Anatomic Pathology / P504S/34βE12/p63 COCKTAIL IN LIMITED PROSTATE CANCER

Using an AMACR (P504S)/34βE12/p63 Cocktail for the Detection of Small Focal Prostate Carcinoma in Needle Biopsy Specimens

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Abstract

We assessed the usefulness of immunohistochemical analysis with a 3-antibody cocktail (α-methylacyl coenzyme A racemase [AMACR, or P504S], 34βE12, p63) and a double-chromogen reaction for detection of limited prostate cancer in 138 needle biopsy specimens, including 82 with small foci of prostatic adenocarcinoma and 56 benign prostates. When carcinoma was present, red cytoplasmic granular staining (AMACR) in the malignant glands and cells and dark brown nuclear (p63) and cytoplasmic (34βE12) staining in basal cells of adjacent nonmalignant glands were found. Of 82 cases of small foci of prostatic adenocarcinoma, 78 (95%) expressed AMACR; all malignant glands were negative for basal cell staining. All benign glands adjacent to malignant glands were recognized easily by basal cell marker positivity and little or no AMACR expression. No benign glands were simultaneously positive for AMACR and negative for basal cell markers (specificity, 100%). There were no differences in intensity and numbers of positive glands with double-chromogen staining compared with using 1-color staining. Our results indicate that immunohistochemistry with a 3-antibody cocktail and double chromogen is a simple and easy assay that can be used as a routine test, which overcomes the problems of studying small lesions in prostate needle biopsies with multiple immunohistochemical stains.

With the major effort in the early detection of prostate cancer by mass screening of men, there have been an increasing number of small foci of cancer encountered on prostate needle biopsy specimens.1-3 The diagnosis of these small foci of prostate cancer in needle biopsy specimens is one of the major diagnostic challenges in surgical pathology. Underdiagnosis of a small focus of prostatic adenocarcinoma or the overdiagnosis of a benign lesion mimicking cancer is not uncommon and can cause unfortunate consequences for patients and is a potential liability for pathologists.

It has been shown that using α-methylacyl coenzyme A racemase (AMACR, or P504S) as a positive marker in association with the traditional basal cell–specific 34βE12 and/or p63 as negative markers can help to confirm the diagnosis when small atypical glands are identified by routine H&E staining.4-6 It is crucial to use a combination of positive and negative markers for immunohistochemical analysis to assist in the diagnosis of prostate cancer. The limitation of using a negative marker for diagnosis of the carcinoma is that basal cells can be patchy or discontinuous in some benign lesions. Consequently, negative staining for high-molecular-weight cytokeratin in a few glands suggestive of cancer is not proof of their malignancy because benign glands might not show uniform positivity with this marker.7

Using AMACR as a positive marker alone might be misleading because weak expression of AMACR might be seen in benign glands, and expression of AMACR is seen in high-grade prostatic intraepithelial neoplasia (PIN)8-10 and atypical adenomatous hyperplasia (AAH).11 Therefore, using AMACR as a positive marker along with basal cell–specific negative markers (34βE12, p63) will enhance the diagnostic accuracy in limited prostate cancer and reduce the chance of misdiagnosis.
However, a major restriction of using multiple immunohistochemical stains for small focal cancer is that the atypical glands might disappear on further sectioning. It is not uncommon in clinical practice to be unable to make a definitive diagnosis of a small focal carcinoma because only 1 of the 2 immunostained sections actually shows the lesion in question.

We report the usefulness of a 3-antibody cocktail (P504S + 34BE12 + p63) and 2 chromogens in single paraffin sections to detect limited prostate cancer in prostate needle biopsy specimens. We demonstrate that this technique provides a simple, easy, and sensitive test for helping to confirm or rule out small focal prostatic carcinoma in limited biopsy material.

