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A New and Highly Sensitive Uroplakin III Monoclonal Antibody [BC17]: An Immunohistochemical Comparison Study with Clone [AU1]

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Introduction

Uroplakins (UPs) comprise a group of 4 transmembrane proteins (UPs Ia, Ib, II, and III) expressed in the luminal surface of normal urothelial superficial (umbrella) cells, which are specific differentiation products of urothelial cells. Uroplakin III (UP III) is a 47 kDa glycoprotein that may be a useful marker in cancer diagnosis. A mouse monoclonal antibody to Uroplakin III was developed [clone AU1] and offered commercially by PROGEN, Heidelberg, Germany. In a study by Kaufmann et. al., AU1 was shown to be a moderately sensitive and highly specific antibody for urothelial tumors.[1] This study demonstrated an overall sensitivity of 57% for AU1 staining of primary and metastatic urothelial carcinomas. Importantly, this sensitivity was determined using a cut-off value of 1% of tumor cells staining positive. Consequently, the conclusion amongst practicing pathologists is that AU1 is not sufficiently sensitive to be a useful marker in the diagnosis of urothelial carcinoma and it is not commonly used. It is generally known amongst pathologists that the poor sensitivity of anti-UP III [AU1] prevents its use as a reliable marker for bladder transitional cell carcinoma (TCC) and a more sensitive anti-UP III antibody is desired in the field.The staining expression of AU1 clone in our laboratory is generally focal and less than 10% of the tumor cells.

A clear need exists for an anti-Uroplakin III antibody with greater sensitivity than AU1 for use in cancer diagnosis. A new anti-Uroplakin III antibody with increased staining sensitivity, while preserving equal or superior staining specificity compared to clone AU1, has been developed and was tested for sensitivity on urothelial bladder cancers and tested for specificity on various normal and neoplastic tissues.

Materials and Methods

Antibody Production and Purification

UP III antibody clone BC17 was obtained by immunizing Balb/C mice with a recombinant human UP III protein. The immune reactivity to UP III was assessed by direct ELISA on recombinant UP III protein. Mice with the highest titer were chosen for developing hybridomas by cell fusion. A hybridoma clone demonstrating the best reactivity to UP III on human tissues was chosen and designated as BC17. The BC17 clone tested for isotype as a mouse IgG2a/kappa vs. AU1 isotype of IgG1. The BC17 antibody was be produced by large scale tissue culture of the hybridoma cells and by ascites in BALB/c mice. The supernatant and antibody ascites were collected and the antibody was purified by Protein A affinity column. BC17 demonstrated specific reactivity to human UP III protein by ELISA and Western blotting.

Immunohistochemistry (IHC) method with anti-UP III BC17

Sections (~4µm) of formalin-fixed paraffin-embedded tissues were deparaffinized and rehydrated through a series of alcohol/water solutions, followed by blocking of endogenous peroxidases with a 3% hydrogen peroxide solution. Tissues were subjected to heat-induced antigen retrieval using a modified citrate buffer in a pressure cooker (Decloaking Chamber; Biocare Medical) and were heated to 125°C for 30 seconds. The UP III antibodies BC17 and AU1 were applied to target tissues for 30 minutes. Detection of the UP III antibody was accomplished using a MACH 4 Universal HRP-Polymer detection system (Biocare Medical). In a final detection step, 3,3'-diaminobenzidine (DAB) was applied, for visualization. Slides were briefly counterstained in a modified Mayer's hematoxylin.

Results

A variety of normal and neoplastic tissues were evaluated for UP III expression using BC17 and compared to staining patterns using the mouse monoclonal anti-UP III antibody (AU1, PROGEN). Both antibodies were optimized for maximum staining intensity, while minimizing or eliminating background staining. For each antibody, the titer that provided the maximum staining intensity, with the minimal background staining, was used.

Figures 1-4 shows several examples of staining of bladder transitional cell carcinoma by BC17, in comparison to staining with AU1, on a serial section of the same specimen.

