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A Comparison of a Newly Developed Mouse Monoclonal p40 vs. p63 in Basal Cells of Benign Prostate Glands, Prostatic Intraepithelial Neoplasia and Prostate Cancer

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Background

An adjunctive study for the diagnosis of adenocarcinoma of the prostate, particularly when minimal adenocarcinoma may be present, has been the use of immunohistochemistry (IHC) with antibodies directed against basal cells (CK5/14 or 34βE12 (cytoplasmic) and p63 (nuclear)), and p504S (also known as AMACR) directed at prostate cancer. In the past, the IHC staining of basal cells used a single stain and also a negative marker. Currently, many laboratories combine the use of basal cell markers and p504S in an antibody cocktail; thus both negative and positive markers can be observed on a single section.[1]

It has been suggested that p40, a p63 isoform (ΔNp40) is a potential marker for lung squamous cell carcinoma and also a basal cell marker for prostate cancer.[2, 3] Recently, a mouse monoclonal p40 [BC28] has been developed for formalin-fixed paraffin-embedded tissues. In this study, we compare staining patterns and sensitivity/specificity of p40 vs. p63 in basal cells in benign prostate glands, in prostatic intraepithelial neoplasia, (PIN) and in prostate cancers.

Double stain cocktails containing p63 have also been used routinely in the clinical setting for the assessment of PIN and prostatic cancers; therefore, monoclonal mouse p40 will also be evaluated in a double stain cocktail with high-molecular-weight cytokeratins (HMW CK) and p504S for identification of benign glands, PIN and prostate adenocarcinoma.

Design

Immunohistochemistry (IHC)

All tissue sections were deparaffinized in the usual manner and hydrated down to water. Slides were placed in a modified citrate buffer and heated in a pressure cooker (Decloaking Chamber) at 110°C for 12 minutes. Antibodies p63 [4A4] and p40 [BC28] (Biocare Medical, Concord, CA) were titered for optimum dilutions, using a 30 minute incubation time for all antibodies tested. Slides were rinsed in Tris-buffer saline wash buffer (TBS), and incubated for 30 minutes with a biotin-free, goat anti-mouse anti-horseradish peroxidase (HRP) polymer detection system. Tissue sections were visualized with diaminobenzidine (DAB) and briefly counterstained in modified Mayer's hematoxylin. For double stains, an antibody cocktail of HMW CK, p40 or p63, and p504S was applied for 30 minutes, followed by a double stain kit using a goat anti-mouse HRP and a goat anti-rabbit alkaline phosphatase polymer kit (Biocare Medical). Visualization was achieved with DAB and Fast Red chromogens.

Scoring Method

Twenty-five simple and/or radical prostatectomy cases were selected, and in a direct comparison, each case was stained with both p63 and p40. Microscopically, areas of 5 benign glands and 5 PIN glands were selected in each prostatectomy case; and 12 cases of whole mount prostatectomy cases with prostate cancer were selected, based on the presence of cancer. The percentage of positive staining of the basal cells in the circumference of benign and PIN glands, and the nuclear staining in adjacent prostate cancers was recorded for each case.

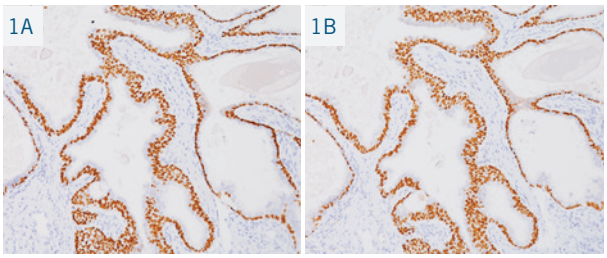
Results

In benign prostate and PIN glands, virtually identical staining was achieved with p40 vs. p63 (Table 1) (Figure 1A-H). In Figure 2A, p40 did not stain striated muscle, however in Figure 2B, p63 did stain the cytoplasm of striated muscle. Nuclear staining with p40 or p63 was not observed in any cases of prostate cancer (Table 2) (Figure 3A-D); however other reports have demonstrated nuclear staining with p63 and with a rabbit polyclonal p40 in prostate cancer (1.4% and 0.6%, respectively).[2] In this study, p40 showed negative staining in cases of prostate cancer (Figure 3A), and displayed no background staining; whereas, p63 also showed negative staining for prostate cancer, but displayed non-specific background staining (Figure 3B).

