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A New and Innovative Antibody p16 INK4a [BC42]: An IHC Comparative Analysis with Clone [E6H4]

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Introduction

p16 INK4a is a protein member of the family of cyclin-dependent kinase inhibitor (CKIs). It is a specific inhibitor of CDK4 and CDK6. It is involved in cellular senescence, apoptosis and DNA repair. This protein is a tumor suppressor and any mutation or deficiency in functionality may lead to carcinogenesis. A mouse monoclonal antibody to p16 was developed [E6H4] and offered commercially by ROCHE, Basel, Switzerland. Recent analyses of the p16INK4a gene revealed homozygous deletions, nonsense, missense, or frameshift mutations in several human cancers[1]. Although the frequency of p16 INK4a abnormalities is higher in tumor-derived cell lines than in unselected primary tumors, significant subsets of clinical cases with aberrant p16 INK4a gene have been reported among melanomas, gliomas, esophageal, pancreatic, lung, and urinary bladder carcinomas[2]. p16 immunoreactivity in paraffin-embedded tissues has also been shown to be an independent predictor in minimally invasive urothelial bladder cancer; a prognostic factor in non-small cell lung carcinoma; and has been shown to predict a positive response to chemoradiotherapy in Stage IV head and neck squamous cell carcinoma[3-6].

A new anti-p16 antibody was needed as an alternative to the current E6H4 for use in cancer diagnosis. Anti-p16 INK4a antibody [clone BC42] has been developed with equivalent high staining sensitivity and specificity compared to clone E6H4, and was tested for sensitivity on cervical cancers and specificity on various normal and neoplastic tissues.

Materials and Methods

Antibody Production and Purification

p16 INK4a antibody clone BC42 was obtained by immunizing Balb/C mice with a recombinant human p16 protein. The immune reactivity to p16 was assessed by direct ELISA on recombinant p16 protein. Mice with the highest titer were chosen for developing hybridomas by cell fusion. A hybridoma clone demonstrating the best reactivity to p16 on human tissues was chosen and designated as BC42. The BC42 clone tested for isotype as a mouse IgG1/kappa vs. E6H4 isotype of IgG2a. The BC42 antibody was 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. BC42 demonstrated specific reactivity to human p16 protein by ELISA and Western blotting.

Immunohistochemistry (IHC) method with anti-p16 BC42

Sections (~4µm) of formalin-fixed paraffin-embedded tissues were deparaffinized, 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 110°C for 15 minutes. The p16 antibodies BC42 and E6H4 were applied to target tissues for 60 minutes. Detection of the p16 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 (30; Table 1) and neoplastic (12; Table 2) tissues were evaluated for p16 expression using BC42 and compared to staining patterns using the mouse monoclonal anti-p16 antibody (E6H4, ROCHE). 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.

Table 1 - BC42 and E6H4 comparison staining of various normal tissues

Normal Tissue Cross-Reactivity					
Tissue	**Total Cases**	**BC42 Positive**	**% Positive**	**E6H4 Positive**	**% Positive**
Cerebrum	3	3	100.00%	3	100.00%
Cerebellum	3	3	100.00%	3	100.00%
Adrenal	3	3	100.00%	3	100.00%
Ovary	3	2	66.70%	2	66.70%
Pancreas	3	3	100.00%	3	100.00%
Parathyroid	3	1	33.30%	1	33.30%
Pituitary	3	3	100.00%	3	100.00%
Testis	3	0	0.00%	0	0.00%
Thyroid	3	0	0.00%	0	0.00%
Breast	3	3	100.00%	3	100.00%
Spleen	3	3	100.00%	3	100.00%
Tonsil	3	3	100.00%	3	100.00%
Thymus	3	3	100.00%	3	100.00%
Bone Marrow	3	3	100.00%	3	100.00%
Lung	3	0	0.00%	0	0.00%
Heart	3	0	0.00%	0	0.00%
Esophagus	3	2	66.70%	2	66.70%
Stomach	3	3	100.00%	3	100.00%
Small Intestine	3	2	66.70%	3	100.00%
Colon	3	1	33.30%	1	33.30%
Liver	3	1	33.30%	1	33.30%
Salivary Gland	3	3	100.00%	3	100.00%
Kidney	3	2	66.70%	2	66.70%
Prostate	3	3	100.00%	3	100.00%
Uterus	3	2	66.70%	2	66.70%
Cervix	3	1	33.30%	1	33.30%
Skeletal Muscle	2	0	0.00%	0	0.00%
Skin	3	3	100.00%	2	66.70%
Peripheral Nerve	3	1	33.30%	3	100.00%
Linging Cells	3	*	N/A	*	N/A

