Immunophenotype of High-Grade Prostatic Adenocarcinoma and Urothelial Carcinoma

Elizabeth M. Genega, M.D., Brian Hutchinson, B.S., Victor E. Reuter, M.D., Paul B. Gaudin, M.D.*

Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York and Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania.

Morphologic features alone can usually be used to distinguish prostatic adenocarcinoma and urothelial carcinoma of the urinary bladder. Poorly differentiated tumors, however, can occasionally have features of both neoplasms, making determination of site of origin difficult. No study has provided a panel of antibodies to assist in the distinction of these two tumors. For this study, 73 examples of moderately and poorly differentiated prostatic adenocarcinoma and 46 examples of high-grade urothelial carcinoma were obtained from radical resection specimens. Immunohistochemical studies were performed using the following panel of antibodies: cytokeratin (CK) 7, CK 20, 34βE12, Leu M1, carcinoembryonic antigen (CEA)m, CEAp, p53, Leu 7, prostate-specific acid phosphatase (PSAP), prostate-specific antigen (PSA), and B72.3. Mucicarmine was also performed. Intermediate and high-grade prostatic carcinoma were compared and then high-grade prostatic carcinoma was compared with high-grade urothelial carcinoma. PSA and PSAP each stained 94% of prostatic adenocarcinomas, but no urothelial carcinomas. Leu 7 stained 94% of prostate and 17% of urothelial carcinomas. Over half of the urothelial carcinomas showed positivity for 34βE12 (65%), as did two cases of prostatic carcinoma (6%). Eighty-three percent of urothelial carcinomas and 12% of prostatic adenocarcinomas stained with CK 7. Forty-one percent of urothelial carcinomas and 12% of prostatic carcinomas were reactive for CEAm, and p53 stained 33% and 3% of urothelial and prostatic adenocarcinomas, respectively. No significant difference was seen in the expression of CEAp, CK 20, B72.3, Leu M1, or mucicarmine between prostate and urothelial carcinoma. We propose a panel of six antibodies to assist in the distinction of high-grade prostatic adenocarcinoma from high grade urothelial carcinoma: PSA, PSAP, 34βE12, Leu 7, CK 7, and p53. The first three antibodies should be used initially; if results are negative, the remaining antibodies may be employed.

KEY WORDS: 34βE12; CK 7; immunohistochemistry; Leu 7; prostatic adenocarcinoma; PSA; PSAP; urothelial carcinoma.

Prostate adenocarcinoma and urothelial carcinoma of the urinary bladder are the most common and fifth most common cancers in men, respectively (1). Both neoplasms usually are found in patients older than 50 years; in a subset of patients, both neoplasms occur synchronously or metachronously. Indeed, for patients who undergo cystoprostatectomy for urothelial carcinoma of the bladder, the reported incidence of concurrent prostatic adenocarcinoma is reported to be from 46 to 70% (2–4).

At Memorial Sloan-Kettering Cancer Center (MSKCC), we frequently encounter patients with bladder neck tumors that clinically and pathologically are not clearly urothelial carcinoma or prostatic adenocarcinoma. Cystoscopy typically reveals a mass at the bladder neck, whereas the remainder of the bladder is unremarkable. Biopsies show a tumor that is often high grade and has morphologic features of both urothelial carcinoma and prostatic adenocarcinoma.

The purpose of this study was to compare the immunohistochemical (IHC) profile of intermediate-grade with that of high-grade prostatic adenocarcinoma. The purpose was also to compare the IHC profile of high-grade prostatic adenocarcinoma with that of high-grade urothelial carcinoma. The latter was done in an attempt to determine an IHC panel, which enables us to distinguish prostatic adenocarcinoma from urothelial carcinoma.

