CK5/6 in UC involving the prostate, positivity for CK5/6 has been demonstrated in approximately 50% of cases of UC primary to the bladder.[18] Our results were comparable for both in situ and invasive UC, with the latter showing an increased staining intensity. No published data have addressed the expression of CK5 in UC.

Double-stain IHC has been used to aid in the differential diagnosis of atypical prostatic glands vs prostatic adenocarcinoma.[16] However, this technique has not been described for evaluating challenging foci of UC in the prostate. CK5/6 has been used in double-stain IHC as a prostatic basal cell marker; however, CK5 was favored over CK5/6 in our study because it had slightly less reactivity with in situ UC.[16] CK7 and p53 were known to be reliable single markers for UC in the bladder.[19-25] We demonstrated that the novel combinations of CK7 (red)/CK5 (brown) and p53 (brown)/CK5 (red) consistently highlighted basal cells surrounding in situ UC in the prostate. The use of 2 IHC markers in a double-stain allowed for clear discrimination of in situ UC from invasive UC on one slide. Every case of in situ UC expressed both CK7 and p53 with these double-stains. CK5 had variable reactivity in the invasive tumor and did not show circumferential staining around invasive tumor nests. All invasive UC specimens were positive for CK7 and more than 80% expressed p53, similar to the expression reported for UC localized to the bladder.[19-25] Therefore, the use of CK7 (red)/CK5 (brown) or p53 (brown)/CK5 (red) is recommended to aid in the interpretation of foci of UC equivocal for invasion in the prostate.

Multiple IHC antibody markers have shown reactivity either in prostate tissue or UC in the prostate. Several were evaluated for their reliability in differentiating in situ and invasive UC in the prostate, but were not found to be helpful. HMWK and p63 are expressed in both basal cells and UC, and no differential staining was appreciated.[16,26-28] Weak reactivity was observed in prostatic basal cells of nonneoplastic prostate using PSA and PSAP, but no reactivity was noted in basal cells surrounding in situ UC. CD44s, reported to be positive in prostatic basal cells, did demonstrate expression in basal cells, yet components of the fibromuscular stroma were also positive and had high background, impairing interpretation.[29] Although AR was weakly expressed in basal cells, stronger reactivity was found in interglandular fibromuscular stroma, making identification of basal cells difficult.[30] Laminin, a basement membrane marker, was circumferentially positive surrounding in situ tumor, but was difficult to interpret because of widespread reactivity in vascular smooth muscle and nerve fiber tissue.[31] As other authors indicate, CD141 and CK20 did have high sensitivity for UC, but were suboptimal for our purpose because of inadequate specificity.[19,32] Consequently, the aforementioned IHC markers afforded no clinical usefulness in differentiating in situ from invasive UC in the prostate.

In conclusion, we demonstrate that IHC markers were efficacious in differentiating in situ UC in the prostatic ducts from invasive UC involving the prostatic fibromuscular stroma. Specifically, CK5/6 and CK5 as well as the novel double-stain preparations CK7 (red)/CK5 (brown) and p53 (brown)/CK5 (red) were effective. Additional efforts should be made to evaluate IHC markers to discriminate in situ from invasive UC in the prostate in cases with equivocal histologic features to improve staging accuracy.

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