* + in lung; - in muscle & fat

Table 2 - BC42 and E6H4 comparison staining of various neoplastic tissues

Diseased Tissue Specificity and Sensitivity					
Tissue	**Total Cases**	**BC42 Positive**	**% Positive**	**E6H4 Positive**	**% Positive**
Cervical intraepithelial neoplasia	24	16	**66.70%**	16	**66.70%**
Cervical adenocarcinoma	22	13	**59.10%**	12	**54.50%**
Cervix squamous cell carcinoma	16	16	**100.00%**	16	**100.00%**
Head and neck cancer	12	4	**33.30%**	4	**33.30%**
Bladder Cancer	39	28	**71.80%**	28	**71.80%**
Breast Cancer	29	26	**89.70%**	26	**89.70%**
Colon Cancer	35	28	**80.00%**	28	**80.00%**
Lung Cancer	48	25	**52.10%**	26	**54.20%**
Endometrium cancer	48	42	**87.50%**	43	**89.60%**
Ovarian Cancer	12	10	**83.30%**	10	**83.30%**
Prostate Cancer	12	10	**83.30%**	10	**83.30%**
Renal Cancer	30	21	**70.00%**	21	**70.00%**

Figures 1-6 Shows several examples of staining of Cervical Intraepithelial Neoplasia (CIN) cell carcinoma, Cervical Squamous Cell Carcinoma, Cervical Adenocarcinoma, Head and Neck Cancer, Endometroid carcinoma and Colon Adenocarcinoma by BC42, in comparison to staining with E6H4, on a serial section of the same specimen.

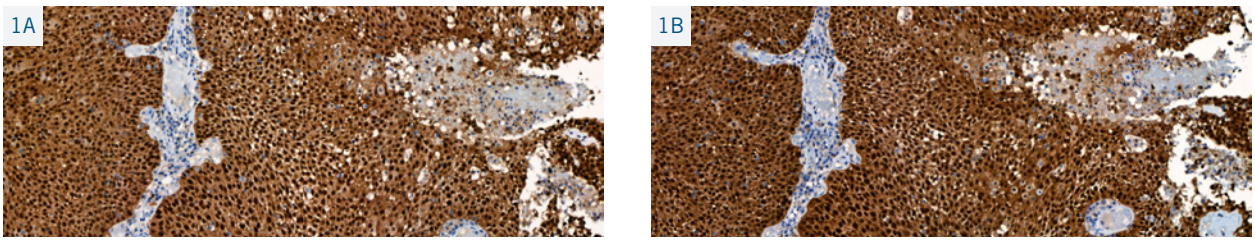

Figure 1: Cervical Intraepithelial Neoplasia (grade 3); 1A: Stained with BC42; 1B: Stained with E6H4 (Serial section of same case, 1A)

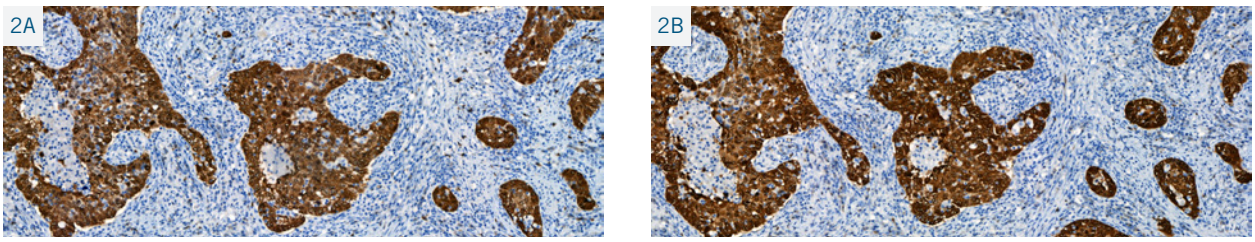

Figure 2: Cervical Squamous Cell Carcinoma (grade 3); 2A: Stained with BC42; 2B: Stained with E6H4 (Serial section of same case, 2A)

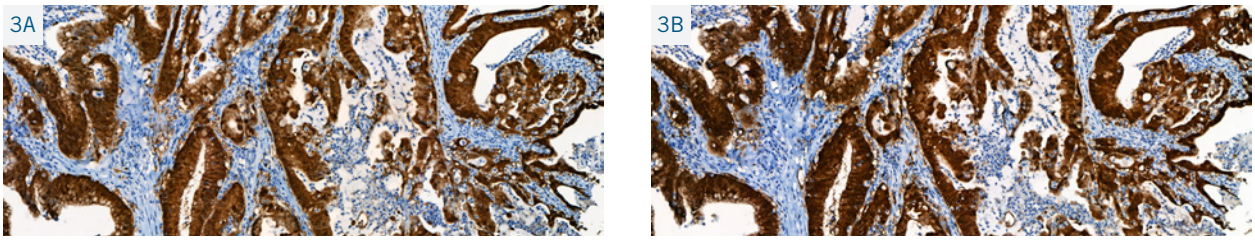

Figure 3: Cervical Adenocarcinoma (grade 2); 3A: Stained with BC42; 3B: Stained with E6H4 (Serial section of same case, 3A)

