ORIGINAL ARTICLE

p63 as a Complimentary Basal Cell Specific Marker to High Molecular Weight-Cytokeratin in Distinguishing Prostatic Carcinoma from Benign Prostatic Lesions

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SUMMARY

The diagnosis of prostatic carcinoma (Pca) on routine biopsies may be challenging, and to date the commonly used marker to distinguish prostate carcinoma from benign prostatic lesions has been High Molecular Weight-Cytokeratin (HMW-CK). However, the antigen of HMW-CK is susceptible to the effect of formalin fixation and causes frequent loss or patchy staining in the obviously benign glands. More recently, antibodies to p63 have been reported to be more sensitive than HMW-CK for the detection of prostatic basal cells. p63, a homologue of tumour suppressor gene p53, is essential for prostate development and is selectively expressed in the nuclei of basal cells of normal prostate glands. The objective of this study is to compare the sensitivity and specificity of HMW-CK and p63 in distinguishing prostatic carcinomas from benign prostatic lesions, as well as determining their positive predictive values. Seventy-two cases from HUKM (comprising 29 prostatic carcinomas and 43 benign prostatic hyperplasias) were stained for both HMW-CK and p63. The sensitivity of p63 and HMW-CK in identifying basal cells in benign glands was 88.37% and 90.70% respectively. The specificity of both reagents was 100%, and the positive predictive value for both reagents was also 100%. Thus, p63 is a useful complementary basal cell specific stain to HMW-CK, and would be very helpful to practicing pathologists in dealing with difficult cases.

KEY WORDS:
Prostatic carcinoma, Benign prostatic hyperplasia, Immunomarker, Sensitivity, Specificity, p63, HMW-CK

INTRODUCTION

Prostate carcinoma (Pca) accounted for 5.7% of total cancers in males who were 50 years old and above in Malaysia in 2002. Pca was rated to be more common in Chinese compared to Malays and Indians[1]. The diagnosis of Pca on routine biopsies can be challenging when pathologists are faced with certain problems such as limited tissue sample, small foci of carcinoma, or benign mimics of prostate cancer like atrophy and atypical adenomatous hyperplasia[2].

The basal cell marker that is commonly used in distinguishing Pca from benign lesions is High Molecular Weight-Cytokeratin (HMW-CK), which demonstrates presence of basal cells in benign glands (positive reaction) and absence of basal cells in carcinomatous glands (negative reaction)[3]. However, even this popular and widely used basal cell specific marker occasionally demonstrates false negative staining in tumor cells due to the susceptibility of the HMW-CK antigen to the effect of formalin fixation, causing frequent loss or patchy staining in benign glands[3].

The discovery of p63 as a basal cell-specific marker makes it a useful stain in difficult cases to distinguish benign lesions from prostatic carcinoma where staining with only HMW-CK gives equivocal results[3]. Thus, the objective of this study is to determine the sensitivity and specificity of p63 antibody as an immunomarker in distinguishing Pca from benign prostatic lesions, in comparison with HMW-CK.

To date no similar studies have been conducted in Malaysia or South East Asia, to the best of this writer's knowledge. It is hoped that this study will further confirm the usefulness of this antibody in our setting to confirm, or to rule out, a diagnosis of prostatic carcinoma in difficult cases.

MATERIALS AND METHODS

Case Selection

To study the immunohistochemical reaction of prostatic basal cells towards HMW-CK and p63, all cases of histologically unequivocal prostatic carcinoma (Pca) and benign prostatic lesions (needle biopsies, transurethral resection of prostatic chips, prostatectomies) were retrieved from the surgical pathology files of Hospital Universiti Kebangsaan Malaysia (HUKM) from 1st January 2003 to 31st December 2004. The exclusion criteria comprised all cases which were diagnosed before and after the time-frame stated above, cases with tissue blocks that were lost or not available, tissue samples which were insufficient or destroyed, and cases in which the diagnosis was disputed.

A total of 72 cases were selected, comprising 29 cases of prostatic carcinoma and 43 cases of benign prostatic hyperplasia, after applying the exclusion criteria.

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Immunohistochemistry

We used the Monoclonal Mouse Anti-Human p63 Protein, code No. M7247, at a dilution of 1:40, which recognizes all known major isotypes of p63. In the first approach, slides were deparaffinised in xylene and rehydrated in alcohol to distilled water. Optimal target retrieval was performed by immersing the slides in sodium citrate at pH 6, then placing them in a microwave for 25 minutes (70% for first 5 minutes and followed by 30% for 20 minutes), followed by a 20-minute cool-down time at room temperature. Immunostaining was performed using Dako autostainer. The slides were quenched with 3% hydrogen peroxide solution for 10 minutes to block endogenous peroxidase, thus ensuring that only primary antibody and antigen reactions were labelled. The primary antibody was incubated for 30 minutes at room temperature, followed by sequential incubations with biotinylated link antibody for 15 minutes, peroxidase-labeled streptavidin for 15 minutes and substrate-chromogen solution for 5 minutes. The slides were then counterstained with hematoxylin.