As Presented at CAP, Poster Session #100, Poster Assignment #85

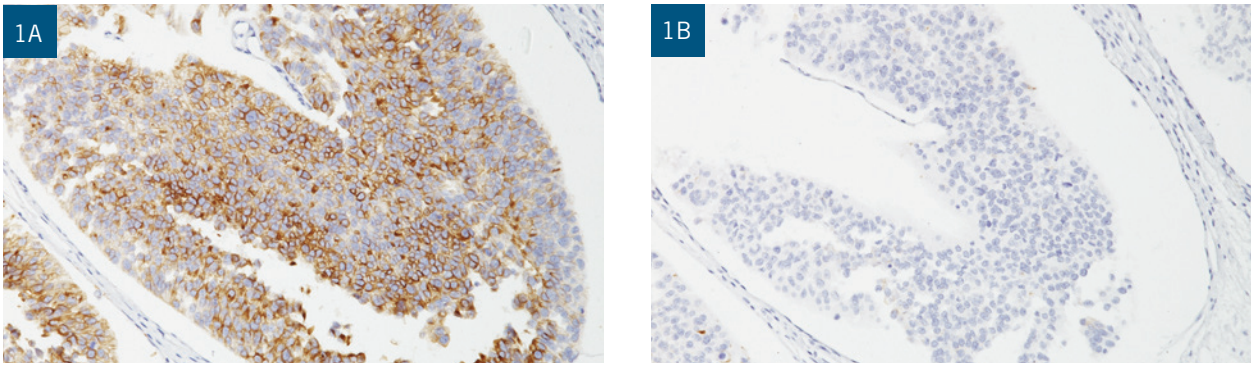

Figure 1: Bladder TCC (grade 2); 1A: Stained with BC17; 1B: Stained with AU1 (Serial section of same case, 1A)

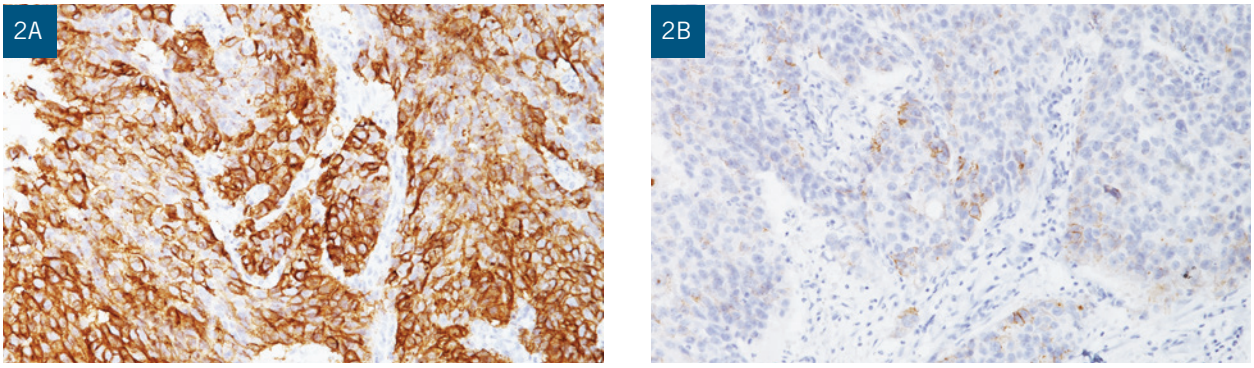

Figure 2: Bladder TCC (grade 2); 2A: Stained with BC17; 2B: Stained with AU1 (Serial section of same case, 2A)

The greater sensitivity of BC17, compared to AU1, was demonstrated by staining the same 59 specimens of TCC of Grades I, II and III with each antibody (Table 1). Using the same criteria, BC17 identified 33 specimens as positive (56%), compared to 18 specimens (31%) determined to be positive with AU1. In Grade II specimens, BC17 and AU1 demonstrated sensitivities of 53% (19 of 36) and 23% (9 of 36), respectively. In Grade III specimens, BC17 and AU1 demonstrated sensitivities of 64% (7 of 11) and 36% (4 of 11), respectively. In each comparison, BC17 was more sensitive than AU1. Importantly, every specimen that was positive with AU1 was also positive with BC17.

