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A Newly Developed Anti-Uroplakin II Monoclonal Antibody with Increased Sensitivity in Urothelial Carcinoma of the Bladder

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Introduction

Bladder cancer is the sixth most commonly occurring cancer in the United States with 73,510 new cases estimated in 2012[1]. This cancer is particularly associated with high recurrence and progression rates. About 70% of superficial bladder cancer patients will experience tumor recurrence, and 10-15% of this sub-population will eventually progress to muscle invasion[2]. Early diagnosis, when the disease is still at a localized stage, increases the chance of successful treatment. The survival rate for *in situ* urinary bladder cancer is 97%[3].

Tissue-based biomarkers for early diagnosis of bladder cancer are of major clinical need. Urothelial carcinoma (UC) of the bladder typically originates in the urothelium and accounts for more than 90% of all bladder tumors. Biomarkers expressed in the urothelium, such as uroplakins, could be predictive markers of UC of the bladder. Pathologists have used UP III [AU1] to establish urothelial origin of the bladder; however use of AU1 is limited due to its poor sensitivity.

Uroplakin II (UP II) is a 15 kDa protein component of urothelial plaques that enhance the permeability barrier of the urothelium[4]. Studies have shown UP II mRNA to be expressed in both bladder cancer tissues, and peripheral blood of patients with primary and metastatic UC[5,6]. Little is known about the immunohistochemical protein expression of UP II in UC of the bladder, possibly due to the absence of a commercially available anti-UP II antibody.

This study evaluated the sensitivity and specificity of a newly developed mouse monoclonal anti-UP II antibody [BC21] in UC of the bladder, and was compared to a previously developed mouse monoclonal UP III [BC17] and UPIII [AU1], a well published clone.

Methods

A mouse monoclonal UP II antibody [BC21](Biocare Medical) was developed by immunizing Balb/C mice with a recombinant human UP II protein corresponding to amino acids 26-155, obtained by *E. coli* expression. Monoclonal mouse UP II and UP III antibodies were optimized for IHC staining FFPE bladder cancer tissues, using an HRP-polymer detection system and visualization with DAB.

Tissue microarrays (TMAs) of 178 cases of UC of the bladder with final diagnosis, grading, staging, various normal and neoplastic tissues were tested with UP II [BC21]. For the comparison between UP II [BC21] and Uroplakin III [BC17] (Biocare Medical), and Uroplakin III [AU1], a TMA containing 56 cases of UC of the bladder was used. The TMAs were either constructed in-house or purchased commercially. UPII was also tested on TMAs and individual cases of various normal and neoplastic tissues for specificity (n=493).

Results

UP II and UP III immunoreactivity were observed in membranous and cytoplasmic staining patterns. Table 1 shows the sensitivity of UP II [BC21] staining 178 specimens of UC of the bladder. 137 of 178 (77%) were found to be positive for BC21. BC21 identified 68 of 83 (82%) of Grade II tumors, and 25 of 44 (57%) of Grade III tumors.

The greater sensitivity of UP II compared to previously developed UP III [BC17] and UP III [AU1] was demonstrated by staining the same 56 specimens with each antibody (Table 2). In all Grades, BC21 identified 44 specimens as positive (79%) compared to 31 specimens (55%) determined to be positive with BC17 ($p<0.002$, BC21 vs. BC17), and 19 cases (34%) positive with AU1 ($p<0.0001$, BC21 vs. AU1). In Grade II specimens, BC21, BC17 and AU1 demonstrated sensitivities of 79% (27 of 34), 53% (18 of 34) ($p<0.02$, BC21 vs. BC17), and 26% (9 of 34) ($p<0.0001$, BC21 vs. AU1), respectively. In Grade III specimens, BC21 and BC17 demonstrated a similar sensitivity of 60% (6 of 10); however, the sensitivity of AU1 dropped to 40% (4 of 10) in Grade III. In many comparisons, BC21 provided a more intense pattern and more tumor cells stained than BC17 and AU1: however the number of total tumor cells was much higher in BC17 and BC21 vs. AU1 (data not shown). UP II antibody was found to be highly specific when evaluated on a variety of normal and neoplastic tissues (Table 3). The staining of UP II was evaluated on 37 cases of 37 FDA normal tissue types (Table 3). Bladder and ureter were the only normal tissues to stain positive . Such staining is expected, considering the known expression of UP II in normal urothelium. UP II did not stain any other normal tissues, thus demonstrating its high specificity.

