Application of combined immunohistochemical panel of AMACR(P504S)/p63 cocktail, cytokeratin 5 and D2-40 to atypical glands in prostatic needle biopsy

Naoto KURODA

Department of Diagnostic Pathology, Kochi Red Cross Hospital, Kochi, Japan

Abstract

Various immunohistochemical studies using cocktail antibodies to elucidate atypical glands in prostatic biopsy specimens have been previously tried. However, there is scantly information on combined cocktail antibodies and other basal cell markers. We investigated the utility of an immunohistochemical panel of AMACR/p63, cytokeratin 5(CK5) and D2-40 for atypical glands in twenty lesions of fourteen patients obtained from prostatic needle biopsy specimens. The final diagnosis of all lesions, including 13 adenocarcinoma demonstrating AMACR+/basal cell(p63, CK5 and D2-40)- pattern, 5 benign lesions noting AMACR-/basal cell+ pattern, and 2 high grade PIN with AMACR+/basal cell+ pattern, were resolved. The immunohistochemical panel of AMACR(P504S)/p63 cocktail, CK5 and D2-40 is useful in deciding the final diagnosis for atypical gland foci in the prostatic needle biopsy specimens and is helpful in the reduction of opportunity of further follow-up or re-biopsy.

Keywords: prostate; needle biopsy; P504S/p63 cocktail

INTRODUCTION

The assessment of small foci of atypical glands in prostatic needle biopsy specimens is a key diagnostic challenge in routine work for pathologists. For this evaluation, a-methyacyl-CoA rasemase (AMACR) is usually used as a cancer-associated positive marker. Additionally, high molecular weight cytokeratin (HMWCK)(Keratin 903), cytokeratin 5/6 and p63 are generally employed as markers of basal cells. Various antibody cocktails have been investigated for the diagnosis of prostatic cancer in needle biopsies, transuretural resections and prostatectomies. We have recently found that D2-40, a lymphatic endothelial cell marker, is also an available basal cell marker of the prostate. We examined the usefulness of an immunohistochemical panel of AMACR/p63 cocktail, cytokeratin 5 (CK5) and D2-40 for atypical gland foci in the prostatic needle biopsy specimens in reaching a definite diagnosis.

MATERIALS AND METHODS

Twenty atypical gland lesions from 14 patients with the initial diagnosis of atypical glands in prostatic needle biopsy specimens between January, 2012 and December 2012 were selected from the archives of the Department of Urology, Kochi Red Cross Hospital. The ages of patients ranged from 61 to 85 years with a mean age of 75.5 years.

Formalin-fixed and paraffin-embedded tissue blocks of the biopsies were retrieved. Histological sections cut were stained with hematoxylin and eosin, and immunostained using the Ventana Benchmark XT autostainer (Ventana Medical Systems, Tucson, AZ), with primary antibodies against AMACR/p63 cocktail (BC4A4(p63), prediluted, Biocare Medical, CA, USA), CK5 (XM26, 1:800, Novocastra, UK) and D2-40 (D2-40, 1:50, DAKO, Glostrup, Denmark). Diaminoenzidine chromogen was used to visualize the antibodies against AMACR/p63 cocktail, CK5 and D2-40.

Immunohistochemical profile

As a rationale for the assessment of immunohistochemical results, the diagnosis of prostatic adenocarcinoma was based on the...
staining pattern of P504S+ for atypical glands and p63-/CK5-/D2-40- for basal cells. In cases showing AMACR+/p63+/CK5+/D2-40+ pattern, the diagnosis of high grade PIN was considered. The AMACR-/p63+/CK5+/D2-40- pattern was suggestive of benign glands. The AMACR-/p63-/ CK5-/D2-40- pattern would indicate atypical glands, namely lesions requiring follow-up.