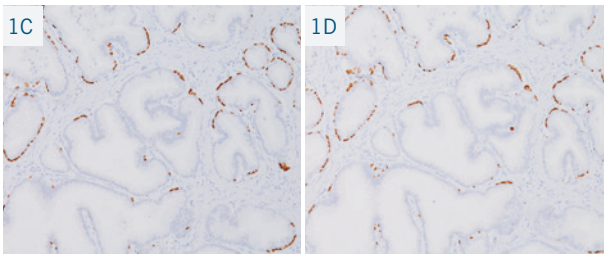
The Multiplex IHC cocktail (double stain) of p40 (DAB) + HMW CK (DAB) + p504S (fast red) provided excellent staining in differentiating prostate cancer from both PIN and benign prostate glands (Figure 4A, B).

As presented at CAP 2013, Poster #151

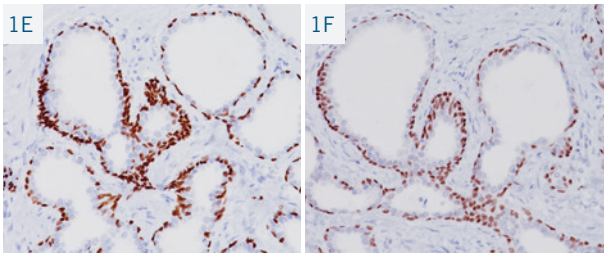
Figures 1-4



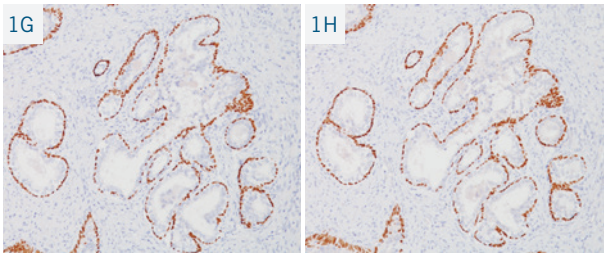
p40 (1A) and p63 (1B) expression in basal epithelium in prostate glands



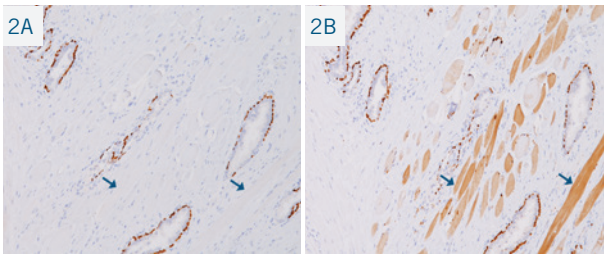
Low expression of p40 (1C) and p63 (1D) in prostate glands



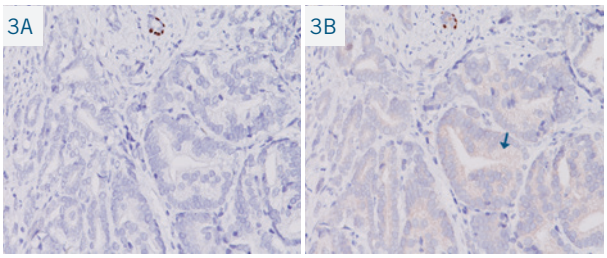
p40 (1E) and p63 (1F) staining in benign prostate glands (sharper staining in Figure 1E)