Table 1
Comparison of UP III antibodies BC17 and AU1 on Bladder cancer (TCC)

Antibody	Grade	Specimens	Positive Specimens	% Positive	Negative Specimens	% Negative
BC17	Grades I, II & III	59	33	56%	26	44%
AU1	Grades I, II & III	59	18	31%	41	69%
BC17	Grade II	36	19	53%	17	47%
AU1	Grade II	36	9	25%	27	75%
BC17	Grade III	11	7	64%	4	36%
AU1	Grade III	11	4	36%	7	64%

Table 2 shows the sensitivity of BC17 staining 178 specimens of bladder cancer (i.e. transitional cell carcinoma (TCC), or urothelial carcinoma), using a tissue microarray (TMA). Employing a cut-off of ≥1% of tumor cells staining as the criteria to determine a case as "positive" for UP III, and conversely <1% of tumor cells staining as the criteria to determine a case "negative," 97 of 178 (54%) were found to be positive for UP III with BC17. Diagnosis of tumors of higher grade can sometimes be a challenge. In these specimens, BC17 identified 46 of 78 (59%) of Grade II tumors, and 18 of 41 (42%) of Grade III tumors.

Table 2

UP III (BC17) on Bladder cancer (TCC)

Grade	Specimens	Positive Specimens	% Positive	Negative Specimens	% Negative
Grades I, II & III	178	97	54%	81	46%
Grade II	78	46	59%	32	41%
Grade III	43	18	42%	25	58%

BC17 was found to be highly specific when evaluated on a variety of normal and neoplastic tissues (Table 3). Bladder was the only normal and neoplastic tissues to stain positive with BC17. Such staining is expected, considering the known expression of UP III in normal urothelium. BC17 did not stain any other normal or neoplastic tissues, demonstrating its high specificity.

Table 3

BC17 staining of various normal and neoplastic tissues

Tissue Types	Cases	Positive Cases
Kidney: Clear cell carcinoma	48	0
Kidney: Granular cell carcinoma	13	0
Normal kidney	3	0
Breast ductal carcinoma	144	0
Breast lobular carcinoma	24	0
Normal breast	40	0
Pancreatic adenocarcinoma	59	0
Pancreatic squamous cell carcinoma	1	0
Normal pancreas	20	0
Seminoma	71	0
B-cell lymphoma	8	0
T-cell lymphoma	1	0
Lung adenocarcinoma	24	0
Lung squamous cell carcinoma	25	0
Lung adenosquamous cell carcinoma	4	0
Small cell lung cancer	10	0
Large cell lung cancer	2	0
Alveolar cell lung cancer	3	0
Lung carcinoid	2	0
Normal lung	3	0
Colon adenocarcinoma	57	0
Colon squamous cell carcinoma	1	0
Normal colon	3	0
Prostate adenocarcinoma	40	0
Normal prostate	8	0
Ovarian serous papillary carcinoma	40	0
Ovarian mucinous papillary carcinoma	1	0
Ovarian clear cell carcinoma	6	0
Ovarian granular cell carcinoma	2	0

Conclusion

The monoclonal mouse anti-UP III antibody BC17 offers distinct advantages with its improved sensitivity, compared to AU1. Clone BC17 demonstrates cases where a pathologist may have been able to definitively identify the presence of urothelial carcinoma with BC17, which would not have been possible with a less sensitive antibody, such as AU1. We suggest using UP III in a panel with p63 and/or GATA3 for differential diagnosis. UP III may also have potential use in a double stain format with GATA3.

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