In addition, Table 3 shows that all cancers were 100% negative with UP II with the exception of the following cases: One prostate cancer case was stained positive with, which was highly likely to be metastatic

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Results Continued

bladder cancer that had spread to prostate, and three kidney cancer cases, which were diagnosed as transitional cell carcinomas from the upper ureters (Table 3). This was to be expected because of the cellular make up of transitional cell carcinomas. UP II did not stain any other cancers, indicating its high specificity.

The mouse monoclonal anti-UP II antibody exhibited improved sensitivity compared to mouse anti-UP III [BC17 and AU1]. Figures 1-3 showed comparisons of BC21 with BC17 and AU1 staining in serial sections of the same bladder cancer specimens that demonstrated the greater sensitivity of UP II. Staining with UP II exhibited strong membrane and cytoplasmic expression, while the staining of BC17 was minimal, and AU1 was completely negative (Figure 1). The case shown in Figure 2 displayed strong staining with BC21 and BC17, but only limited staining with AU1. Figure 3 shows a case that also exhibited strong staining with BC21; in contrast, BC17 and AU1 were completely negative on the same case.

Discussion

The anti-UP II antibody exhibited an increased sensitivity and wider localization pattern compared to anti-UP III antibodies. This is probably due to the superior sensitivity of the UP II antibody, and the two different uroplakin isoforms may have distinct roles in the formation of urothelial plaques. The difference in their function is not fully known; however, mice lacking the UP II gene showed no urothelial plaque formation, while mice lacking the UP III gene still retained small urothelial plaques[7]. If UP II and UP III indeed exhibit non-overlapping functions, determination of either isoform may not be sufficient for the most effective diagnosis of UC of the bladder. In some cases, we did observed much stronger staining for UP III when compared to UP II; however, we did not observed UP II negative or UP III positive staining in the same case. As UP II and UP III [BC17] were both 100% specific for bladder cancer, the combination of these two antibodies in an antibody cocktail may further increase the sensitivity for detection of bladder cancer.

Figures

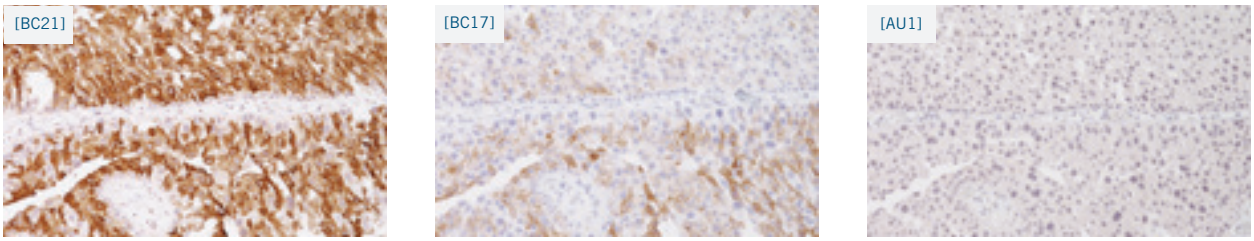

Figure 1: UP II [BC21] and UP III [BC17 and AU1] staining on serial sections of the same bladder cancer tissue (Grade II)

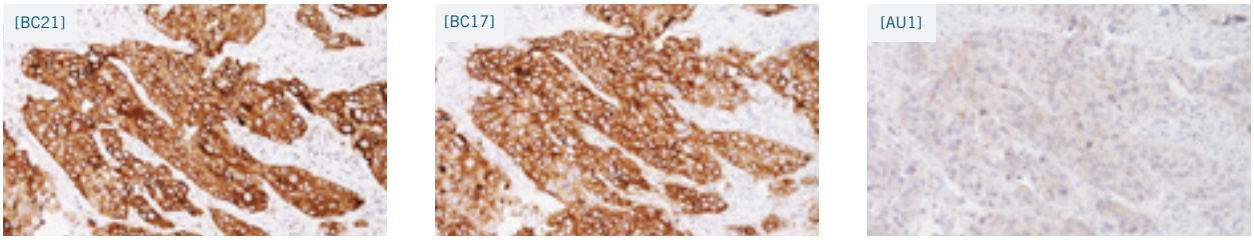

Figure 2: UP II [BC21] and UP III [BC17 and AU1] staining on serial sections of the same bladder cancer tissue (Grade III)

