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Uroplakin II (UPII), GATA-3, and p40 are Highly Specific Markers for the Differential Diagnosis of Urothelial Carcinoma of the Bladder

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Background

Urothelial carcinoma (UC) of the bladder is often associated with high rates of recurrence and progression. About 70% of superficial bladder cancer patients will experience tumor recurrence, and 10–15% of this subpopulation will eventually progress to muscle invasion.[1] Establishing urothelial origin is important for the detection of both primary and metastatic UC of the bladder. Moreover, as UC exhibits a broad range of morphologies, the distinction between UC and other genitourinary carcinomas, such as prostatic and renal carcinomas, can be difficult. Tissue-based markers for the differential diagnosis of UC are essential for the detection of primary bladder tumors.

In the past, Uroplakin III (UPIII) and p63 have been used for the differential diagnosis of UC.[2] Recently, a mouse monoclonal GATA-3 has been reported to identify a high percentage of UCs and breast carcinomas.[3, 4] Mouse monoclonal Uroplakin II (UPII) [BC21] and mouse monoclonal p40 [BC28] antibodies have been recently developed and shown great promise in UC. UPII stained the majority of high grade UCs, and was shown to be negative in various neoplastic tissues.[5] This study evaluated and compared the immunohistochemical staining sensitivity of UPII, GATA-3, p40 and p63 in the detection of UC.

Design

UPII, GATA-3, p40, and p63 (Biocare Medical, Concord, CA) were optimized for IHC staining using an HRP-polymer detection system and visualization with DAB. The four antibodies were tested on a tissue microarray containing 48 cases of UC with confirmed diagnosis, grade, and stage. For each antibody, cases were considered positive if more than 5% of tumor cells were stained. A semi-quantitative scoring system for staining intensity was applied: 0=negative, 1+=weak, 2+=medium, 3+=strong and intensely strong.

Results

In tumors of all grades, UPII, GATA-3, p40, and p63 exhibited sensitivities for UC of 75%, 85%, 85%, and 83%, respectively (Table 1). As isoforms of one another, it is not surprising that p40 and p63 achieved comparable sensitivities (85% and 83%, respectively) (Table 1). However, when comparing staining scores, p40 achieved higher staining intensity than p63. Of the 48 UC cases, 10 cases had a score of 3+ when stained with p40; whereas only 6 cases had a score of 3+ with p63. There were 26 cases stained with p40 had a score of 2+, compared to 15 cases determined with p63 (Table 2). The increase in the number of higher intensity cases observed with p40 was statistically significant when compared with the intensity distribution observed for p63 ($P<.0001$).

The combination of UPII, GATA-3, and p40 increased the staining sensitivity to 94% (45/48) in UC of all grades in both sexes ($P=.02$, combined expression of UPII, GATA-3, and p40 vs. UPII alone), including 88% (14/16) of grade III UC (Table 1). The combination of all 3 antibodies also stained 100% (36/36) of UC cases in men. Importantly the combination of p40, GATA-3, and UPII provided a sensitivity of 75% (9/12) in UC cases detected in women, which was notably higher than the sensitivity of each antibody alone (Table 1).

In tumor of all grades, the co-expression p40 and GATA3 in the same cases was 77.1% (37/48). This co-expression ruled out most breast and lung squamous cell carcinomas, since p40 was negative in breast carcinomas (data not shown) and GATA-3 was negative in lung squamous cell carcinomas (data not shown).

When evaluating UPII, GATA-3, and p40 on prostatic adenocarcinomas (n=84) and renal cell carcinomas (n=70), all tumors were negative for all three antibodies, with the exception of the following cases: one prostatic adenocarcinoma was UPII positive, and one prostatic adenocarcinoma was p40 positive, which was negative with PSA/UPII/GATA-3 and was a poorly differentiated tumor (Table 3).

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Table 1: Comparison of UPII, GATA-3, p40, and p63 in UC cases

Parameters	UPII (+)	GATA-3 (+)	p40 (+)	p63 (+)	Combined expression p40, UPII, GATA-3	Co-expression of p40, GATA-3 in the same case
Grade I (n=15)	14 (93%)	13 (87%)	14 (93%)	13 (87%)	15 (100%)	12 (80%)
Grade II (n=17)	13 (76%)	16 (94%)	16 (94%)	15 (88%)	16 (94%)	16 (94%)
Grade III (n=16)	9 (56%)	12 (75%)	11 (69%)	12 (75%)	14 (88%)	9 (56%)
Grades I, II, III (n=48)	36 (75%)	41 (85%)	41 (85%)	40 (83%)	45 (94%)	37 (77%)
Men (n=36)	29 (81%)	34 (94%)	33 (92%)	33 (92%)	36 (100%)	31 (86%)
Women (n=12)	7 (58%)	7 (58%)	8 (67%)	7 (58%)	9 (75%)	6 (50%)

Table 2: Staining intensity of p40 and p63 in UC cases

Antibody	Positive	Score 0	Score 1+	Score 2+	Score 3+
p40 (n=48)	41 (85%)	7 (15%)	5 (10%)	26 (54%)	10 (21%)
p63 (n=48)	40 (83%)	8 (17%)	19 (40%)	15 (31%)	6 (13%)