The Monoclonal Mouse Anti-Human Cytokeratin HMW Clone 34βE12 was used at 1:60 dilution. The target retrieval and immunostaining processes were performed in a similar manner as to that for p63.

Evaluation of IHC

In the majority of benign prostatic glands, both antibodies demonstrated intense positive basal cell-specific immunostaining with HMW-CK (localized to the cytoplasm) and p63 (localized to the nucleus). For both stains, positive staining was taken as an evidence of benignity whereas negative staining was taken as evidence of malignancy.

The density of basal cell staining and percentage of basal cell-positive glands were evaluated for HMW-CK and p63 in all of the cases. Basal cell staining density was scored as 3+ (strong), 2+ (moderate), or 1+ (weak) while percentage of basal cell staining was scored from 0% to 100%. Basal cell staining was considered positive only if the density was 2+ or more and >10% of the glands were stained, and negative if the score was 1+ or less and ≤10% of the glands were stained.

Statistical Analysis

The sensitivity, specificity and positive predictive values of HMW-CK and p63 were calculated using the following formulae:

Sensitivity = True Positive / True Positive + False Negative x 100%
Specificity = True Negative / True Negative + False Positive x 100%
Positive Predictive Value = True Positive / True Positive + False Positive x 100%

In addition, Epi-info version 3.01 was used to compare the sensitivity between HMW-CK and p63.

RESULTS

From Tables I and II, it is apparent that all of the malignant glands showed total absence of HMW-CK and p63 staining leading to a specificity of 100% for both HMW-CK and p63.

The number of benign glands showing absence of basal cell staining (false negativity) for HMW-CK and p63 were small, accounting for 9.3% of cases (4 cases) for HMW-CK and 11.6% of cases (5 cases) for p63, in which four of these cases were the same. In the remaining case, p63 did not stain the benign glands, but HMW-CK demonstrated strong cytoplasmic staining in a basal cell distribution.

The sensitivity in identifying basal cells in benign glands was 90.70% and 88.37% for HMW-CK and p63 respectively. The positive predictive value was 100% for both HMW-CK and p63.

EpiInfo showed that there is no significant difference in the sensitivity of p63 as compared to the sensitivity of HMW-CK ($\chi^2 = 0.12$, d.f. = 1 and p = 0.7)

DISCUSSION

The diagnosis of Pca on routine biopsies can be challenging when pathologists are faced with certain problems such as limited tissue sample, small foci of carcinoma, or benign mimics of prostate cancer like atrophy and atypical adenomatous hyperplasia[2]. It has been well documented that that benign prostatic glands retain their basal cells while infiltrating adenocarcinomas do not[2,4,5]. Therefore histologically, absence of a basal cell layer provides supportive evidence for prostatic carcinoma (Pca).

Immunohistochemical (IHC) detection of basal cells is widely used to help in the diagnosis or exclusion of Pca. HMW-CK

Table I: Results of HMW-CK Staining in Benign and Malignant Prostatic Glands

Results of HMW-CK Staining	Benign	Malignant	Total
Positive	39	0	39
Negative	4	29	33
Total	43	29	72

Sensitivity of HMW-CK	=	90.70%
Specificity of HMW-CK	=	100%
Positive Predictive Value of HMW-CK	=	100%

Table II: Results of p63 Staining in Benign and Malignant Prostatic Glands

Results of p63 Staining	Benign	Malignant	Total
Positive	38	0	38
Negative	5	29	34
Total	43	29	72

Sensitivity of p63	=	88.37%
Specificity of p63	=	100%
Positive Predictive Value of p63	=	100%

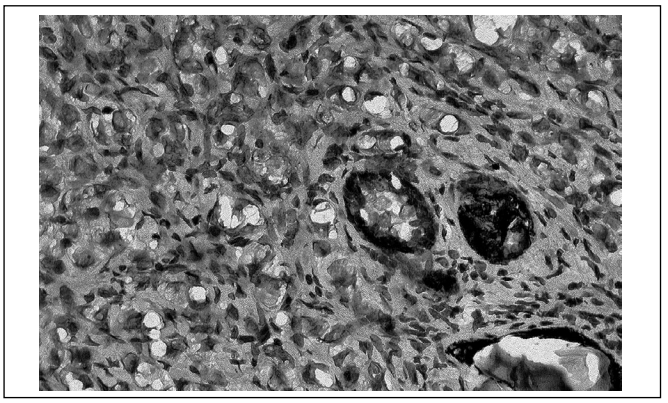

Fig. 1: Prostatic carcinoma showing negativity for HMW-CK within carcinomatous glands (left) and cytoplasmic positivity within the basal cells of a few benign glands (right). (x200)