Materials and Methods

Cases

A total of 138 prostate needle biopsy specimens, including 82 prostate needle biopsy specimens with small foci (≤1 mm or <5% of a core) of prostatic adenocarcinoma and 56 benign prostates were obtained from the surgical pathology files at the University of Massachusetts Medical Center, Worcester. A small focus of prostatic adenocarcinoma was defined as a tumor focus of 1 mm or less in diameter that was present as a single focus in the entire biopsy specimen (n = 68) or in one side of the prostate in addition to carcinoma present in the other biopsy sites (n = 14). The diagnosis of prostate cancer was established from examination of multiple levels of H&E-stained sections by at least 2 surgical pathologists (Z.J. and C.L.) and was confirmed by absence of basal cell staining and/or positivity for AMACR (P504S). All radical prostatectomy specimens from cases with a small focus of prostatic carcinoma in needle biopsy specimens showed residual prostate cancer. No false-positive cases were found in follow-up radical prostatectomy specimens. This study was conducted with institutional review board approval.

Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded tissue blocks were cut into 5-µm sections, transferred to glass slides, and treated with a 0.1-mol/L concentration of citrate buffer, pH 6.0, in an 800-W microwave oven for 15 minutes for antigen retrieval before immunostaining. The slides were stained on an automated immunostainer (DAKO, Carpinteria, CA) using an avidin-biotin complex staining procedure. A cocktail of the 3 antibodies, including a mouse monoclonal antibody (34BE12, DAKO) using an avidin-biotin complex staining procedure. A cocktail of the 3 antibodies, including a mouse monoclonal antibody (34BE12, DAKO) to high-molecular-weight cytokeratin at a dilution of 1:50, a mouse monoclonal antibody to AMACR (P504S, Corixa, Seattle, WA) at 0.5 µg/mL, and a rabbit monoclonal antibody to AMACR (P504S, Corixa, Seattle, WA) at 0.5 µg/mL dilution was mixed and applied to the tissue sections for 45 minutes. After a buffer rinse, the polymer-based secondary antibodies with a mixture of antirabbit–alkaline phosphatase and antimouse–horseradish peroxidase conjugates (Biocare Medical, Walnut Creek, CA) were applied for 25 minutes. For the single-color reaction, all enzymes were developed in the substrate diaminobenzidine for 5 minutes. For double-color reactions, each enzyme was developed separately, beginning with the application of the antimouse–horseradish peroxidase substrate, diaminobenzidine, for 5 minutes for 34BE12 and p63, then the antirabbit–alkaline phosphatase substrate, fast red, for 20 minutes for P504S. After development, the slides were rinsed in distilled water, counterstained with hematoxylin, and rinsed again. The slides were allowed to air dry and were coverslipped with permanent mounting media. In addition, a duplicate set of slides was stained with an irrelevant rabbit monoclonal antibody, anti–von Willebrand factor, for a negative control slide. The irrelevant monoclonal antibody did not stain benign or malignant prostate glands.

Morphologic Evaluation

The Gleason scores were 3 + 3 (6) in 79 specimens, 4 + 3 (7) in 1, and 4 + 4 (8) in 2. Positive AMACR staining was defined as continuous, dark red cytoplasmic staining or apical granular staining patterns in the epithelial cells that could be observed easily at low-power magnification (<×100). Positive basal cell staining was defined as dark brown nuclear and/or cytoplasmic staining in the basal cells.

Results

Prostatic carcinoma showed a red cytoplasmic granular staining pattern of AMACR in the malignant glands and cells and dark brown nuclear (p63) and cytoplasmic (34BE12) staining in basal cells in the adjacent nonmalignant glands Image 11. Of 82 cases of small foci of prostatic carcinoma, 78 (95%) expressed AMACR (P504S), whereas all malignant glands were negative for basal cell staining Table 11. Of 78 cases, AMACR positivity was detected in more than 75% of the malignant glands in 71, 51% to 75% of malignant glands in 4, and 25% to 50% of malignant glands in 3. All benign glands adjacent to the malignant glands were recognized easily by the positivity of basal cell markers (34BE12 and p63) and absence or very low levels of AMACR expression Image 2B (Image 1, Table 1). No benign glands were found simultaneously negative for basal cell markers (34BE12 and p63) and were coverslipped with permanent mounting media. In addition, a duplicate set of slides was stained with an irrelevant rabbit monoclonal antibody, anti–von Willebrand factor, for a negative control slide. The irrelevant monoclonal antibody did not stain benign or malignant prostate glands.