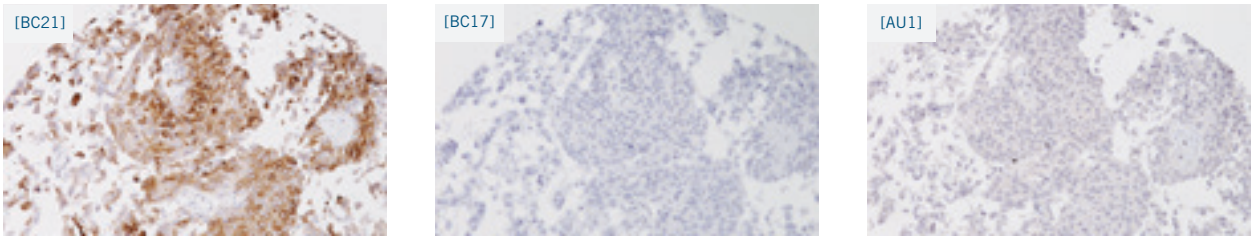

Figure 3: UP II [BC21] and UP III [BC17 and AU1] staining on serial sections of the same bladder cancer tissue (Grade III)

Table 1: UP II [BC21] on UC of the bladder

Grade	Specimens	+ Specimens	% Positive	- Specimens	% Negative
Grades I, II & III	178	137	77%	41	23%
Grade II	83	68	82%	15	18%
Grade III	44	25	57%	19	43%

Table 2: Comparison of UP II [BC21] and UP III [BC17 and AU1] on UC of the bladder

Antibody	Grade	Specimens	+ Specimens	% Positive	- Specimens	% Negative
UP II [BC21]	Grades I, II & III	56	44	79%	12	21%
UP III [BC17]	Grades I, II & III	56	31	55%	25	45%
UP III [AU1]	Grades I, II & III	56	19	34%	37	66%
UP II [BC21]	Grade II	34	27	79%	7	21%
UP III [BC17]	Grade II	34	18	53%	16	47%
UP III [AU1]	Grade II	34	9	26%	25	74%
UP II [BC21]	Grade III	10	6	*60%	4	40%
UP III [BC17]	Grade III	10	6	60%	4	40%
UP III [AU1]	Grade III	10	4	40%	6	60%

*Total number of positive tumors cells stained was much higher in BC21 vs. BC17 and AU1.

Table 3: UP II antibody [BC21] staining of various normal and neoplastic tissues (n=493)

Tissue Types	Cases	Positive	Negative	% +	% -
37 FDA Normal Organ Tissue Array	37	*2	35	5%	95%
Prostate cancer	88	**1	87	1%	99%
Lung cancer (various phenotypes)	20	0	20	0%	100%
Kidney cancer (various phenotypes)	75	***3	72	4%	96%
Colon cancer	63	0	63	0%	100%
Brain cancer	13	0	13	0%	100%
Lymphoma	25	0	25	0%	100%
Melanoma	19	0	19	0%	100%
Ovarian cancer	11	0	11	0%	100%
Seminoma	14	0	14	0%	100%
Breast cancer	74	0	74	0%	100%
Adrenal gland cancer	2	0	2	0%	100%
Thyroid cancer	2	0	2	0%	100%
Pancreas cancer (various phenotypes)	10	0	10	0%	100%
Head & neck cancer (various phenotypes)	10	0	10	0%	100%
Soft tissue cancer (various phenotypes)	10	0	10	0%	100%
Liver cancer (various phenotypes)	10	0	10	0%	100%
Cervix cancer (various phenotypes)	10	0	10	0%	100%

*1 normal ureter and 1 normal bladder, **1 positive case, which could be metastatic bladder cancer that spread to prostate, ***3 positive cases that were transitional cell carcinomas from upper ureters

Conclusion

The monoclonal mouse anti-UPII antibody [BC21] demonstrated increased sensitivity in UC of the bladder when compared to mouse monoclonal UPIII antibodies [BC17 and AU1]. This antibody exhibited superior specificity, thus making it useful in the identification of tumors of urothelial origin. The highly sensitive and specific UP II may serve as a promising tissue-based biomarker in the differential diagnosis of UC and in the detection of tumor of unknown origin, specifically in cases of metastatic bladder cancer that has spread to the prostate.

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