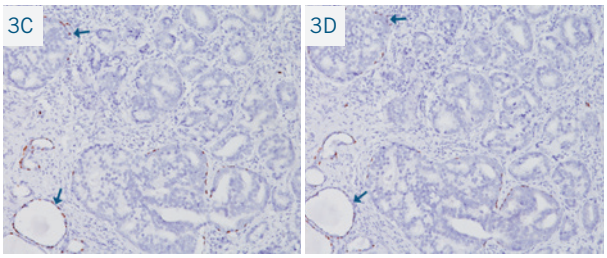
p40 (1G) and p63 (1H) expression in PIN



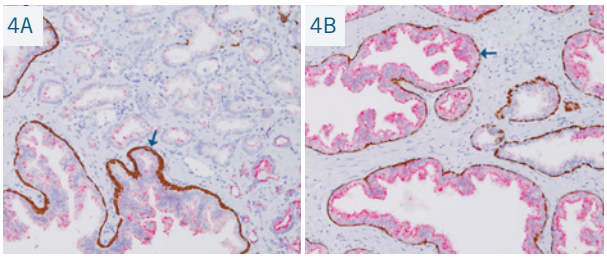
p40 (2A) expression in prostate cancer (no striated muscle staining)(arrows);
p63 (2B) expression in prostate cancer (p63 staining striated muscle)(arrows)



p40 (3A) and p63 (3B) is negative in prostate cancer (Note: p63 cytoplasmic staining)(arrow)



p40 (3C) and p63 (3D) negative in prostate cancer (Note: p40 has sharper staining in PIN and benign glands vs. p63)(arrows)



(4A) PIN40 antibody cocktail in high grade PIN adjacent to prostate cancer (arrow);
(4B) PIN40 antibody cocktail in high grade PIN.

Table 1: Prostate gland circumference staining (% of basal cell staining)

Benign gland (n=125)		PIN (n=125)	
p40	p63	p40	p63
68.20%	67.60%	71.10%	70.20%

Abbreviations: BPH (benign prostate hyperplasia), PIN (prostatic intraepithelial neoplasia)

Table 2: Prostate Adenocarcinoma: Whole-Section Radical Prostatectomies (n=12)

Primary Antibody	Number of Specimens	Number of (+) Specimens	% Positive
p63 [4A4]	12	0	0%
p40 [BC28]	12	0	0%

Discussion

In a direct comparison of basal epithelial cell staining in prostate tissues, p40 provided equivalent staining to p63, and was virtually background free in all cases. In addition some cases showed sharper, more specific and/or cleaner staining using p40 vs. p63 (Figure 1E, F; Figure 2A, B; and Figure 3A, B).

This is the first study using a p40 mouse monoclonal (patent pending) in prostate cancer, and represents an alternative to p63. In the past, polyclonal p40 antibodies were commercially available, and were shown to have superior specificity in lung cancers, when compared to p63.[3] Sailer *et al,* demonstrated equivocal staining of p40 vs. p63 in the basal cells of prostate; however, the authors concluded that the additional cytoplasmic immunoreactivity (non-specific background) of the rabbit polyclonal p40 narrowed its eligibility for use in antibody cocktails (e.g. with HMWCK and p504S).[2] Thus, development of a mouse monoclonal antibody was needed to potentially reduce non-specific background staining and also eliminate the lot-to-lot variability found using polyclonal antibodies.

A multiplex double stain (PIN40) was developed for prostate cancer that included mouse monoclonal p40, HMWCK and rabbit monoclonal p504S (PIN40). Twenty cases of prostate cancer were compared to the antibody cocktail of p63 + HMWCK + p504S. The PIN40 was equivalent to the analogous cocktail containing p63 (data not shown) (Figure 4A, B).

Conclusion

Mouse monoclonal p40 antibody is highly sensitive for prostate basal epithelial cells and can be substituted for p63; and p40 can also be used in an antibody cocktail with HMW CK and p504S, which is suitable for simultaneous examination on a single section.

Acknowledgement

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References

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