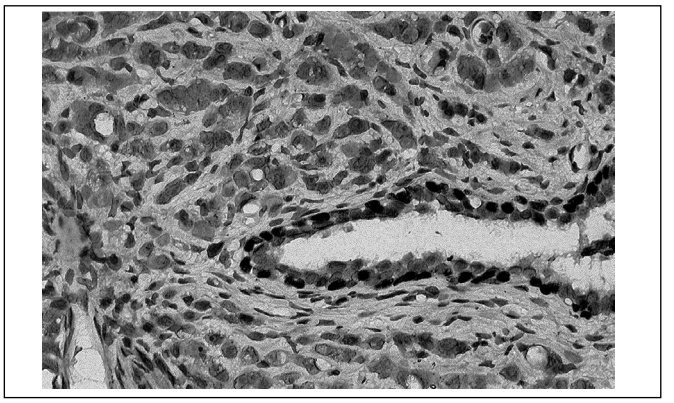

Fig. 2: Prostate carcinoma (Pca) showing negativity for p63 within carcinomatous glands (left), and nuclear positivity within the basal cells of a benign gland (right). (x 200)

has thus far been the most commonly used IHC stain in the diagnosis of Pca. When dealing with small foci of atypical glands with some suspicious (but not diagnostic) architectural or cytologic features of Pca, negative HMW-CK staining in the atypical glands would favour a diagnosis of adenocarcinoma. However, occasional false-negative staining in benign glands and false positive staining in cancer cells have also been reported [6, 7-9].

p63 has recently generated much interest due to its expression in the basal cells of the prostate, and it is essential for prostate development. The critical role of the p63 gene with specific relation to the human prostate gland was first reported by Yang *et al*[10]. Signoretti *et al* also highlighted the role of p63 in the development of prostate gland, and showed that p63 is expressed in virtually all the basal cells of prostatic glands, including a subset negative for HMW-CK[11].

The results of our study demonstrates that p63, like HMW-CK, is specific for basal cells in the prostate gland. None of the 29 cases of prostate needle biopsies with histologically unequivocal Pca demonstrated immunoreactivity for either HMW-CK or p63 (100% specificity).

Our study also revealed that HMW-CK staining (Figure 1) is more eye-catching than p63 (Figure 2) under low magnification. This is because the volume of cytoplasm is larger than nuclei in the same circumference of a benign gland, and many nuclei of basal cells are not seen because of the thin section of the slides. However p63, when positive, showed a consistently strong nuclear signal, which is easier to interpret.

It is common for some benign glands show absence of basal cell staining due to the effects of prolonged formalin fixation, as extended formalin fixation decreases the HMW-CK antigenicity[7,12,13]. Shah *et al* reported that absence of basal cell staining in more than two benign glands occurred in 23% and 9% of needle biopsies stained with HMW-CK and p63, respectively[13]. Similarly, our study also identified rare benign glands showing lack of basal cell staining in nine cases. The overall number of false negative staining of HMW-CK and p63 accounted for only four cases (9.3%) and five cases (11.6%), respectively. This absence of basal cell staining may be attributed to the true absence of basal cells, or diminished or absent gene expression of basal cell markers. Technical variabilities, including those resulting from surgical procedures and antigen retrieval methods could be another important source of negative basal cell IHC reactions. Prostatic glands in the transition zone are especially susceptible to such variability. Multhaupt *et al*. found that 88% of benign glands in the transition zone obtained by transurethral resections of the prostate lost their HMW-CK antigenicity if antigen retrieval was not used[7].

Our study showed that the sensitivity in identifying basal cells in benign glands is 90.70% and 88.37% for HMW-CK and p63, respectively. Hence, HMW-CK is slightly more sensitive in identifying basal cells than p63. However, statistical analysis showed that this difference of sensitivity between HMW-CK and p63 is not significant. Wu et al similarly found that HMW-CK is more sensitive in identifying basal cells than p633. Cost wise, the average cost per test for HMW-CK was noted to be slightly less than p63.

CONCLUSION

This study has demonstrated that p63 has the same specificity and almost the same sensitivity as HMW-CK, and may also be utilized in distinguishing Pca from benign prostatic lesions. In addition, p63 staining which shows a nuclear reaction is easier to interpret than HMW-CK which shows a cytoplasmic reaction. On the basis of our observations, we recommend p63 use for histologically difficult prostate needle biopsies as a supplement stain for equivocal HMW-CK results. However, using p63 also has its disadvantages. Some pathologists refuse to use p63 because it similarly uses a negative reaction to confirm malignancy. Ultimately, understanding the potential pitfalls of IHC stains and paying careful attention to morphologic details are crucial to prevent the false-positive and false-negative diagnoses of Pca in needle biopsies.

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