There was no difference in staining patterns between single- and double-color staining Image 3AI and Image 3BI. Although AMACR positivity was easily separable from basal cell staining with the double-color reactions Image 3CI, it
Image 1 | Immunohistochemical stains of prostate needle biopsy specimens with a triple-antibody cocktail (P504S/34βE12/p63) showing small focal prostatic carcinomas (A, Gleason grade 6, ×200; B, Gleason grade 7, ×200) with a red cytoplasmic granular staining pattern of α-methylacyl coenzyme A racemase (P504S) and basal cells with dark brown nuclear (p63) and cytoplasmic (34βE12) staining in adjacent benign glands in the same slides.

Table 1 | Expression of P504S and Basal Cell Markers (34βE12/p63) in Minimal Small Foci of Prostatic Carcinomas and Benign Prostates in Needle Biopsy Specimens

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Cases</th>
<th>P504S+ and 34βE12/p63+</th>
<th>P504S− or ± and 34βE12/p63+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal prostatic carcinoma</td>
<td>82</td>
<td>78 (95)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Benign prostates</td>
<td>56</td>
<td>0 (0)</td>
<td>56 (100)</td>
</tr>
<tr>
<td>Benign glands or prostatic intraepithelial neoplasia adjacent to cancer</td>
<td>82</td>
<td>0 (0)</td>
<td>82 (100)</td>
</tr>
</tbody>
</table>

+, positive; −, negative; ±, focal and weakly positive.

* Data are given as number (percentage).

Image 2 | Comparison of H&E (A, ×200) and a triple antibody cocktail (P504S/34βE12/p63) staining (B, ×200) in a small focal prostatic carcinoma with adjacent high-grade prostatic intraepithelial neoplasia (PIN) on a biopsy slide. The tumor glands show only red cytoplasmic staining (α-methylacyl coenzyme A racemase/P504S, black arrows), whereas adjacent benign glands show only dark brown cytoplasmic and nuclear staining (basal cell, 34βE12 and p63, white arrow), and high-grade PIN shows red cytoplasmic and dark brown cytoplasmic and nuclear staining (arrowheads).
was difficult to distinguish whether the positivity was from the AMACR or from basal cell stains in some malignant glands with a single-color reaction. Four cases with Gleason score 6 were negative for AMACR.

Discussion

We have demonstrated that immunohistochemical analysis with a triple-antibody cocktail (P504S/34βE12/p63) and double color detects small foci of prostatic adenocarcinoma in needle biopsy specimens with several attractive features that make the technique useful as a clinical assay.

First, immunohistochemical analysis with a triple-antibody cocktail and double color is a sensitive assay for the confirmation of small foci of prostate carcinoma. Previous studies have shown that small foci of carcinoma in the needle biopsy specimens express AMACR with 80% to 100% sensitivity. Determination of the sensitivity of AMACR with a triple-antibody cocktail and 2 chromogens is an important issue. Our study showed that AMACR is expressed in 95% of small focal adenocarcinoma.
carcinomas with triple-antibody cocktail staining. The positive rate of AMACR with the triple-antibody cocktail in immunohistochemical analysis is similar to that found in a previous study using single-antibody (P504S) staining.\(^4\) When small atypical glands identified by routine H&E staining are negative for basal cell markers and positive for AMACR/P504S, a malignant diagnosis is established. Although the false-negative rate of P504S staining is very low, it is important to recognize that a negative P504S stain in small “suspicious” glands does not necessarily indicate a benign diagnosis. In suspicious cases with negative AMACR staining, the diagnosis of prostate cancer should be based on architectural and cytologic changes in combination with the absence of basal cells.