MATERIALS AND METHODS

The surgical pathology files of MSKCC were searched electronically for radical cystectomy, cystoprostatectomy, and prostatectomy specimens containing prostatic adenocarcinoma or urothelial carcinoma. Specimens subjected to neoadjuvant therapy were excluded from further study. Seventy-three examples of known prostatic adenocarcinoma and 46 examples of known urothelial carcinoma were included in this study. Prostatic adenocarcinomas were graded using the Gleason grading system (5, 6). All invasive urothelial carcinomas were considered to be high grade, as is the convention at our institution for locally advanced, high-stage tumors. We do not employ a grading system for invasive urothelial carcinoma, because we have found the pathologic stage and not the grade of the tumor to be an important predictor of outcome (unpublished data).

Tissue sections were assessed for high-grade areas that were then matched to the corresponding formalin-fixed, paraffin-embedded tissue block. One 3-mm punch biopsy was obtained of high-grade tumor from each tissue block, and the punch biopsies were then re-embedded as tissue multiblocks. Each multiblock contained 20 to 24 tumor samples. We chose multiblocks for this study because each punch biopsy approximates the amount of tumor seen in routine needle biopsies or transurethral resections, and the multiblocks allow for many tumor samples to be analyzed on a single slide. The multiblock technique has been shown previously to be a useful, low cost method for studying IHC antibodies (7, 8).

From each multiblock, 4-μm sections were stained with hematoxylin and eosin (H&E), mucicarmine, and the 11 IHC stains listed in Table 1. The immunohistochemical reactions were performed using the streptavidin-biotin horseradish peroxidase conjugation method, as described previously (9), and the antigen retrieval methods as listed in Table 1.

The IHC reactions were scored as positive for any percentage of cell reactivity. The p53 and mucicarmine stains were scored differently. For p53, at least 20% of the tumor cell nuclei had to stain to be considered positive. For mucicarmine, only intracytoplasmic staining was scored as positive. Staining intensity was scored as weak or strong with all antibodies. The intensity was considered strong if immunoreactivity could be detected at 10× magnification.

Statistical analyses were performed using Fisher's Exact Test. All comparisons were made at a significance level of $P < 0.05$. The calculations were performed using Prism software (ver. 3.0; Graph Pad, San Diego, CA, USA).

RESULTS

The combined Gleason score distribution of our 73 cases of prostatic adenocarcinoma is shown in Table 2. Of the 73 cases, 27% were classified as Gleason score 6, 26% as Gleason score 7, and 46% as Gleason score ≥ 8.

Intermediate- versus High-Grade Prostatic Adenocarcinoma

We initially assessed the immunophenotype of prostatic adenocarcinoma, and compared intermediate-grade prostatic adenocarcinoma (Gleason 6 & 7) to high-grade prostatic adenocarcinoma (Gleason ≥8) (Table 2). Carcinoembryonic antigen (CEAp; $P < 0.0001$) and cytokeratin (CK) 7 ($P = 0.0280$) showed a statistically significant decrease in the amount of staining in high-grade tumors, whereas Leu M1 ($P = 0.0150$) showed significantly greater staining in high-grade tumors. Prostate-specific acid phosphatase (PSAP) and Leu 7 showed a smaller but insignificant decrease in the percentage of staining in high-grade tumors. Two cases of high-grade prostatic adenocarcinoma expressed 34βE12.

High-Grade Prostatic Adenocarcinoma versus High-Grade Urothelial Carcinoma

The immunohistochemical profile of high-grade prostatic adenocarcinoma was compared with that of urothelial carcinoma (Table 3). Prostate-specific

Table 1. Immunohistochemical Antibodies

Antibody	Clonality	Antigen retrieval	Manufacturer
CK 7	Monoclonal	Pronase	Dako (Carpinteria, CA)
CK 20	Monoclonal	Protease 24	Dako
34βE12	Monoclonal	Protease 24	Dako
Leu M1 (CD15)	Monoclonal	None	Dako
CEAm	Monoclonal	Citric acid	Dako
CEAp	Polyclonal	Trypsin	Dako
p53 (D07)	Monoclonal	Citric acid	Dako
Leu 7 (HNK-1)	Monoclonal	Pronase	Becton Dickinson (Mountain View, CA)
PSAP	Monoclonal	None	Becton Dickinson
PSA	Monoclonal	Citric acid	Biogenex (San Ramon, CA)
B72.3	Monoclonal	Citric acid	Biogenex

Table 2. Immunophenotype of Intermediate- and High-Grade Prostate Cancer.