RESULTS

The final diagnosis after immunohistochemistry is summarized in Table 1. The final diagnosis of all lesions was resolved with immunohistochemical panel of AMACR/p63, CK5 and D2-40. The final diagnosis of the twenty lesions included 13 adenocarcinoma with AMACR+/basal cell- (Figure. 1A-D), 5 benign lesions showing AMACR-/basal cell+ and 2 high grade PIN expressing AMACR+/basal cell+ (Figure. 2A-D). The Gleason score of nine of 13 adenocarcinomas was 3+3=6. The tumour amount of carcinoma cases was usually less than 10%. However, a foamy gland adenocarcinoma (case no.14) had a tumour amount of 70%.

DISCUSSION

When pathologists encounter a small amount of atypical cells or benign mimickers of carcinoma in prostatic needle biopsies, a diagnostic difficulty occurs. Such cases comprise 1.5-9% of prostatic biopsies. In such a circumstance, immunohistochemistry can be a powerful adjuvant for diagnosing limited prostatic cancer. On the one hand, a new marker, a-methyacyl-CoA rasemase, has been identified and many pathologists use the antibody against AMACR in the diagnostic challenging cases now as a positive marker.1,2 On the other hand, antibodies against basal cell markers have traditionally been tried. Keratin 903 is a high molecular weight cytokeratin (HMWCK) that is expressed in the cytoplasm of normal prostatic basal cells. However, benign lesions often show the discontinuous staining for this antibody. p63 is

Table 1: Summary of twenty lesions of atypical glands

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Site</th>
<th>Final diagnosis</th>
<th>Gleason score and amount</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83</td>
<td>rt middle PZ</td>
<td>postatrophic hyperplasia</td>
<td></td>
<td>2mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rt TZ</td>
<td>postatrophic hyperplasia</td>
<td></td>
<td>2mm</td>
</tr>
<tr>
<td>2</td>
<td>71</td>
<td>lt apex PZ</td>
<td>adenocarcinoma</td>
<td>3+3=6, 3%</td>
<td>2mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lt middle PZ</td>
<td>adenocarcinoma</td>
<td>3+3=6, 3%</td>
<td>1mm</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>lt TZ</td>
<td>high grade PIN</td>
<td></td>
<td>1mm</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>lt TZ</td>
<td>postatrophic hyperplasia</td>
<td></td>
<td>2mm</td>
</tr>
<tr>
<td>5</td>
<td>77</td>
<td>lt base PZ</td>
<td>adenocarcinoma</td>
<td>3+5=8, 10%</td>
<td>1mm</td>
</tr>
<tr>
<td>6</td>
<td>82</td>
<td>lt apex PZ</td>
<td>adenocarcinoma</td>
<td>3+3=6, 3%</td>
<td>1mm</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>lt middle PZ</td>
<td>postatrophic hyperplasia</td>
<td></td>
<td>2mm</td>
</tr>
<tr>
<td>8</td>
<td>68</td>
<td>rt apex PZ</td>
<td>adenocarcinoma</td>
<td>3+3=6, 5%</td>
<td>1mm</td>
</tr>
<tr>
<td>9</td>
<td>77</td>
<td>lt TZ</td>
<td>adenocarcinoma</td>
<td>3+3=6, 2%</td>
<td>1mm</td>
</tr>
<tr>
<td>10</td>
<td>81</td>
<td>rt TZ</td>
<td>adenocarcinoma</td>
<td>3+3=6, 3%</td>
<td>1mm</td>
</tr>
<tr>
<td>11</td>
<td>85</td>
<td>rt base PZ</td>
<td>adenocarcinoma</td>
<td>4+5=9, 1%</td>
<td>1mm</td>
</tr>
<tr>
<td>12</td>
<td>76</td>
<td>lt apex PZ</td>
<td>adenocarcinoma</td>
<td>3+3=6, 5%</td>
<td>2mm</td>
</tr>
<tr>
<td>13</td>
<td>75</td>
<td>lt middle PZ</td>
<td>adenocarcinoma</td>
<td>3+3=6, 2%</td>
<td>1mm</td>
</tr>
<tr>
<td>14</td>
<td>79</td>
<td>lt middle PZ</td>
<td>adenocarcinoma</td>
<td>3+3=6, 2%</td>
<td>2mm</td>
</tr>
<tr>
<td>14</td>
<td>79</td>
<td>lt apex PZ</td>
<td>adenocarcinoma, foamy gland</td>
<td>3+3=6, 70%</td>
<td>10mm</td>
</tr>
</tbody>
</table>
PIN COCKTAIL IN PROSTATIC BIOPSY