Table 3: UPII, GATA-3, p40 and p63 in UC cases

Tissue Types	UPII (+)	GATA-3 (+)	p40 (+)
Prostatic adenocarcinoma (n=84)	1 (1.2%)	0 (0%)	1(1.2%)
Renal cell carcinoma (clear cell, papillary, & various other phenotypes) (n=70)	0 (0%)	0 (0%)	0 (0%)

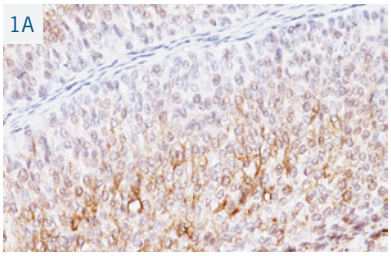
Discussion

Several studies have suggested that p40 is a more reliable marker than p63 for diagnosing pulmonary squamous cell carcinoma.[6] However, it was not fully known if p40 exhibits superior staining characteristics in the detection of UC of the bladder. This study demonstrated that p40 and p63 exhibit comparable sensitivities; however, p40 outperformed p63 in staining intensity, as it stained more cases with scores of 2+ and 3+ than p63 (Table 2).

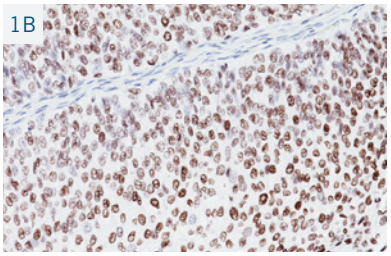
Of 84 prostatic carcinomas, one stained positive with UPII (Table 3), and was likely to be metastatic UC that has spread to the prostate. Shen *et al*. reported that prostatic carcinoma involvement by UC was detected in 32% of prostatic carcinoma cases.[7] Since metastatic UC into the prostate is not uncommon, it is reasonable to speculate that one case of out of 84 cases of prostatic carcinomas could be metastatic UC.

The p40 positive prostatic adenocarcinoma was a poorly differentiated case (Table 3) and was PSA/UPII/GATA-3 negative; however, prostatic squamous cell carcinomas make up about 0.5-1% of all prostatic carcinomas and were reported to be negative for PSA[8]; and thus we could not rule out this possibility.

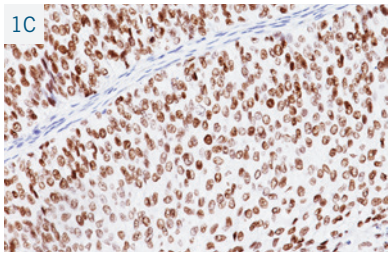
Figures



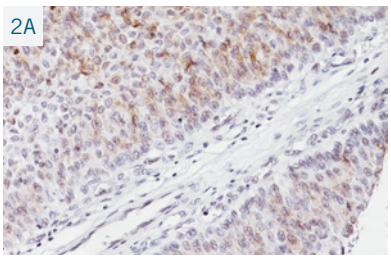
UPII positive staining on serial sections of UC



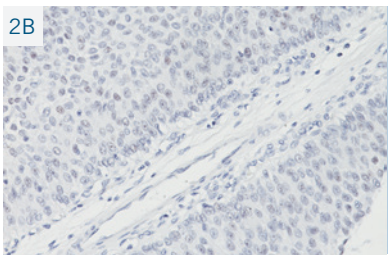
GATA-3 positive staining on serial sections of UC



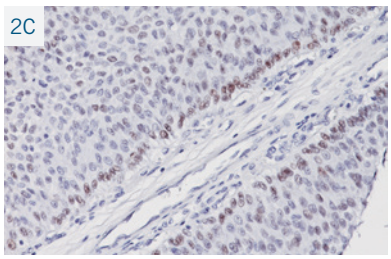
p40 positive staining on serial sections of UC



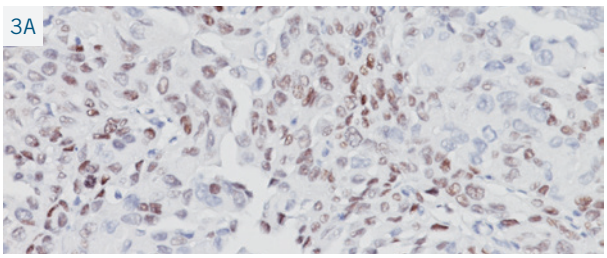
UPII positive staining on serial sections of UC



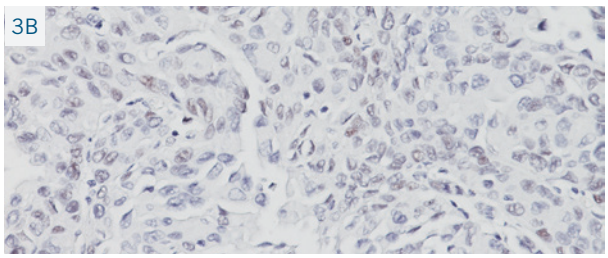
GATA-3 positive staining on serial sections of UC



p40 positive staining on serial sections of UC



p40 positive nuclear staining (2+) on serial sections of UC



p63 marginally positive nuclear staining (1+) on serial sections of UC

Conclusion

The new and novel mouse monoclonal p40 and UPII antibodies when used together in a panel with GATA-3 are very efficient in detecting UC, especially when prostatic and renal carcinomas are also suspected. The combination of these three antibodies provides a very high staining sensitivity, and most important, very high specificity for UC. Therefore, this panel of antibodies is highly recommended for the differential diagnosis of UC.

References

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