Second, immunohistochemical analysis with a triple-antibody cocktail is an assay with high specificity for prostate carcinoma. Although expression of AMACR might increase the confidence level in the definitive diagnosis of small focal prostate cancer, high-grade PIN, AAH, and some benign glands might exhibit some reactivity for AMACR. Because benign glands usually are lined by basal cells, the combination of AMACR/P504S and 34βE12 or p63 can be used to recognize benign glands if both markers are positive in the same gland. Because P504S (cytoplasmic staining) is a rabbit monoclonal antibody and p63 (a basal cell marker, nuclear staining) is a mouse monoclonal antibody, a P504S/p63 cocktail with a single chromogen has been used to detect prostate cancer in tissue sections.\(^1\)\(^3\)-\(^5\) However, Zhou et al.\(^6\) reported that basal cell cocktail (34βE12/p63) stains show the basal cell layers more intensely than p63 or 34βE12 alone and benign glands lack basal cell lining in 2% of glands with cocktail staining. Consequently, 34βE12/p63 cocktail staining not only increases the sensitivity of the basal cell detection but also reduces the staining variability and, therefore, provides more reliable immunostaining results.\(^6\)

Our data show that the triple-antibody cocktail is 100% specific in the detection of small focal cancer because benign glands or high-grade PIN are not P504S+/34βE12–/p63– (Table 1). Because P504S and 34βE12 are cytoplasmic stains, the positive marker P504S cannot be separated clearly from the basal cell marker 34βE12 by a single-color reaction (Image 3D), whereas 2-color immunohistochemical analysis is able to avoid this problem (Images 2B, 3A, and 3C). Although AAH, which was not included in this study, is a lesion that might express AMACR, it retains a basal cell layer\(^7\) that will be positive for 34βE12 and p63. Therefore, the P504S/34βE12/p63 staining cocktail is able to rule out AAH as a cancer diagnosis.

Third, single-step immunohistochemical analysis with triple-antibody staining is applicable to small biopsy samples that contain small lesions requiring immunohistochemical study. A major restriction of using different slides to identify basal cell markers and P504S is that the limited tissue may disappear as the block is cut. If the stains fail or the tissue falls off, performance of additional stains might be difficult because the small lesion might be absent in the recuts. It also might be difficult for pathologists to match up the same glands or population of cells in different histologic sections. Thus, immunohistochemical analysis with a single slide and triple-antibody staining obviates these shortcomings.

Fourth, this is a simple and easy assay that can be applied in routine clinical practice because triple-antibody and double-color immunohistochemical analysis can be used in automated immunostainers and the entire staining process takes only approximately 2 hours.

Although immunohistochemical analysis with the triple-antibody cocktail is a very powerful tool to confirm or rule out prostate cancer in clinical practice, 2 cautions should be exercised in interpreting the results: (1) If atypical glands are too few, even with P504S+/34βE12–/p63– results, a malignant diagnosis should be made with caution because rare small glands adjacent to high-grade PIN with AMACR staining and absence of basal cells might represent an outpouching of the PIN glands because PIN might show discontinuous basal cells. (2) Nephrogenic adenoma (NA) might be positive for AMACR.\(^1\)\(^8\) Although nephrogenic adenoma is a lesion along the urothelial lining with expression of 34βE12 in more than 50% of cases\(^9\) and is rarely present in prostate biopsy specimens, it is possible that a P504S+/34βE12–/p63– staining pattern might be seen in nephrogenic adenoma.\(^1\)\(^8\)

Immunohistochemical analysis with a P504S/34βE12/p63 cocktail and double-color staining provides an assay with high sensitivity and specificity for the detection of small focal prostate carcinoma and can increase diagnostic accuracy. It is a simple and easy test that can be used as a routine assay in clinical practice and will reduce the number of stained slides required in limited biopsy materials.

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References