FIG. 1. Microscopic findings of case 6. (A) HE stain. Crowded glands contain no crystalloid or blue mucin in the glandular lumens. Nuclear enlargement is slight and no prominent nucleoli are evident. It is difficult to judge definitely whether this lesion is adenocarcinoma or benign lesion. After immunohistochemical study, the diagnosis of adenocarcinoma is rendered. (B) AMACR/p63 cocktail. Atypical glands are positive for AMACR, but basal cells in this area are absent. (C) Cytokeratin 5 (CK5). In contrast to normal glands having CK5-positive basal cells, atypical glands possess no basal cells. (D) D2-40. In the area of atypical glands, no D2-40-positive basal cells are seen. Lymphatic endothelial cells are strongly positive for D2-40 and normal glands are surrounded by D2-40-positive basal cells.

a homologue of the \( p53 \) tumor suppressor gene and localized in the nuclei of normal prostatic basal cells.

Previously, various cocktail antibodies including AMACR/p63, AMACR/p63/HMWCK or AMACR/p63/CK5/6 using one or two chromogens have been investigated by some researchers.\(^4\)\(^{14}\) Dabir et al have reported that the specificity of three antibody cocktail including AMACR/p63/CK5 using two chromogens is superior to that of two-antibody cocktail including AMACR/p63 using one chromogen.\(^14\) Ng et al have also reported that the specificity of three cocktail including AMACR/p63/HMWCK using one chromogen is superior to the antibodies used individually. However, the sensitivity of three cocktail is lower than when the antibodies were used separately but not differ substantially.\(^10\) We suggest that it may be difficult for pathologists to interpret results of three cocktail using one chromogen. AMACR/p63 cocktail seems to be widely used in the routine diagnostic procedure now and we routinely employ this double staining cocktail in the pathological diagnosis for prostatic needle biopsy specimens. In our experience, we do not feel the difficulty in the diagnostic evaluation of results of AMACR/p63 cocktail using one chromogen. As it is known that Keratin 903 occasionally shows aberrant expression in prostatic cancer cells, CK5 is superior to Keratin 903.\(^14\) Keratin 903 may be affected by duration of tissue fixation and antigen retrieval.\(^12\) Additionally, we previously reported the diagnostic utility of
D2-40 as a basal cell marker in atypical small acinar proliferation or sclerosing adenosis of the prostate. However, D2-40 is also expressed in fibroblasts or myofibroblasts. Fortunately, prostatic adenocarcinoma seldom shows the desmoplastic reaction in the invasive process. In the present study, there are no lesions to remain unresolved by immunohistochemical panel using AMACR/p63 cocktail, CK5 and D2-40. The use of three basal cell markers can compensate above described individual defects. Accordingly, we usually employ this immunohistochemical panel in the evaluation of atypical glands in the prostatic needle biopsy specimens. However, AMACR/p63 cocktail should take precedence over CK5 or D2-40 because small lesions may disappear in the process of additional specimen cutting for immunohistochemistry. At contrast, we can expect that additional specimens including CK5 and D2-40 may cause the appearance of more plenty carcinoma cells than only AMACR/p63 specimen. The present study included one foamy gland adenocarcinoma. Pathologists need to pay attention to the interpretation of immunohistochemical results of AMACR because the expression rate of AMACR in foamy gland and pseudohyperplastic prostatic cancer is lower than usual adenocarcinoma.

Finally, the immunohistochemical panel of AMACR(P504S)/p63 cocktail, CK5 and D2-40 is available for the decision of final diagnosis for the atypical gland foci in the prostatic needle biopsy specimens. We suggest that this panel can
reduce the opportunity of unnecessary re-biopsy due to the retained atypical glands.

**ACKNOWLEDGEMENT**

No potential conflict of interest relevant to this article was reported. This study was not supported by any funding agency.

**REFERENCES**