Gleason score	No. positive (%)										
	PSA	PSAP	Leu 7	CEAm	CEAp	CK 7	CK 20	34βE12	B72.3	Leu M1	p53
6 (n = 20)	19 (95)	20 (100)	20 (100)	1 (5)	19 (95)	4 (20)	5 (25)	0	9 (45)	2 (10)	0
7 (n = 19)	19 (100)	19 (100)	19 (100)	0	19 (100)	10 (53)	6 (32)	0	11 (58)	1 (5)	0
≥8 (n = 34)	32 (94)	32 (94)	32 (94)	4 (12)	16 (47)	4 (12)	8 (24)	2 (6)	18 (53)	11 (32)	1 (3)

PSA, prostate-specific antigen; PSAP, prostate-specific acid phosphatase; CEA, carcinoembryonic antigen; CK, cytokeratin.

Table 3. Immunophenotype of High-Grade Urothelial Carcinoma and High-Grade Prostatic Adenocarcinoma.

	No. positive (%)										
	PSA	PSAP	Leu 7	CEAm	CEAp	CK 7	CK 20	34βE12	B72.3	Leu M1	p53
UCa (n = 46)	0	0	8 (17)	19 (41)	29 (63)	38 (83)	10 (22)	30 (65)	20 (43)	14 (30)	15 (33)
PCa (n = 34)	32 (94)	32 (94)	32 (94)	4 (12)	16 (47)	4 (12)	8 (24)	2 (6)	18 (53)	11 (32)	1 (3)

PSA, prostate-specific antigen; PSAP, prostate-specific acid phosphatase; CEA, carcinoembryonic antigen; CK, cytokeratin; UCa, urothelial carcinoma; PCa, prostatic adenocarcinoma.

antigen (PSA) and PSAP only stained prostatic adenocarcinoma. Leu 7 also stained a significant percentage of prostatic adenocarcinoma, as well as a smaller percentage of urothelial carcinoma. This was found to be statistically significant ($P < 0.0001$). 34βE12, CK 7, and p53 were found to be preferentially expressed by urothelial carcinoma ($P < 0.0001$). CEAm was also preferentially expressed by urothelial carcinoma, but to a less significant degree ($P = 0.0055$). No significant difference was seen between high-grade prostatic adenocarcinoma and urothelial carcinoma in the expression of CEAp, CK 20, B72.3, Leu M1, or mucicarmine.

PSA, PSAP, Leu 7, 34βE12, CK 7, and p53 were determined to be the most useful antibodies for distinguishing between high-grade prostatic adenocarcinoma from high-grade urothelial carcinoma. PSA, PSAP, and Leu 7 were more often expressed in prostatic adenocarcinoma. On the other hand, 34βE12, CK 7, and p53 were more often expressed in urothelial carcinoma. The sensitivity and specificity for each antibody are shown in Tables 4 and 5.

We also evaluated the staining intensity for these six antibodies (Tables 4 & 5). The majority of prostatic adenocarcinoma that expressed PSA, PSAP, and Leu 7 showed strong reactivity with these an-

Table 4. Sensitivity, Specificity, and Staining Intensity of Immunohistochemical Antibodies in High-Grade Prostatic Adenocarcinoma.

Antibody	Sensitivity	Specificity	Staining Intensity	
			Strong	Weak
		%		
PSA	95.8	100	68.8	31.2
PSAP	97.2	100	84.4	15.6
Leu 7	97.2	82.6	96.9	3.1

PSA, prostate-specific antigen; PSAP, prostate-specific acid phosphatase.

Table 5. Sensitivity, Specificity, and Staining Intensity of Immunohistochemical Antibodies in High-Grade Urothelial Carcinoma

Antibody	Sensitivity	Specificity	Staining Intensity	
			Strong	Weak
		%		
34βE12	65.2	97.2	83.3	16.6
CK 7	82.6	75.3	36.8	55.2
p53	32.6	98.6	80	20

CK, cytokeratin.

tibodies. Most urothelial carcinoma that reacted with 34βE12 demonstrated strong positivity, whereas expression of CK 7 was weak for the majority of the tumors.