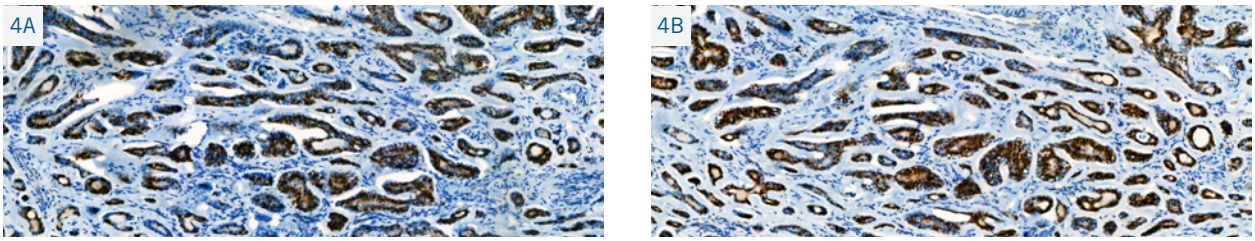

Figure 4: Salivary Gland (adenoid cystic carcinoma); 4A: Stained with BC42; 4B: Stained with E6H4 (Serial section of same case, 4A)

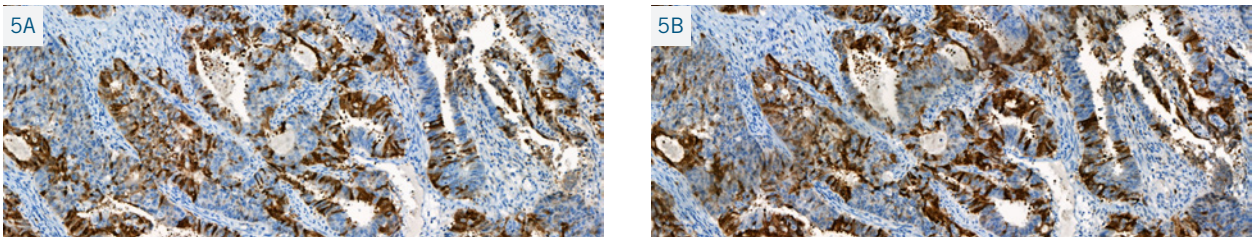

Figure 5: Endometroid Carcinoma (grade 2); 5A: Stained with BC42; 5B: Stained with E6H4 (Serial section of same case, 5A)

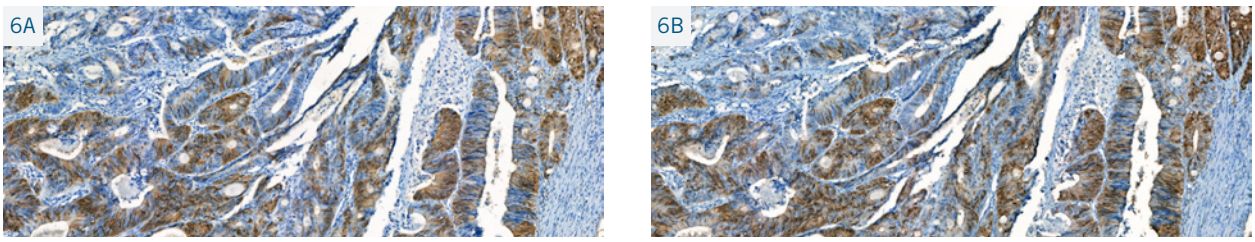

Figure 6: Colon Adenocarcinoma (grade 2); 6A: Stained with BC42; 6B: Stained with E6H4 (Serial section of same case, 6A)

Conclusion

Results show concordant staining regarding sensitivity and specificity of both clones on all tissue tested, whether normal or neoplastic. Notably, clone BC42 showed nearly identical specificity and sensitivity, along with staining pattern, in: cervical adenocarcinoma (22 cases); cervical intraepithelial neoplasia (CIN) (24 cases); cervical squamous carcinoma (16 cases); head and neck cancer (12 cases) and endometrium carcinoma (48 cases). In conclusion, BC42 clone of p16 INK4a antibody offers equally exceptional staining results as the current antibody offered on the market.

References

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