Several of the known cases of prostatic adenocarcinoma and urothelial carcinoma showed unexpected results. One case of prostatic adenocarcinoma was negative for both PSA and PSAP; it was also negative for CK 7 and 34βE12 but positive for Leu 7 (Figure 1). Two other cases of prostatic adenocarcinoma were negative for Leu 7 but positive for PSA and/or PSAP. With regard to urothelial carcinoma, 4 cases showed no reactivity for CK 7, 34βE12, PSA, PSAP, or Leu 7. Two of these four cases were positive for p53 (Figure 2, A and B). Eight urothelial carcinomas expressed Leu 7, but also expressed CK 7 and/or 34βE12. All seven of these cases were negative for PSA and PSAP.

DISCUSSION

Our results show that a panel of six IHC antibodies is useful in distinguishing high-grade prostatic adenocarcinoma from urothelial carcinoma. These antibodies include PSA, PSAP, Leu 7, 34βE12, CK 7, and p53. A positive result with the antibodies listed in Table 4 supports a diagnosis of prostatic adeno-
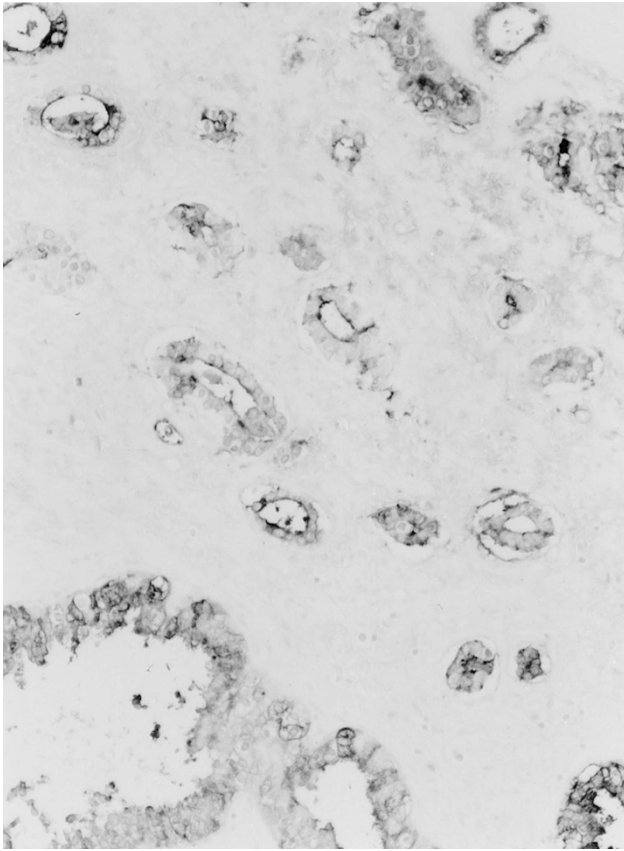
FIGURE 1. Prostatic adenocarcinoma: Positive staining of benign duct and tumor cells with Leu 7.

carcinoma, whereas a positive result with an antibody in Table 5 supports a diagnosis of urothelial carcinoma.

Our study supports the results of others showing PSA and PSAP to be highly sensitive and specific for prostatic adenocarcinoma (10–12). Indeed, we found both PSA and PSAP to be 100% specific for prostatic adenocarcinoma. Like others, we found a rare case of high-grade prostatic adenocarcinoma that was negative for both PSA and PSAP (10, 11, 13–15). Similar to previous reports, none of our cases of urothelial carcinoma of the urinary bladder expressed PSA or PSAP (11, 12).

We found Leu 7 to be a sensitive and relatively specific marker of prostatic adenocarcinoma when compared with urothelial carcinoma. Leu 7 was initially characterized as a human natural killer cell-binding antibody and subsequently was found to bind a variety of carcinomas including prostatic adenocarcinoma (16–19). May *et al.* (17) reported consistent Leu 7 immunoreactivity in primary and metastatic prostatic adenocarcinoma, and Wahab and Wright (19) found Leu 7 immunoreactivity in 95% of prostatic adenocarcinomas. In contrast, we found only 17% of urothelial carcinomas to be Leu 7-positive.

In contrast to previous studies, we found only a minority urothelial carcinomas to be CK 20 positive

(20–22). Reasons for this apparent discrepancy between studies is unclear. Wang *et al.* (22) reported CK 20 expression in 17 of 19 urothelial carcinomas (89%) and used a cutoff similar to ours (more than 1% positive cells). Also using a percentage cutoff similar to ours, Miettinen (20) found CK 20 expression in 58% of urothelial carcinomas. Like Wang *et al.* (22), we found the majority of urothelial carcinomas and a minority of prostatic adenocarcinomas to be positive for CK 7.

Our data show that the high-molecular-weight cytokeratin antibody 34βE12 is an insensitive but highly specific marker in distinguishing urothelial carcinoma from prostatic adenocarcinoma. 34βE12 binds to high-molecular-weight cytokeratins 1, 5, 10, and 14 and has been previously shown to be highly expressed in urothelial carcinoma and only rarely expressed in prostatic adenocarcinoma (23, 24). It is most commonly used to label prostate basal cells and thus distinguish benign prostate lesions from prostatic adenocarcinoma (25–27). Recently, Googe *et al.* (28) reported a high frequency of 34βE12 expression in primary and metastatic prostatic adenocarcinoma. However, our data and that of Yang *et al.* (24), which collectively includes 183 cases of prostate cancer, shows that 34βE12 expression is rare in prostatic adenocarcinoma.

Similar to previous studies, we found almost half of the cases of high grade prostate and urothelial carcinomas (53% and 43%, respectively) to be immunoreactive to B72.3, a monoclonal antibody that binds tumor-associated glycoprotein-72 (TAG-72) (29–33). Although Mazur *et al.* (31) found that the majority of prostatic adenocarcinomas were B72.3 positive, most were either weakly or focally positive. Myers *et al.* (32), on the other hand, found that 31% of prostatic adenocarcinomas were "moderately" or "strongly" B72.3 positive.

The monoclonal antibody Leu M1 is a myelomonocytic cell marker that has been reported to stain a subset of urothelial carcinomas, particularly *in situ* carcinoma (34). For example, Hoshi *et al.* (34) found Leu M1 expression in 50% of *in situ* urothelial carcinomas compared to only 7% invasive carcinomas. We, however, found 30% of invasive urothelial carcinomas to be Leu M1 positive.

Similar to previous studies, we found CEAp to be commonly expressed in urothelial carcinoma (35–37). However, because CEAp is also frequently expressed in prostatic adenocarcinoma, it is not useful in distinguishing prostatic adenocarcinoma from urothelial carcinoma (13). We found urothelial carcinomas to express CEAm more often than prostate carcinomas.

In a subset of cases, p53 immunohistochemistry may help to distinguish urothelial carcinoma from prostatic adenocarcinoma. Mutations of the p53 gene, as determined by p53 immunohistochemis-
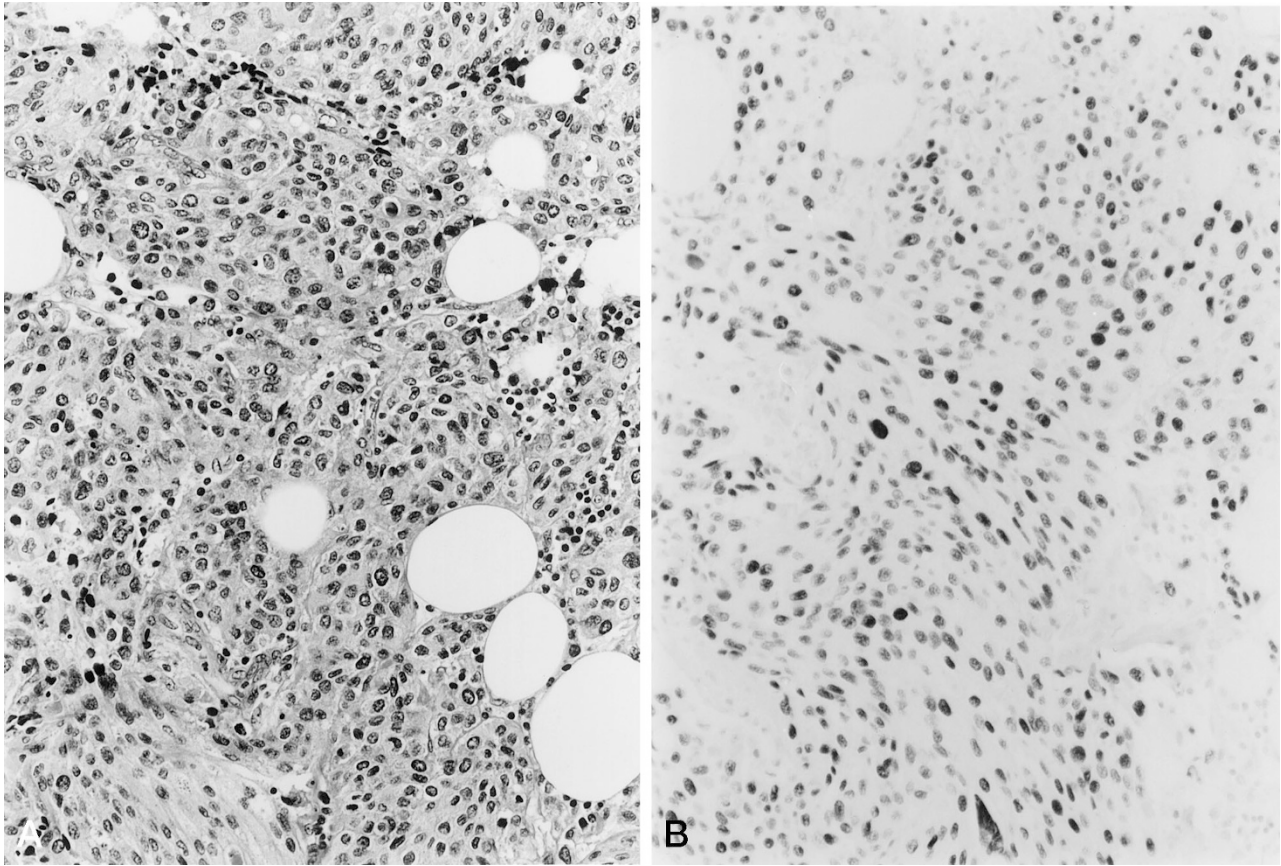
FIGURE 2. Urothelial carcinoma: **A**, high grade urothelial carcinoma involving perivesical adipose tissue. **B**, tumor nuclei show immunoreactivity with p53.

try, are common in invasive urothelial carcinoma and androgen-independent prostatic adenocarcinoma but are uncommon in hormone-sensitive, localized prostatic adenocarcinoma. For example, p53 accumulation or overexpression has been reported in 33 to 81% of urothelial carcinomas, 60% of androgen-independent prostatic adenocarcinomas, and 1 to 19% of hormone-sensitive prostatic adenocarcinomas (38–41).

In conclusion, we describe an immunohistochemical panel including PSA, PSAP, Leu 7, 34βE12, CK 7, and p53, that may be helpful in distinguishing high-grade prostatic adenocarcinoma from high grade urothelial carcinoma in diagnostically difficult cases. A positive stain with PSA or PSAP confirms the diagnosis of prostate carcinoma, and a positive stain with Leu 7 favors this same diagnosis. Currently, there are no markers entirely specific for urothelial carcinoma; however, a positive reaction with 34βE12, CK 7, or p53 supports the diagnosis of urothelial carcinoma. We recommend initially performing PSA, PSAP, and 34βE12. In the event that these three stains are negative, Leu 7, CK 7, and p53 could be used.

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